Noble metal-dithiocarbamates precious allies in the fight against cancer

Eszter Márta Nagy, Luca Ronconi, Chiara Nardon and Dolores Fregona*

Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy

Abstract: To date, cisplatin and its analogs are among the most effective chemotherapeutic agents for cancer treatment. However, high systemic toxicity and the propensity for patients to develop tumor resistance remain the main challenges in the clinical application. Therefore, the discovery and development of novel active chemotherapeutic agents are largely needed and the research of new metal-based anticancer drugs continues to be a very active international field. In this review paper we aim to give a detailed overview on our research work devoted to the design of novel dithiocarbamato complexes with different noble metals (such as palladium, platinum, copper, ruthenium and gold), which have gained considerable interest in both the development and the treatment of cancer. In particular, we summarize the results of the metal complexes achieved so far, focusing on the gold(III) compounds, that show outstanding *in vitro* and *in vivo* antitumor properties and reduced, or even no, systemic and renal toxicity, compared to the reference drug cisplatin.

Keywords: Anticancer agents, copper, dithiocarbamato complexes, metal-based drugs, gold, ruthenium, platinum, palladium.

INTRODUCTION

The discovery of the anticancer properties of cisplatin (*cis*-[$PtCl_2(NH_3)_2$] Fig. **1**) in the late 1960s, the first inorganic compound to block DNA replication and cell division, has demonstrated that metal complexes can play a significant role in the treatment of cancer opening up new perspectives in the anticancer research based on metallopharmaceuticals. Owing to the intrinsic nature of metal centres, characteristic coordination modes and kinetic properties, metallodrugs act through mechanisms that cannot be mimicked by organic agents. At present, cisplatin is one of the most effective drugs employed to treat testicular and, in combination with other chemotherapeutic agents, ovarian, small-cell lung, bladder, cervical, brain, lung and breast cancer [1]. In spite of having a major role in current oncology, chemotherapy with platinum complexes is frequently accompanied by severe side effects including gastrointestinal symptoms (nausea, vomiting, diarrhea, abdominal pain), renal tubular injury, neuromuscular complications and ototoxicity. In addition, their activity is limited in many widespread tumors due to resistance to the treatment, either acquired during cycles of therapy (as occurs in patients with, for example, ovarian cancer) or intrinsic resistance (such as in patients with, for instance, colorectal, prostate, lung or breast cancer) [2-4]. However, almost forty years after the first report on the antitumor activity of cisplatin, only its analogs, carboplatin and oxaliplatin (Fig. **1**) are in clinical use worldwide, while nedaplatin (Fig. **1**) is used exclusively in Japan [3]. Furthermore, cisplatin and its analogs continue to enjoy the status of the world's best-selling anticancer drugs [3].

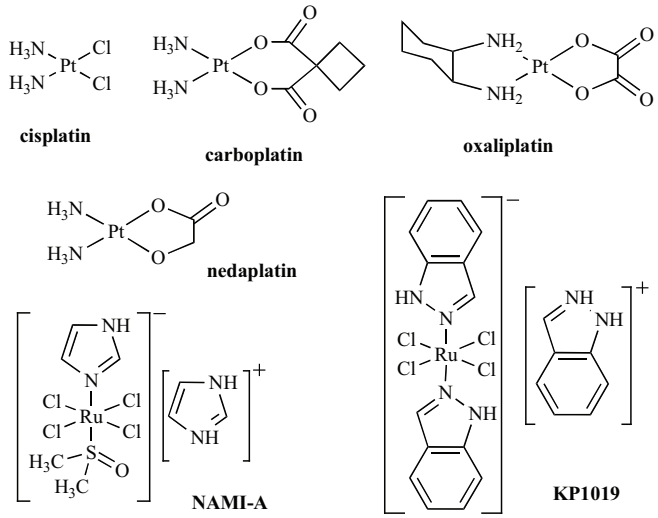

Fig. (1). Platinum(II) complexes in clinical use and ruthenium(III) complexes in clinical trials.

According to the above, the main aims of the modern medicinal chemistry and drug design for the development of novel anticancer metallodrugs are the reduction in the side effects of platinum-based drugs, as well as the enhancement of their therapeutic index and effectiveness against cisplatin-resistant tumors [5]. Therefore, the research has continued to try to elucidate structure–activity relationships, especially in the area of improving new inorganic pharmaceuticals as potential anticancer drugs. To date, a series of platinum- and non platinum-based chemotherapeutic agents have been developed to treat or cure a variety of cancers, but the most of them demonstrate restricted efficacy due to problems of delivery and penetration, and low selectivity for the tumor cells, thus causing severe damage to healthy tissues [5, 6]. Among the non-platinum antitumor agents, gold, ruthenium

*Address corrrespondence to this author at the Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy; Tel: +39-(0)49-8275159; Fax: +39-(0)2-700500560;
E-mail: dolores.fregona@unipd.it

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and copper complexes have recently gained increasing attention due to their strong tumor cell growth inhibiting effects generally achieved by exploiting non cisplatin-like pharmacodynamic and pharmacokinetic properties and mechanisms of action [7-9]. It is well-known that copper is a co-factor essential for tumor angiogenesis process and its levels are increased in various human cancer tissues [10]. Consequently, many copper complexes were synthesized and tested for their anticancer activity *in vitro* and *in vivo* [9]. Gold compounds are natural candidates as potential alternatives to platinum drugs owing to their traditional use in medicine (so called chrysotherapy). However, because of its limited medical application, gold compounds are presently used only for the treatment of severe rheumatoid arthritis [11]. A number of reviews on the use of gold complexes have been published in the recent years, highlighting the special interest in this class of metal complexes as anticancer agents [7, 12-15]. Ruthenium-based complexes have raised great interest in the design and development of novel active chemotherapeutic agents, since several ruthenium compounds display promising antitumoral and antimetastatic properties [16-18]. Furthermore, up to now two ruthenium complexes, NAMI-A (imidazolium *trans*-[tetrachloro(*S*-dimethylsulfoxide)(1H-imidazole) ruthenate(III)]) [19] and KP1019 (indazolium [*trans*-tetrachlorobis(1H-indazole)ruthenate(III)]) [20, 21] (Fig. **1**) have successfully completed phase I and entered phase II clinical trials.

In this context, during the last decade our research group has been designing a new class of metal-dithiocarbamato complexes containing platinum, palladium, gold, ruthenium and copper, potentially able to combine the cytotoxic activity of the metal centres with the lack of nephrotoxicity [22]. The complexes have been tested, at least preliminarily, for their *in vitro* cytotoxic activity toward a panel of human tumor cell lines. Among all, gold(III) complexes have shown outstanding *in vitro* and *in vivo* antitumor properties and reduced or no systemic and renal toxicity, compared to the reference drug [23, 24].

The choice of dithiocarbamato ligands was not accidental but well-considered, since sulfur-containing biomolecules play a significant role in the pharmacodynamic and pharmacokinetic profile of platinum-based drugs. In fact, cysteine-, methionine-containing molecules and glutathione, metallothionein and albumin are characterized by high affinity to the soft platinum(II) ion. The platinum–sulfur interactions are ubiquitous in the human body and many occurrences encountered during the chemotherapy such as uptake, excretion, resistance and toxicity are related to them [25]. However, these interactions are generally believed to have negative effects on the therapeutic efficacy of the platinum drugs, as strong and irreversible binding of the metal to intracellular thiolato ligands is considered as a major inactivation step. In particular, renal toxicity (nephrotoxicity) may result from either too high administered doses or accumulation of platinum in the body. The effects of cisplatin on renal functions are not fully understood, but recent studies have hypothesized that renal failure may be induced by platinum binding to and, consequently, inactivation of thiol-containing renal enzymes [26]. Due to these observations, a number of sulfur-containing nucleophiles have been tested as chemoprotectants to modulate cisplatin nephrotoxicity, and some of them showed promising for clinical use [27]. The chemoprotective effect results from the capability to remove platinum from the thiol groups of proteins without reversal of platinum–DNA adducts, responsible for the antitumor activity. Among the tested compounds, sodium diethyldithiocarbamate was shown to provide protection against renal, gastrointestinal and bone marrow toxicity induced by cisplatin without decreasing the antitumor activity [28]. Nevertheless, the overall nephroprotective benefits of sodium diethyldithiocarbamate are somewhat limited by the acute toxicity profile of dithiocarbamates themselves (*i.e.* not complexed) [29]. Nevertheless, upon coordination to a metal ion, the resulting complexes are expected to be quite stable due to the so-called "chelate effect", and possible decomposition with subsequent loss of the dithiocarbamato ligand is unlikely to occur.

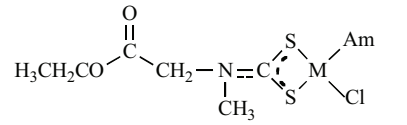
The aim of this review article is to highlight up-to-date results on the anticancer activity of noble metal-dithiocarbamates discussing the complexes with platinum, palladium, copper, ruthenium and gold. In particular, we focalize on the latest in-depth mechanistic studies of the gold(III) complexes that have provided insights into their mechanism of action, thus opening up new prospects for further pharmacological testing and, hopefully, to enter clinical trials.

PLATINUM(II)- AND PALLADIUM(II)-DITHIOCARBAMATES

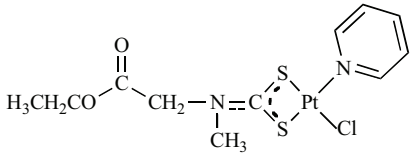
As previously mentioned, the wide success of cisplatin has led to the continuous increase of interest in platinum drugs, promoting even now the development of alternative platinum-based anticancer agents [3]. Several platinum analogs had entered clinical trials but none had provided significant benefit compared with cisplatin, carboplatin or oxaliplatin [30]. In order to obtain compounds with superior chemotherapeutic index in terms of increased bioavailability, higher cytotoxicity, and lower side effects than cisplatin, we have designed a number of Pt(II)- and, given the strict similarity, Pd(II)-dithiocarbamato derivatives ([M^{II}Cl(ESDT)(Am)], Fig. **2**) [31-35]. These complexes were developed in such a way to combine the antineoplastic activity of the metal centre with the desirable reduction of the nephrotoxic side-effects by coordinating dithiocarbamates. The synthesized complexes generally contain: an aliphatic or aromatic amino ligand as most bioactive Pt(II) complexes; chloride as a good leaving group that may undergo hydrolysis and allow subsequent DNA binding, thus resembling the mechanism of action of cisplatin; and a dithiocarbamate ligand potentially able to prevent interactions of the metal centre with sulfur-containing proteins, therefore reducing renal toxicity. All the synthesized complexes were evaluated for their *in vitro* cytotoxic activity on a panel of selected human tumor cell lines.

Among all, the pyridine derivative of Pt(II) ([PtCl(ESDT)(py)], from now on referred to as PTL1, Fig. **2**) showed the most promising antitumor properties. PTL1

showed *in vitro* cytotoxic activity higher or, at least, comparable to cisplatin toward human leukaemic promyelocytes (HL60), human squamous cervical adenocarcinoma (HeLa), human ovarian carcinoma cisplatin-sensitive (2008) and -resistant (C13*), human Burkitt's lymphoma (Daudi), human colon adenocarcinoma (LoVo), human non-small lung adenocarcinoma (A549) and human malignant melanoma (MeWo) cells. Results reported for Daudi, LoVo, A549 and MeWo cells are particularly interesting since those cell lines represent tumor types intrinsically resistant to cisplatin. Furthermore, PTL1 induced a greater inhibition of cell growth than cisplatin on both cisplatin-sensitive (2008) and -resistant (C13*) human ovarian carcinoma cells, and a complete lack of cross-resistance was observed in C13* cells. The resistance factor (R.F. = IC_{50} (C13*)/IC_{50} (2008)) of cisplatin and PTL1 were 19.70 and 1.42, respectively. As decreased drug uptake is often associated with cisplatin resistance, [36] firstly its accumulation in both cisplatin-sensitive and -resistant human ovarian carcinoma cell lines were measured, finding that the uptake of PTL1 by 2008 and C13* cells was five and eight times higher than cisplatin, respectively [37]. Moreover, current data indicate that cisplatin cell uptake is mediated by transmembrane channels and/or high-capacity facilitated transport [38]. Since PTL1 entered both parent cell lines with similar efficiency, this indicates that its uptake is not influenced by modifications in the plasma membrane, thus suggesting a possible alternative uptake mechanism, such as passive diffusion.



[MCl(ESDT)(Am)]



[PtCl(ESDT)(py)] (PTL1)

Fig. (2). Selected Pt(II)- and Pd(II)-dithiocarbamato derivatives: M^{II} = Pt^{II}, Pd^{II}; Am = *n*-pra (*n*-propylamine), *c*-bua (*c*-butylamine), py (pyridine), 2-pic (2-picoline), 3-pic (3-picoline), nor (norphenylephrine), syn (synephrine); ESDT = ethylsarcosinedithiocarbamate.

Cellular DNA is generally accepted as the major biological target of platinum drugs [39]. For this reason, the DNA-binding affinity of PTL1 has been investigated [37]. It was shown to bind calf thymus DNA two-fold more efficiently than cisplatin, with a marked preference for GG-rich sequences. However, when binding studies were carried out *in vivo*, PTL1 induced DNA platination to a lower extent than cisplatin. This result does not correlate with both the *in vitro* cytotoxicity and DNA binding studies. Such a finding may be due to a mechanism of action that involves intracellular targets other than DNA. Furthermore, DNA interstrand and DNA-protein cross-links, as other possible mechanisms of DNA platination have also been investigated [37].

On the basis of the observed remarkable cytotoxic activity of PTL1, further *in vitro* and *in vivo* studies were carried out. PTL1 was shown to overcome acquired resistance to cisplatin when tested towards human squamous cervix carcinoma cisplatin-sensitive (A431) and cisplatin-resistant (A431-R), as well human osteosarcoma cisplatin-sensitive (U2OS) and cisplatin-resistant (U2OS-R) cells, whose resistance is associated with reduced cell uptake, increased tolerance to cisplatin-induced DNA lesions, and defects in the DNA mismatch repair pathway [40]. The results support the occurrence of a different mechanism of action compared to the "classical" platinum-based drugs, possibly involving different types of DNA lesions rather than overcoming specific alterations. In addition, mutagenicity data obtained on human peripheral blood lymphocytes showed that PTL1 induces different DNA repair responses and much lower genotoxicity than cisplatin.

Consequently, *in vivo* experiments were performed on Ehrlich carcinoma-bearing mice. PTL1 resulted to be much more active than cisplatin in the treatment of Ehrlich ascitic carcinoma increasing the life span of treated animals with %T/C (*i.e.* mean survival time of treated tumor bearing mice *vs.* control) of 190% and 129%, respectively. In Ehrlich solid tumor-inoculated mice both PTL1 and cisplatin induced similar tumor size reduction (*ca*. 70%), but treatment with the Pt(II)-dithiocarbamato species was significantly better tolerated, and treated mice rapidly recovered lost body weight, whereas cisplatin-treated animals kept on suffering from weight loss, anorexia and dehydration all treatment long [40].

PTL1 was also tested for *in vitro* and *in vivo* nephrotoxicity. *In vitro* nephrotoxicity was studied by means of a renal cortical slice model in which the kidney slices of young male Wistar rats where incubated with different doses of either PTL1 or cisplatin [41]. Cisplatin was shown to induce five-fold higher lipid peroxidation than PTL1, a clear evidence of a marked renal damage. Moreover, incubation with cisplatin caused a massive reduction of the activity of the enzyme glutamine synthetase (GS) in the kidney cortex, a commonly accepted marker for renal damages occurring in the S3 segment (*pars recta*) of the proximal tubules [42]. On the other hand, PTL1 induced significant lipid peroxidation and slight reduction of GS activity in the renal tissue only at the highest dose ($5.0x10^{-4}$ M). Afterwards, *in vivo* nephrotoxicity of both PTL1 and cisplatin was investigated by measuring some specific biomarkers in either the urine or the renal cortical slices [40]. Increase of GS, total urinary proteins (TUP) and *N*-acetyl-β-D-glucosaminidase (NAG) in the urine of treated rats is a sign of a general kidney injury, whereas reduced activity of GS and uptake of *p*-aminohippuric acid (PAH) in renal cortical slices of the sacrificed treated rats identify specific injuries affecting S3 and S1-S2 segments, respectively [42]. As expected, cisplatin induced a significant increase of GS, NAG and TUP excretion in urine. Moreover, inhibition of GS activity in renal cortical slices of treated rats (without affecting PAH uptake) was detected, due to a dose- and time-dependent

severe diffuse tubular necrosis of the S3 segment of proximal tubules in the outer stripe of the outer medulla. On the contrary, PTL1 caused no significant changes in both urinary and renal cortical biomarkers, confirming the total lack of nephrotoxic side-effects.

COPPER(II)-DITHIOCARBAMATES

Copper is the third most abundant transition metal ion in human body [43] with a daily intake of 1-2 mg in healthy adults [44]. Once entered organisms, Cu(II) is reduced to Cu(I) and then transferred into cells by various transmembrane transporters (*e.g.* Ctr1 and Ctr3) [45]. Copper(II) with an electronic configuration d^9 plays a major role in proper folding and stabilization of some proteins. In addition to the structural function, it is involved in the catalytic activity of many enzymes such as superoxide dismutase [43]. However, copper homeostasis is significantly altered in serum and in neoplastic tissues [46-49]. In fact, copper accumulation in tumors has been related to angiogenesis (formation of new blood vessels) [50, 51]. The increase of copper levels has been shown to trigger the proliferation and the integrin-mediated migration of endothelial cells as well [52]. Fox *et al.* reported that copper is an essential cofactor in the extracellular matrix degradation and in the cytokine production [53]. Interestingly, the copper chelator tetrathiomolybdate (used to treat Wilson's disease) suppresses the tumor growth and angiogenesis in two animal models of breast cancer [54]. This work has demonstrated innovative, and other compounds have recently been studied as antiangiogenic agents. In fact, organic molecules as prodrugs were used to remove copper ions by coordination to the metal centre. The corresponding complexes showed cytotoxic effects as well [55, 56].

On the basis of these considerations, novel copper(II)-dithiocarbamato complexes have been designed and investigated by our research group from biological point of view. Complexes involving different types of ligands, such as *N,N*-dimethyl dithiocarbamate (DMDT), pyrrolidine dithiocarbamate (PyDT) and ester derivatives of sarcosinedithiocarbamate (*tert*-butyl (TSDT), ethyl (ESDT) and methyl (MSDT)) (Fig. **3**) have been synthesized and characterized [57]. In all compounds the metal-ligand stoichiometry is 1:2 with symmetrical bidentate (chelate) coordination of the ligands. The complexes were tested as chemotherapeutics on human ovarian and cervix carcinoma cell lines (2008 and A431) and the corresponding cisplatin-resistant subclones (C13 and A431Pt). Accordingly, cisplatin was used as a reference drug. All the investigated compounds inhibited tumor cell growth in a dose-dependent manner both in wild-type and in cisplatin-resistant cells.

As expected, cisplatin was more potent in wild-type cancer cells than in resistant subclones. Remarkably, regarding the latter subclones, all the copper complexes showed lower IC_{50} values than cisplatin, some of them reported at least one order of magnitude lower values, thus ruling out the occurrence of cross-resistance with cisplatin. In wild-type cancer cells $[Cu(DMDT)_2]$, $[Cu(MSDT)_2]$ and $[Cu(ESDT)_2]$ proved the most potent compounds with IC_{50} values comparable or lower than the reference drug. Among all, $[Cu(ESDT)_2]$ was the most promising with IC_{50} values about three- to twenty-fold lower than cisplatin in 2008 and A431 cell lines, respectively. Its higher stability and solubility in physiologic-like media may account for the observed greater cytotoxic activity toward the investigated cell lines [57]. $[Cu(PyDT)_2]$ was further investigated since the ligand PyDT alone has been reported to inhibit the activation of the nuclear factor κB [58]. Such compound was tested on the androgen receptor-independent human prostate cancer cells PC-3, two estrogen receptor α-positive human breast cancer cell lines (MCF-7 and MCF10dcis.com) and the triple negative highly metastatic MDA-MB-231 breast cancer cells, thus inducing 90%, 85%, 95% and more than 80% growth inhibition, respectively [59]. Notably, $[Cu(PyDT)_2]$ proved a potent tumor cell proteasome inhibitor. In fact, for each cell line, already after a few hours of treatment at 20 μM about 80% of intracellular proteasome inhibition was detected by recording the fluorescence of a proper peptide substrate for the proteasomal chymotrypsin-like (CT-like) activity [59]. Consistently, Western Blot analyses highlighted over time accumulation of ubiquitinated proteins and the proteasome target protein IκB-α. On the contrary, $[Cu(PyDT)_2]$ inhibited only 40% of the CT-like activity of the purified rabbit 20S proteasome at 50 μM, thus leading the authors to hypothesize that a metabolic activation may take place within the cell. An *in situ* change in the chemical structure of the parent compound could account for the reactivity enhancement towards the target, thus inducing apoptosis in these cell lines since PARP (poly (ADP-ribose) polymerase) cleavage was observed. In addition, upon treatment apoptotic morphological changes (shrunken cells and characteristic apoptotic blebbing) along with up to 3-fold increase in caspase-3/-7 activity were observed. A TUNEL assay confirmed that the cell death occurred by apoptosis [17]. In another study, the complex $[Cu(DEDT)_2]$, involving the ligand *N,N*-diethyldithiocarbamate, was also tested in the highly malignant and metastatic MDA-MB-231 cells. Its cytotoxicity was shown to be associated with inhibition of cellular 26S proteasome (>90% inhibition at 20 μM). Likewise to $[Cu(PyDT)_2]$, $[Cu(DEDT)_2]$ inhibited the purified 20S proteasome to a less extent (35% inhibition at 50 μM) [60].

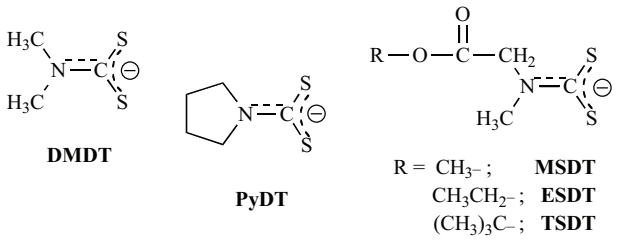


Fig. (3). Chemical drawings of the dithiocarbamato ligands: dimethyldithiocarbamate (DMDT), pyrrolidinedithiocarbamate (PyDT), methylsarcosinedithiocarbamate (MSDT), ethylsarcosinedithiocarbamate (ESDT) and *tert*-butylsarcosinedithiocarbamate (TSDT).

The other aforementioned strategy relies on using copper chelators in antiangiogenic therapy. Based on the high levels

of copper detected in several human cancers, including prostate, [61,62] breast, [63, 64] lung, [65] colon, [62] and brain tumors [66] compared to normal tissues, an innovative targeting approach has been recently developed. Organic molecules such as the aforesaid PyDT, DEDT and disulfiram (tetraethylthiuram disulfide, DSF) have been widely investigated as potential prodrugs able to bind to endogenous copper, thus forming metal complexes within the cell [55, 67]. Interestingly, DSF was approved by FDA for alcoholism treatment, [68] while its metabolite DEDT is an agent for HIV-1 infection treatment [69]. Both showed low or no toxicity in clinical trials [70, 71]. The *in situ* reaction of such chelators with the pro-angiogenic copper converts the tumor cell copper to a selective anti-cancer weapon. D. Chen *et al.* reported indeed that DSF significantly inhibited tumor growth *in vivo* by 74% after 30 days of treatment (mice bearing MDA-MB-231 breast cancer xenografts) by selectively inhibiting tumor proteasome and thus inducing apoptosis [67]. Furthermore, melanoma cells treated with DSF in combination with Cu(II) showed reduced *in vitro* proliferation and decreased cyclin A expression [72]. In a similar study, in addition to the antimelanoma activity of DSF mixed with $CuCl_2$, Cu(II) was demonstrated to react with DSF in the extracellular milieu forming the complex $[Cu(DEDT)_2]$, thus suggesting such compound to be the active species [73]. DEDT is indeed one of the metabolites of DSF [70] and in combination with copper(II) was reported to induce apoptosis in both human breast and prostate cancer cells and strongly inhibit proteasomal chymotrypsin-like activity [74]. Likewise, suppression of proliferation, induction of apoptosis and inhibition of proteasomal chymotrypsin-like activity were observed in human breast [75] and human prostate [76] cancer cells treated with PyDT and $CuCl_2$. Interestingly, histone acetyltransferase proved to be another target of $[Cu(PyDT)_2]$ since the mixture PyDT-Cu(II) markedly inhibited the proliferation of human leukemia cells HL-60 by histone acetylation inhibition [77]. All these findings show that high copper levels *in vivo* may be targeted by a ligand, resulting in formation of a cell death inducer specifically in tumor tissues, but not normal cells.

RUTHENIUM(III)-DITHIOCARBAMATES

Ruthenium-based complexes are believed to be the most promising alternatives to platinum complexes, as numerous ruthenium compounds display antitumoral and antimetastatic properties [16-18, 78]. Two representatives of this class of compounds, NAMI-A (imidazolium *trans*-[tetrachloro(*S*-dimethyl sulfoxide)(1H-imidazole)ruthenate(III)]) and KP1019 (indazolium [*trans*-tetrachlorobis(1H-indazole)ruthenate(III)]) (Fig. **1**), after extensive preclinical tests have entered human clinical trials so far [20, 79]. Despite their structural and chemical similarities, in preclinical experiments the two compounds showed different antitumor properties. While NAMI-A has demonstrated inhibitory effects against the formation of cancer metastases but appears to lack direct *in vitro* cytotoxic effects, KP1019 has exhibited activity against primary tumors by inducing apoptosis, including colorectal carcinomas and a variety of primary explanted human tumors [19-21]. Although many aspects of the tumor-inhibiting action displayed by ruthenium compounds are still unknown, ruthenium-based chemotherapies are making significant advances in clinical trials because of its supposed selectivity to cancer cells, as well as its low systemic toxicity. On the other hand, the results obtained with NAMI-A are really important, as metastases of solid tumors still represent the main reason of failure in cancer therapy [19].

Ruthenium shows a number of chemical and structural differences with platinum [80]. However, it has been considered to be an attractive alternative to platinum because of the similar ligand exchange kinetics in aqueous solution of both Ru(II) and Ru(III) complexes to those of Pt(II) complexes. Ligand exchange is an important determinant of biological behaviour being crucial for the anticancer activity. In fact, only a few metal drugs reach the biological target without being modified (such as interactions with macromolecules or small S-donor compounds and/or water) [4]. On the other hand, probably due to a different mechanism of action compared to platinum containing drugs, several ruthenium compounds have been shown to be active against cisplatin-resistant tumors [18, 81].

Under physiological conditions Ru(III) is predominant, while Ru(II) and Ru(IV) oxidation states are readily accessible in the presence of biological reductants (such as glutathione, ascorbate and single electron transfer proteins) or oxidants (molecular oxygen and cytochrome oxidase) [17]. In these oxidation states the ruthenium centre is predominantly hexacoordinate with essentially octahedral geometry. Accordingly, ruthenium complexes likely remain in their relatively inert +3 oxidation state until they reach the tumor site [78]. As cancer cells have lower oxygen concentration, higher level of glutathione and lower pH than normal tissues creating a strongly reducing environment, the redox potential of ruthenium can be modified by varying the ligands. Thus, the inactive Ru(III) complex can be activated by reduction to the more reactive Ru(II) complex [80, 82]. However, a better understanding of chemical transformations of ruthenium complexes in biological media is required for the design of new compounds with outstanding antineoplastic activity.

Furthermore, the low toxicity of ruthenium drugs is also believed to be due to the ability of ruthenium to mimic iron in binding to many biomolecules, including serum transferrin and albumin [17]. As rapidly dividing cells (including cancer cells) have a greater requirement for iron, this results in up-regulation of the number of transferrin receptors on the cell surface. In fact, ruthenium appears to accumulate preferentially in neoplastic masses rather than normal tissues, possibly by using transferrin to be shuttled into tumors. Accordingly, a significant increase in the ruthenium concentration in cancer cells compared to healthy cells has been shown in *in vivo* studies [83].

Based on the above mentioned promising anticancer activity of some ruthenium compounds, several Ru(III)–dithiocarbamato complexes were synthetized and fully characterized by our research group, using *N*,*N*-dimethyl- (DMDT), pyrrolidine- (PyDT), methylsarcosine- (MSDT), ethylsarcosine- (ESDT) and *tert*-butylsarcosine- (TSDT) dithiocarbamates as ligands (Fig. **3**) [84, 85]. It is worthy to be mentioned that ruthenium−dithiocarbamate chemistry is

extremely rich in the number and nature of compounds that can be formed because of the possibility to obtain several distinct stereoisomers with peculiar biological profiles. In all cases we have obtained two different complexes: the paramagnetic neutral monomer $[RuL_3]$ and the antiferromagnetically coupled ionic dinuclear α-$[Ru_2L_5]Cl$ species (Fig. **4**). In both the mono- and dinuclear complexes the ruthenium is in the +3 oxidation state and the metal centres are characterized by a distorted octahedral coordination attained by six sulphur atoms. In the dinuclear species each metal ion is seven coordinated counting the Ru–Ru bond (~2.74 Å) with two bridged sulphurs between them (Fig. **4**) [86, 87]. Subsequently, the NAMI-A-like species *mer*-$[RuCl_3(DMSO)(PyDTM)]$ and *mer*-$[RuCl_3(DMSO)(DMDTM)]$ were synthesized using the S-methyl ester derivative of DMDT and PyDT ligands (Fig. **4**).

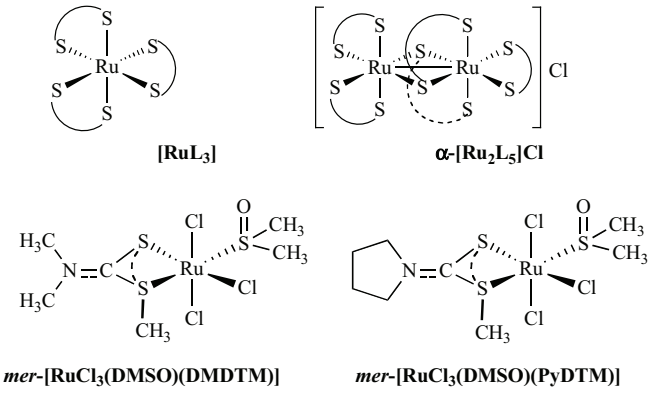


Fig. (4). Chemical drawings of the Ru(III)-dithiocarbamato complexes.

In vitro cytotoxicity assays of the complexes have been performed on different human tumor cell lines, such as esophageal squamous carcinoma (OE-19 and OE-21), pancreas adenocarcinoma (PT-45), ovary adenocarcinoma (Igrov and Ovcar-3), breast cancer (MCF-7), adenocortical carcinoma (NCI-I4295R), lung carcinoma (A549), cervix carcinoma (HeLa), leukemia wild type (CEM wt) and vimblastin-resistant leukemia (CEM vim). The results pointed out the greater anticancer activity of the ionic dinuclear complexes, in particular α-$[Ru_2(DMDT)_5]Cl$ and α-$[Ru_2(TSDT)_5]Cl$, showing higher cytotoxicity than cisplatin at very low concentrations (IC_{50} < 1 μM) in OE-19, OE-21, HeLa, PT-45, MCF-7, Ovcar-3 and CEM wt cell lines. On the contrary, the monomer species, in particular $[Ru(DMDT)_3]$ and $[Ru(ESDT)_3]$ were more selective toward leukemic cells (IC_{50} ~ 3 μM) [84]. Furthermore, the lack of *in vitro* cytotoxicity towards both the cisplatin sensitive and intrinsically resistant cells for *mer*-$[RuCl_3(DMSO)(PyDTM)]$ and *mer*-$[RuCl_3(DMSO)(DMDTM)]$ (structurally similar to NAMI-A) resemble NAMI-A's behavior, which could constitute an *in vitro* screening strategy for ruthenium analogs [85].

In order to elucidate the mechanism of action of the ruthenium(III)-dithiocarbamato complexes, the redox behaviour of the compounds has intensely been studied [7]. Electrochemical studies showed that both the mono- and the dinuclear complexes undergo reversible reduction process, which is assigned to the Ru(III)/Ru(II) step. While one-electron reduction step was observed for $[RuL_3]$, two separate reduction processes with the formation of $[Ru^{III}Ru^{II}L_5]^0$ and $[Ru^{II}{}_2L_5]^-$ species were detected for α-$[Ru_2L_5]Cl$. Furthermore, two oxidation processes for the monomer complexes were recognized and ascribed to the ligand oxidation apparently irreversible, and to the Ru(III)/Ru(IV) step with partial chemical reversibility. The oxidation processes of the dimers were not discussed being not well-defined.

Moreover, preliminary solution studies were carried out to investigate the stability of the ruthenium complexes in physiological-like conditions [86]. The *in vitro* cytotoxicity studies have been performed in phosphate buffered saline (PBS) dissolving the metal complexes in dimethyl sulfoxide beforehand. For this reason, the stability of the complexes was checked in dimethyl sulfoxide and in PBS solution. Both the mono- and the dinuclear species were stable in dimethyl sulfoxide, whilst they had a low solubility in aqueous solution. However, interaction of both the $[Ru(ESDT)_3]$ and α-$[Ru_2(ESDT)_5]Cl$ complexes with bovine serum albumin (BSA), thus mimicking the human counterpart (HSA), has shown that such protein could firstly interact with these compounds, and it might correlate with the antitumoral effect of the complexes. In the presence of BSA a significant increase of the solubility and stability of both the monomer and dinuclear species have been observed. However, no direct coordination between the protein and the Ru(III) centre was found. On the other hand, conformational changes of BSA induced by the metal complexes were evident and probably based on secondary interactions (electrostatic and/or steric). This result may confirm a possible role of albumin in the stabilization and the transport of the complexes in the body.

GOLD(III)-DITHIOCARBAMATES

The use of gold derivatives in medicine (the so-called chrysotherapy) dates back into history, perhaps as far back as 2500 BC, the earliest therapeutic application being reported in China [88]. The modern interest in the medical use of gold compounds originates from the discovery in 1890 that gold cyanide inhibits the growth of *Tubercle bacillus*, and the subsequent development of gold(I)-thiolato salts to treat tuberculosis, despite a lack of experimental evidence for any antitubercular benefits [89]. At the same time, gold-based therapy was found to significantly reduce joint pain in a group of non-tubercular patients, thus opening up new perspectives for the treatment of rheumatoid arthritis. Antirheumatic gold complexes belong to the class of disease-modifying antirheumatic drugs (DMARDs), which are used to halt or slow down disease progression with lower bone and cartilage damage. In this regard, late-stage rheumatoid arthritis was treated with various gold drugs, including aurothioglucose (solganol), sodium aurothiomalate (myocrisin), aurothiosulfate (sanocrysin), aurothiopropanol sulfonate (allocrysin), and tetraacetyl-β-D-thioglucose gold(I) triethylphosphine (auranofin) (Fig. **5**), and some are still in clinical use [90]. The antiarthritic activity of gold compounds arises from their known immunosuppressive and anti-inflammatory actions.

Interestingly, auranofin (Fig. **5e**) was shown to have antiarthritic properties and also to inhibit the growth of tumor cells *in vitro*, but further evaluation as potential anticancer agent was dismissed because of its relatively poor effectiveness against *in vivo* tumor models [91].

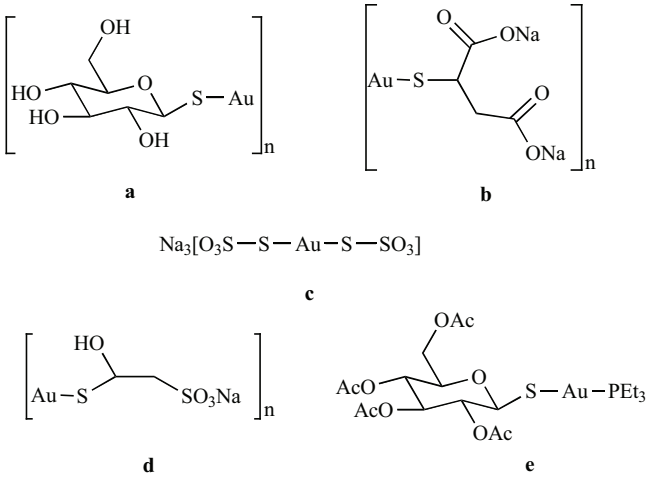


Fig. (5). Examples of gold(I) drugs used for the treatment of rheumatoid arthritis: solganol (**a**), myocrisin (**b**), sanocrysin (**c**), allocrysin (**d**) and auranofin (**e**).

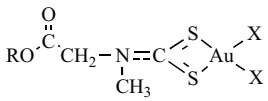
Notwithstanding the initial unsatisfactory outcomes, among the metal-based non-platinum antitumor agents, gold compounds have been gaining increasing attention as a new class of chemotherapeutics owing to their potentially strong tumor cell growth inhibiting effect, generally achieved by exploiting non-cisplatin-like pharmacodynamic and pharmacokinetic properties and mechanisms of action. In this regard, a number of review papers on the therapeutic use of gold complexes have been published in recent years, highlighting the special interest in this class of metal complexes as anticancer agents [7, 12, 14, 92, 93].

Gold compounds with reported cell proliferation inhibitory properties comprise a number of different ligands coordinated to the metal center in the +1 or +3 oxidation states. On account of the wide structural variety of the ligands, a unique mode of action or pharmacological profile of gold complexes is unlikely to be identified, and several biological targets, other than intracellular DNA (generally acknowledged as the major biological target of cisplatin and its analogs), [94] have to be considered to elucidate the pharmacology of gold metallodrugs. Gold(III) derivatives were first investigated as potential antitumor agents with the idea that square-planar gold(III) complexes (being d^8 and hence isoelectronic with platinum(II)) could mimic the activity of cisplatin. In general, they are not very stable under physiological conditions because of the high reduction potential and fast rate of hydrolysis. However, in recent times, a range of strategies have been exploited to stabilize the metal center in the +3 oxidation state and a large variety of different classes of gold(III) compounds proved cytotoxic toward cancer cell lines *in vitro* but very few turned out to be active *in vivo* as well [12].

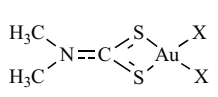
In this context, during the last decade, a number of gold(III)-dithiocarbamato derivatives have been developed and tested by our research group as potential anticancer agents, showing promising and outstanding anticancer properties both *in vitro* and *in vivo*, as well as reduced toxicity levels [18, 95]. As above mentioned, the rationale of our designing strategy is based on the use of dithiocarbamates as both intrinsic chemoprotectants and stabilizing moieties due to the so-called "chelate effect". In fact, the presence of a chelating dithiocarbamate in a square-planar complex should make the coordination of additional *S*-donor biomolecules *trans* to the –NCSS moiety less favored because of the rather strong *trans*-influencing effect of the dithiocarbamato sulfur atoms, thus potentially avoiding the interaction of the metal center with thiol-containing biologically-relevant macromolecules and, consequently, reducing side-effects.

First Generation Compounds: the Gold(III)-Dithiocarbamato Challenge

Following the promising results obtained for some platinum(II) analogs (see above), a number of gold(III)-dithiocarbamato derivatives of the type $[AuX_2(dtc)]$ (X = Cl, Br; dtc = various dithiocarbamato ligands) (Fig. **6**) have been synthesized. All compounds were fully characterized by means of several techniques, confirming the near square-planar geometry achieved through the bidentate symmetric coordination of the dithiocarbamato moiety *via* the sulfur atoms, and the presence of two *cis*-halides occupying the remaining coordination positions. Coordination by dithiocarbamates was proved to induce a substantial stabilization of the metal ion in the +3 oxidation state. In fact, electrochemical studies performed under physiological-like conditions showed that both DMDT and ESDT derivatives undergo irreversible stepwise reduction processes (leading to the corresponding dinuclear gold(I) species $[Au(DMDT)]_2$ and $[Au(ESDT)]_2$) at *ca.* –0.300 V and –0.180 V (*vs.* saturated calomel electrode, SCE), respectively [96]. Remarkably, the recorded potentials are considerably lower than the typical values for the Au(III)/Au(I) couple reported for the corresponding $K[AuX_4]$ (X = Cl, Br) precursors (*ca.* +1.29 V) [97]. Moreover, these gold(III) complexes hydrolyze in a physiologically-relevant environment by delivering two moles of halide ions per mole of starting complex, thus leading to the corresponding gold(III)-diaquo counter-parts within 30-40 min. Intriguingly, the hydrolyzed species appeared to be reasonably stable in physiological solution, reduction to gold(I) having occurred after 12-24 h [96].



R = CH_3, X = Cl, [$AuCl_2$(MSDT)]
R = CH_3, X = Br, [$AuBr_2$(MSDT)]
R = CH_3CH_2, X = Cl, [$AuCl_2$(ESDT)] (AUL13)
R = CH_3CH_2, X = Br, [$AuBr_2$(ESDT)] (AUL12)
R = $C(CH_3)_3$, X = Cl, [$AuCl_2$(TSDT)]
R = $C(CH_3)_3$, X = Br, [$AuBr_2$(TSDT)]



X = Cl, [$AuCl_2$(DMDT)] (AUL10)
X = Br, [$AuBr_2$(DMDT)] (AUL14)

Fig. (6). Selected gold(III)-dithiocarbamato derivatives.

The capability of the gold(III)-dithiocarbamato derivatives [$AuCl_2$(DMDT)], [$AuBr_2$(DMDT)], [$AuCl_2$(ESDT)] and [$AuBr_2$(ESDT)] (from now on referred to as AUL10, AUL14, AUL13 and AUL12, respectively) to inhibit cell proliferation was evaluated toward a panel of human tumor cell lines and compared to the reference drug cisplatin. These compounds proved much more cytotoxic *in vitro* than cisplatin even toward human tumor cells intrinsically resistant to cisplatin itself, such as Burkitt's lymphoma (Daudi), malignant melanoma (MeWo), non-small cells lung adenocarcinoma (A549), and colon adenocarcinoma (LoVo) cells, with IC_{50} values in the low micromolar/nanomolar range. Moreover, they appeared to be equally active on cell lines made resistant to cisplatin treatment, compared to the corresponding cisplatin-sensitive parent cell lines, ruling out the occurrence of cross-resistance phenomena [98]. The compounds overcame the resistance to cisplatin also in murine leukemia cisplatin- resistant L1210-R cells [96]. From comparative *in vitro* cytotoxicity studies on platinum(II)-, palladium(II)-, and gold(III)-dithiocarbamato analogs on human squamous cervical adenocarcinoma (HeLa) cells and human leukemic promyelocytes (HL60), gold(III) complexes resulted to be significantly more active than both cisplatin and the platinum(II) and palladium(II) counter-parts (IC_{50} *ca.* 1 μM *vs. ca.* 2, >15, and 5 μM, respectively), inducing apoptosis especially in HL60 cells [99]. These gold(III) compounds were also proved to suppress, in a dose-dependent way, cell growth on a panel of acute myelogenous leukemia cell lines with IC_{50} values *ca.* ten-fold lower than the reference drug by inducing only modest cell cycle perturbations but high DNA fragmentation and, consequently, apoptosis [100].

Altogether, these results support the hypothesis that the mechanism of action of the described gold(III)-dithiocarbamato derivatives differs from that of the classical platinum(II)-based anticancer drugs. Although they were proved to bind DNA to a greater extent than cisplatin (that is, 100% binding to calf thymus DNA after less than 3 h, compared to 51% binding by cisplatin over 24 h.), [96] this evidence is likely to be a consequence of their extremely high reactivity toward isolated biologically-relevant macromolecules rather than undoubtedly stating DNA as their ultimate target.

In this regard, recent reports have identified the enzyme thioredoxin reductase (TrxR) as a reliable target for anticancer gold compounds [101]. In order to get insights into their mechanism of action, the four gold(III) complexes AUL10, AUL12, AUL13 and AUL14 were studied in-depth as inhibitors of the thioredoxin system [102]. All the tested compounds were proved to trigger cell death, favor generation of reactive oxygen species (ROS), modify some mitochondrial functions, and to inactivate both cytosolic and mitochondrial thioredoxin reductase. Based on these evidences, we proposed a working model suggesting that deregulation of the TrxR/Trx system is a major mechanism involved in their anticancer activity (Fig. **7**). In particular, we hypothesized that persistent ERK-1/2 activation triggered by accumulation of hydrogen peroxide first, and then by ASK-1 pathway deregulation might be responsible for cell death through both apoptotic and non-apoptotic routes, whereas cisplatin leads to cell death only through an apoptotic pathway.

Together with the thioredoxin system, we have also identified the proteasome as a major *in vitro* and *in vivo* biological target. For example, AUL14 was shown to strongly inhibit the proteasomal chymotrypsin-like activity in the highly metastatic and invasive estrogen receptor α-negative MDA-MB-231 breast cancer cells in a dose-dependent way, as confirmed by the detection of decreased proteasomal activity and increased levels of ubiquitinated proteins and the proteasome target protein p27 [103].

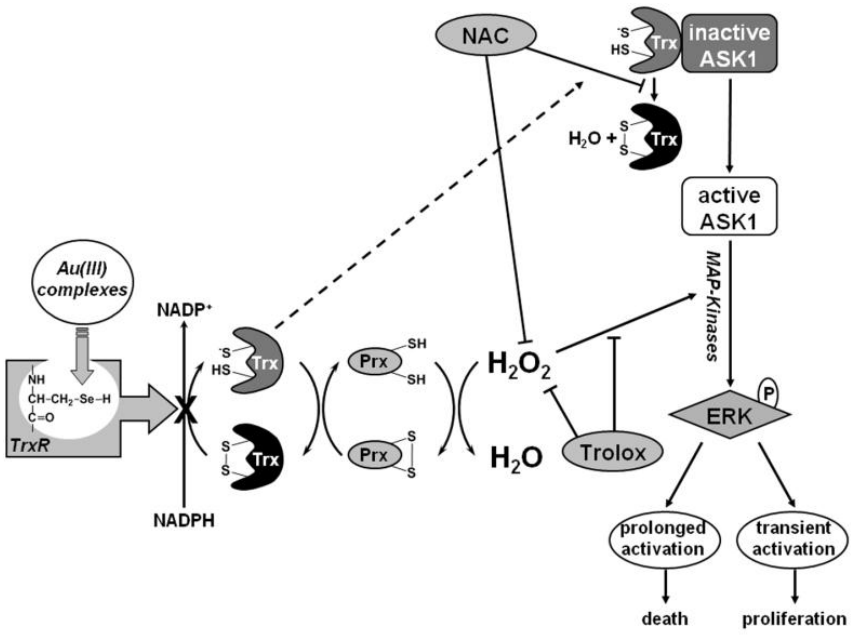


Fig. (7). Proposed working model of the molecular mechanisms involved in the gold(III)-dithiocarbamato derivatives-induced cell death (adapted from ref. [102]).

Additional in-depth studies carried out on the AUL12, provided further evidence that this class of compounds involves (at least) two mechanisms responsible for their antiproteasomal activity, including ROS production and direct metal binding to the proteasome [104]. This hypothesis is not in contrast with the previously discussed model related to the TrxR/Trx system as potential target. In fact, it has been recently reported that the proteasome inhibitor bortezomib induces apoptosis through generation of ROS [105]. Since ROS are produced also in the present case, the observed proteasome inhibition can favor the long-lasting persistence of phosphorylated ERK-1/2 (see above).

Gold compounds whose anticancer activity has been already reported in the literature have often shown promising *in vitro* cytotoxicity that, unfortunately, was not confirmed by subsequent *in vivo* studies [12]. On the contrary, the *in vivo* antitumor activity of our gold(III)-dithiocarbamato derivatives, evaluated against human tumors implanted on immunodepressed nude mice (xenografts), was fully consistent with *in vitro* data. For example, treatment of MDA-MB-231 breast tumor-bearing nude mice with AUL14 resulted in significant inhibition of tumor growth (*ca.* 50% inhibition after daily treatment with 1 mg kg^{-1} along 29 d compared to control), associated with inhibition of proteasome activity (confirming the relevance of such a biological target), accumulation of protein p27 and massive apoptosis induction. Interestingly, during the 29-day treatment, no toxicity was observed, and mice did not display signs of weight loss, decreased activity or anorexia [103]. Analogously, administration of 1 mg kg^{-1} every second day of AUL10 caused an overall 85% reduction of PC3 prostate tumor xenografts in nude mice after a 19-day treatment (compared to control untreated mice) (Fig. **8**). Again, chemotherapy was well tolerated by treated mice which suffered from minimal systemic toxicity only, and histology showed no detectable damage to main animals' organs [19].

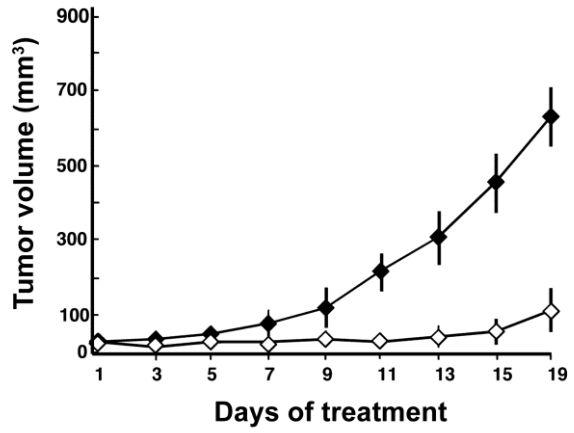


Fig. (8). *In vivo* anticancer activity of AUL10 on PC3 prostate tumor-bearing nude mice (xenografts). Tumor volume was measured in athymic nude mice after subcutaneous injection of either drug-free medium (♦) or containing 1 mg kg^{-1} of AUL10 (◊) every other day (adapted from ref. [19]).

In vivo nephrotoxicity studies were also carried out with the model compound AUL12 by measuring some specific biomarkers in both urines (total urinary proteins (TUP), *N*-acetyl-β-d-glucosaminidase (NAG) and glutamine synthetase (GS)) and renal tissues (*p*-aminohippuric acid (PAH) and glutamine synthetase (GS)) of the treated rats [20]. With reference to (Fig. **9**), cisplatin administration induced a significant increase of all the urinary biomarkers and a significant inhibition of GS activity in renal cortical slices, whereas the gold(III)-dithiocarbamato derivative caused negligible changes compared to untreated control rats (mainly observable at the higher dose, 20 mg kg^{-1}), accounting for a substantial lack of nephrotoxic side-effects.

The favorable toxicity profile was also confirmed by the LD_{50} value of 30 mg kg^{-1} recorded for AUL12 that is, to the best of our knowledge, among the higher ever reported for gold-based therapeutics. Subsequent histopathological investigations showed that no significant histologically detectable toxicity involving treated animals' organs was observed, and SEM evaluation of the surface of all tissues examined were considered compatible with normal conditions when compared to control animals. Surprisingly, no gold was detected in the investigated tissues, thus ruling out the accumulation of the metal in any of the organs taken into account (that is, heart, liver, spleen, kidneys, testicles, pancreas, lungs and brain). Accumulation around the injection site (*i.e.* peritoneal area) was also excluded, and these results are in full agreement with the fact that gold seems to be rapidly (within 48 h) cleared from the body, the large majority being excreted through the feces (>89%) and only about 10% *via* the urinary system [20].

Altogether, these results confirm that the idea of combining the antitumor properties of the gold(III) metal center with the potential chemoprotective function of coordinated dithiocarbamates is a somewhat winning strategy. These "first generation" gold(III)-dithiocarbamato derivatives were proved extremely promising in terms of greater *in vitro* and *in vivo* anticancer activity, reduced nephrotoxicity and acute toxicity compared to cisplatin, by exploiting mechanisms of action different from clinically-established platinum-based drugs. In this regard, from a comparative analysis toward 110 reference substances with known modes of action, AUL12 turned out to be the only gold(III) complex (out of thirteen) covered by this screening study whose mechanism of action did not resemble the mechanism of any of the reference compounds [106].

Second Generation Compounds: Tumor-Targeting Gold(III) Peptidomimetics

Notwithstanding the extremely positive outcomes achieved by these "first generation" gold(III) compounds alternative routes have been explored to improve the therapeutic effectiveness of this class of anticancer agents. Among all, the most intriguing way focuses on the development of dithiocarbamato derivatives of dipeptides as improved intracellular drug transfer and delivery systems supported by transport proteins. Peptide transporters are integral plasma membrane proteins that mediate the cellular uptake of di- and tripeptides and peptide-like drugs (*i.e.* peptidomimetics). Two peptide transporters, namely PEPT1

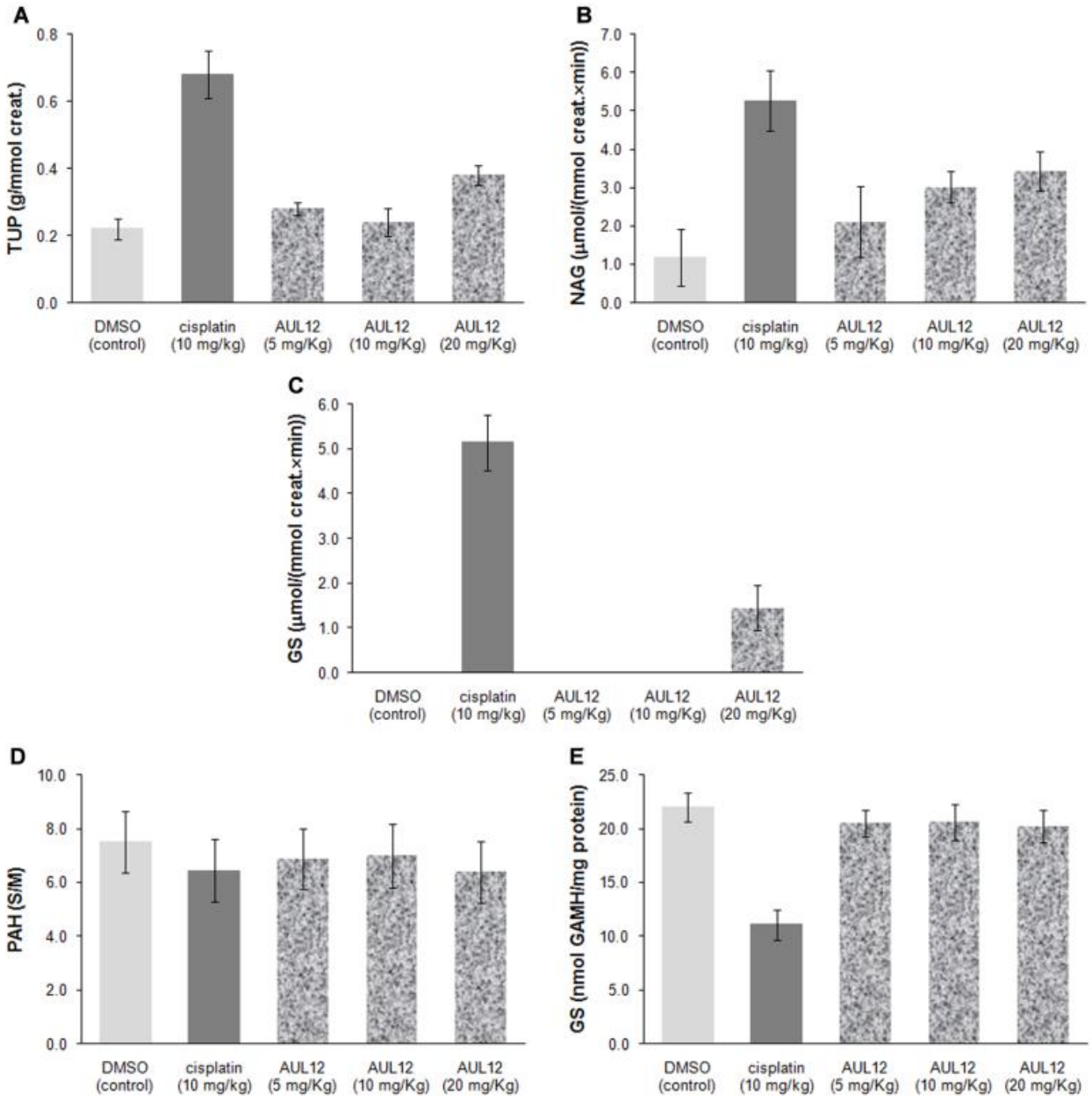
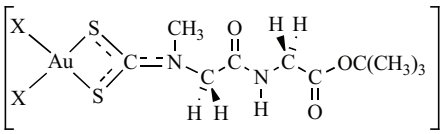


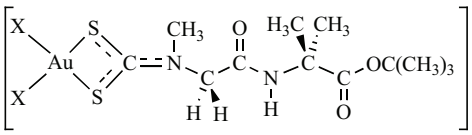
Fig. (9). Urinary and renal cortex profiles of male albino Wistar rats on a single i.p. injection of the tested compounds. Evaluation of (**a**) total urinary proteins (TUP) excretion, (**b**) *N*-acetyl-β-D-glucosaminidase (NAG) activity, and (**c**) glutamine synthetase (GS) activity in urines collected along 24 h after administration, and of (**d**) *p*-aminohippuric acid (PAH) uptake and (**e**) glutamine synthetase (GS) activity in renal cortical slices of rats sacrificed 24 h after administration (adapted from ref. [20]).

and PEPT2, have been identified in mammals. They are present predominantly in epithelial cells of the small intestine, bile duct, mammary glands, lung, choroid plexus and kidney, but are also localized in other tissues (pancreas, liver, gastrointestinal tract) and, intriguingly, seem to be upregulated in some types of tumor [107]. A unique feature is their capability for sequence-independent transport of most possible di- and tripeptides inside the cells. These transporters are stereoselective toward peptides containing *L*-enantiomers of amino acids. Moreover, some peptidomimetic (*e.g.* Bestatin, *L*-Dopa), non-peptidic drugs (*e.g.* Valacyclovir, AZT, 5-Aminolevulinic acid) and prodrugs, β-lactam antibiotics (*e.g.* Cefadrine, Cefadroxil, Cyclacillin, Cefixime, Ceftibuten), and some angiotensin-converting enzyme (ACE) inhibitors (*e.g.* Fosinopril, Captopril and Enalapril) are recognized by either PEPT1 or PEPT2 as substrates because of their resemblance to di- or tripeptides [108]. Therefore, peptide transporters represent excellent targets for the delivery of pharmacologically active compounds because their substrate binding site can accommodate a wide range of molecules with different size, hydrophobicity and charge [109, 111].

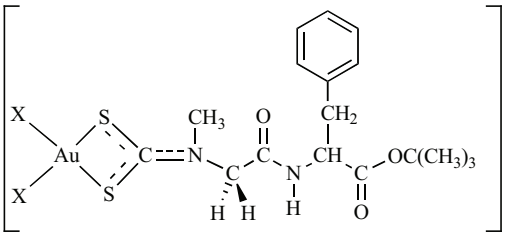
On account of these assumptions, we have been developing some gold(III)-dithiocarbamato peptidomimetics of the type [AuX_2(dpdtc)] (X = Cl, Br; dpdtc = dipeptidedithiocarbamate) which could both preserve the antitumor properties and reduced toxic side-effects of the previously reported gold(III) analogs, together with an improved tumor selectivity by targeting PEPT1 and/or PEPT2 *via* the dipeptide chain. The basic idea relies on the potential recognition of the whole metal complex that, once recognized and transported by PEPTs and delivered inside the tumor cell, could exert its anticancer activity without affecting healthy tissues.



[AuX_2(dtc-Sar-Gly-O(*t*-Bu))]
X = Br (AUD6), Cl (AUD7)



[AuX_2(dtc-Sar-Aib-O(*t*-Bu))]
X = Br (AUD8), Cl (AUD9)



[AuX_2(dtc-Sar-*L*-Phe-O(*t*-Bu))]
X = Br (AUD10), Cl (AUD11)

Fig. (10). Selected gold(III)-dipeptidedithiocarbamato peptidomimetics.

To date, a number of such complexes have been designed and fully characterized, and six of them (of the type [AuX_2(dtc-Sar-AA-O(*t*-Bu))], X = Cl, Br; AA = Gly, Aib, *L*-Phe, Fig. **10**) have been recently reported for their cytotoxic activity [110] Preliminary *in vitro* cytotoxicity tests were carried out on human prostate (PC3 and DU145), ovarian adenocarcinoma (2008) and Hodgkin's lymphoma (L540) cells, and some of the compounds showed promising anticancer activity with IC_{50} values up to five-fold lower than the reference drug cisplatin toward all the investigated cell lines. When cisplatin-resistant ovarian adenocarcinoma C13 cells were tested, activity levels comparable to those observed on the parent sensitive 2008 cells were detected for all the gold(III) derivatives, thus ruling out the occurrence of cross-resistance with cisplatin. Among all, the Aib-containing derivatives AUD8 and AUD9 turned out to be the best performers and, remarkably, apoptosis resulted to be the major mechanism involved in the growth inhibition of PC3, DU145 and cisplatin-resistant C13 cells. Conversely, they exerted their cytotoxic effect on L540 and cisplatin-sensitive 2008 cells mainly by inducing late apoptosis/necrosis over 24 h. As far as we are aware of, this appears to be the first time that metal-based peptidomimetic anticancer agents are designed specifically to target peptide transporters. Eventually, this idea appears to be an original and innovative strategy in order to develop novel and more selective chemotherapeutic agents that could provide a valuable alternative to the platinum-based anticancer drugs in clinical use. Complexes AUD6 and AUD8 are currently under investigation for their *in vivo* anticancer activity as well as for the elucidation of their mechanism of action. Should they really resemble the behavior of their previous analogs, we are expecting the proteasome to be a major biological target, and preliminary outcomes seem to confirm this hypothesis.

These results represent a solid starting point for the recognition of this class of gold(III) peptidomimetics as suitable candidates for further pharmacological testing and, remarkably, allowed us to file an international patent for their use in cancer chemotherapy (just extended in several countries worldwide) [111].

CONCLUSIONS

The discovery of cisplatin was a defining moment which triggered the interest in platinum(II)- and other metal-containing complexes as potential novel anticancer drugs. In fact, up to now many transition metal complexes have been synthesized and assayed for antineoplastic activity. The idea of combining the well-known antitumor properties of the platinum, copper, ruthenium and gold metal centres with the promising chemoprotective function of dithiocarbamates was proved to be a somewhat winning strategy. Some of the studied compounds have shown outstanding anticancer properties, thus justifying the need to carry out further in-depth biological and mechanistic studies. Principally, gold(III)-dithiocarbamates have shown excellent *in vitro* and *in vivo* antitumor properties and reduced, or even no, systemic and renal toxicity, opening up new prospects for further pharmacological testing and, hopefully, to enter clinical trials. However, more extensive *in vivo* investigations are warranted for the complexes to assess their possible efficacy as anticancer agents more thoroughly.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

NAMI-A	=	Imidazolium *trans*-[tetrachloro(*S*-dimethylsulfoxide)(1H-imidazole)ruthenate(III)]
KP1019	=	Indazolium [*trans*-tetrachlorobis(1H-indazole)ruthenate(III)]
PTL1	=	[PtCl(ESDT)(py)]
DMDT	=	*N*,*N*-dimethyldithiocarbamate
PyDT	=	Pyrrolidinedithiocarbamate
MSDT	=	Methylsarcosinedithiocarbamate
ESDT	=	Ethylsarcosinedithiocarbamate
TSDT	=	*tert*-butylsarcosinedithiocarbamate
DMDTM	=	*N*,*N*-dimethylcarbamodithioic acid methyl ester
PyDTM	=	1-pyrrolidinecarbodithioic acid methyl ester
PBS	=	Phosphate buffered saline
BSA	=	Bovine serum albumin
AUL10	=	[$AuCl_2$(DMDT)]
AUL12	=	[$AuBr_2$(ESDT)]
AUL13	=	[$AuCl_2$(ESDT)]
AUL14	=	[$AuBr_2$(DMDT)]
TrxR	=	Thioredoxin reductase
ROS	=	Reactive oxygen species
AUD6	=	[$AuBr_2$(dtc-Sar-Gly-O(*t*-Bu))]
AUD8	=	[$AuBr_2$(dtc-Sar-Aib-O(*t*-Bu))]
AUD9	=	[$AuCl_2$(dtc-Sar-Aib-O(*t*-Bu))]

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