Biometals
DOI 10.1007/s10534-016-9933-5

Metal complexes of 3-(4-bromophenyl)-1-pyridin-2-ylprop-2-en-1-one thiosemicarbazone: cytotoxic activity and investigation on the mode of action of the gold(III) complex

Luciana B. P. Sâmia · Gabrieli L. Parrilha · Jeferson G. Da Silva · Jonas P. Ramos · Elaine M. Souza-Fagundes · Silvia Castelli · Venn Vutey · Alessandro Desideri · Heloisa Beraldo

Received: 15 January 2016 / Accepted: 5 April 2016

Abstract Complexes [Au(PyCT4BrPh)Cl]Cl (**1**), [Pt(PyCT4BrPh)Cl]0.5KCl (**2**), and [Pd(PyCT4BrPh)Cl]KCl (**3**) were obtained with 3-(4-bromophenyl)-1-pyridin-2-ylprop-2-en-1-one thiosemicarbazone (HPyCT4BrPh). Although complexes (**2**) and (**3**) did not exhibit potent cytotoxic activity, HPyCT4BrPh and its gold(III) complex (**1**) proved to be highly cytotoxic against HL-60 (human promyelocytic leukemia) and THP-1 (human monocytic leukemia) cells, and against MDA-MB 231 and MCF-7 (human breast adenocarcinoma) solid tumor cells. Except for HL-60 cells, upon coordination to gold(III) a 2- to 3-fold increase in the cytotoxic effect was observed. An investigation on the possible biological targets of the gold(III) complex was carried out. Complex (**1**) but not the free thiosemicarbazone inhibits the enzymatic activity of thioredoxin reductase (TrxR). The affinity of **1** for TrxR suggests metal binding to a selenol residue in the active site of the enzyme. While HPyCT4BrPh was inactive, **1** was able to inhibit topoisomerase IB (Topo IB) activity. Hence, inhibition of TrxR and Topo IB could contribute to the mechanism of cytotoxic action of complex (**1**).

L. B. P. Sâmia · G. L. Parrilha · H. Beraldo (✉)
Departamento de Química, Universidade Federal de Minas Gerais, Belo Horizonte, MG 31270-901, Brazil
e-mail: hberaldo@ufmg.br

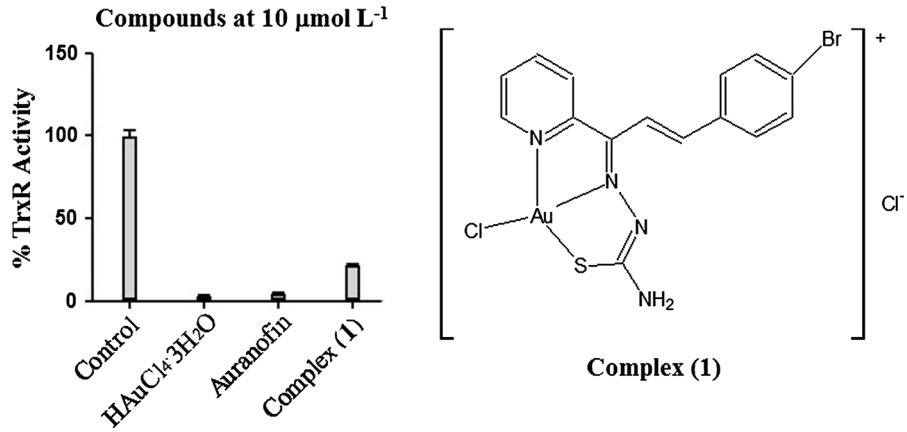
J. G. Da Silva
Departamento de Farmácia, Universidade Federal de Juiz de Fora (UFJF), Governador Valadares, MG 35010-177, Brazil

J. P. Ramos · E. M. Souza-Fagundes
Departamento de Fisiologia e Biofísica, Universidade Federal de Minas Gerais, Belo Horizonte, MG 31270-901, Brazil

S. Castelli · V. Vutey · A. Desideri
Department of Biology, University of Rome "Tor Vergata", Via della Ricerca Scientifica 1, 00133 Rome, Italy

Published online: 18 April 2016

Graphical Abstract



Keywords Chalcone · Thiosemicarbazone · Metal complexes · Cytotoxic activities · Thioredoxin reductase · Topoisomerase IB

Introduction

Cisplatin and the second generation drugs carboplatin and oxaliplatin are among the most important chemotherapeutics used in the treatment of a variety of cancers. However, their side effects and the appearance of resistance to platinum antitumor drugs encourage the development of other metal-based compounds with distinct spectra of activities and lower systemic toxicity (Lippert 1999).

Currently, different studies report a variety of metal complexes with antineoplastic activity, such as palladium(II) (Garbutcheon-Singh et al. 2011), gallium(III) (Lessa et al. 2012a), gold(I, III) (Nobili et al. 2010), antimony(III), bismuth(III) (Tiekink 2002), ruthenium(II) (Bratsos et al. 2007), and copper(II) (Krajčiová et al. 2014) complexes. Since DNA is not the main target for many non-platinum metal-based drug candidates, determining the mechanism of action of these compounds is important to further understand their pharmacological activities.

Thioredoxin reductase (TrxR) is a selenoenzyme essential for antioxidant defense and redox homeostasis. TrxR is overexpressed in several tumor cell lineages. TrxR inactivation has been associated to inhibition of cell growth/proliferation, cell cycle arrest and inhibition of apoptosis. Thus, TrxR may be considered as a promising target for anticancer therapy (Colotti et al. 2013; Powis et al. 2006). The literature reports that many gold(I, III) complexes are potent inhibitors of TrxR, suggesting that the cytotoxic activities of these complexes are mainly due to TrxR inhibition (Nobili et al. 2010; Ott 2009; Ott et al. 2009). Furthermore, TrxR inhibition has also been observed for complexes with metals different from gold, such as ruthenium(II, III) (Mura et al. 2007; Oehninger et al. 2013), tin(IV) (De Oliveira et al. 2013) and antimony(III) complexes (Parrilha et al. 2014).

Topoisomerase is another important enzymatic target for the development of new effective antitumor agents. This family of enzymes is able to control the topological state of DNA during various essential cellular processes as replication, transcription, recombination and chromosomal segregation (Thapa et al. 2015).

Topoisomerases are divided into two subfamilies: type I and type II topoisomerases, which cleave either one or two strands of the DNA helix, respectively, followed by repair and restoration of DNA integrity. In addition, each subfamily contains two different classes: type A enzymes form a covalent bond with the 5′ end of DNA generated at the cleavage site, while type B form a covalent bond with the 3′-phosphate end of the cleaved strand (Castelli et al. 2013; Baikar and Malpathak 2010; Vieira et al. 2015).

Topoisomerase IB (Topo IB) is of great clinical interest and extensive data on the synthesis of Topo IB inhibitors have been reported in the literature (Meng et al. 2003; Baikar and Malpathak 2010; Castelli et al. 2011, 2013). Inhibition of topoisomerase activity has been observed for square planar metal complexes that exhibit cytotoxic activities, such as platinum(II), gold(III) and copper(II) complexes (Che and Siu 2010; Castelli et al. 2011; Zeglis et al. 2011).

Chalcones (1,3-diarylprop-2-en-1-ones) belong to the flavonoid family, in which two aryl rings are linked by an α,β-unsaturated ketone moiety. Their widespread distribution in comestible plants and the ease synthesis of these compounds encourage the investigation on the possible therapeutic applications of chalcone derivatives (Nowakowska 2007). In fact, this class of compounds has been reported to exhibit antimicrobial, anti-inflammatory, antileishmanial, antimalarial, and antitumor activities (Dimmock et al. 1999; Go et al. 2005).

Thiosemicarbazones and their metal complexes also present a wide range of bioactivities as antiviral (Teitz et al. 1994), antimicrobial (Mendes et al. 2008), antiprotozoal (Merlino et al. 2010), and antitumor (Lessa et al. 2010) agents. The main target of α(*N*)-heterocyclic thiosemicarbazones is believed to be ribonucleoside diphosphate reductase (RDR), an essential enzyme involved in the conversion of ribonucleotides into deoxyribonucleotides during DNA synthesis (Beraldo and Gambino 2004). However, inhibition of topoisomerase II by several families of thiosemicarbazones has been reported as well. In addition, upon coordination of a series of thiosemicarbazones to copper(II), inhibition of topoisomerase IIα significantly increased (Zeglis et al. 2011).

Thiosemicarbazones possessing a variety of structural scaffolds have been investigated, but there are few reports in the literature on thiosemicarbazones containing the chalcone skeleton. In previous works we investigated gallium(III), zinc(II) (Da Silva et al. 2013a) and copper(II) (Da Silva et al. 2013b) complexes with chalcone-derived thiosemicarbazones. We demonstrated that the copper(II) complexes exhibit significant cytotoxic activity against different human tumor cell lineages (Da Silva et al. 2013b).

In the context of a research program that aims to contribute to the discovery of new anticancer drug candidates, in the present work we prepared gold(III), platinum(II), and palladium(II) complexes with 3-(4-bromophenyl)-1-pyridin-2-ylprop-2-en-1-one thiosemicarbazone (HPyCT4BrPh) (Fig. 1). The compounds were assayed for their cytotoxic activities against HL-60 and THP-1 leukemia and against MCF-7 and MDA-MB 231 breast cancer cells. As a preliminary investigation on the mode of action of these compounds, the ability of the most cytotoxic gold(III) complex to act as inhibitor of the enzymatic activities of TrxR and Topo IB was evaluated.

Materials and methods

Physical measurements

All common chemicals were purchased from Aldrich and were used without further purification. Partial elemental analyses were performed on a Perkin Elmer CHN 2400 analyzer. An YSI model 31 conductivity bridge was employed for molar conductivity measurements. Infrared spectra were recorded on a Perkin Elmer FT-IR Spectrum GX spectrometer using KBr plates (4000–400 cm^{-1}). NMR spectra were obtained with a Bruker DPX-200 Avance (200 MHz) spectrometer using DMSO-d_6 or MeOD-d_4 as solvent and TMS as internal reference.

Syntheses of the compounds

3-(4-bromophenyl)-1-pyridin-2-ylprop-2-en-1-one thiosemicarbazone (HPyCT4BrPh) was prepared as previously described (Da Silva et al. 2013a). IR and NMR data were compatible with the proposed structure.

*[(3-(4-bromophenyl)-1-pyridin-2-ylprop-2-en-1-en-one thiosemicarbazonato)chlorogold(III)] chloride, [Au(PyCT4BrPh)Cl]Cl (**1**)*

The gold(III) complex was obtained by mixing a 1:1 methanol/acetone solution of HPyCT4BrPh (1.0 mmol) with hydrogen tetrachloroaurate(III) ($HAuCl_4 \cdot 3H_2O$) in 1:1 ligand-to-metal molar ratio at room

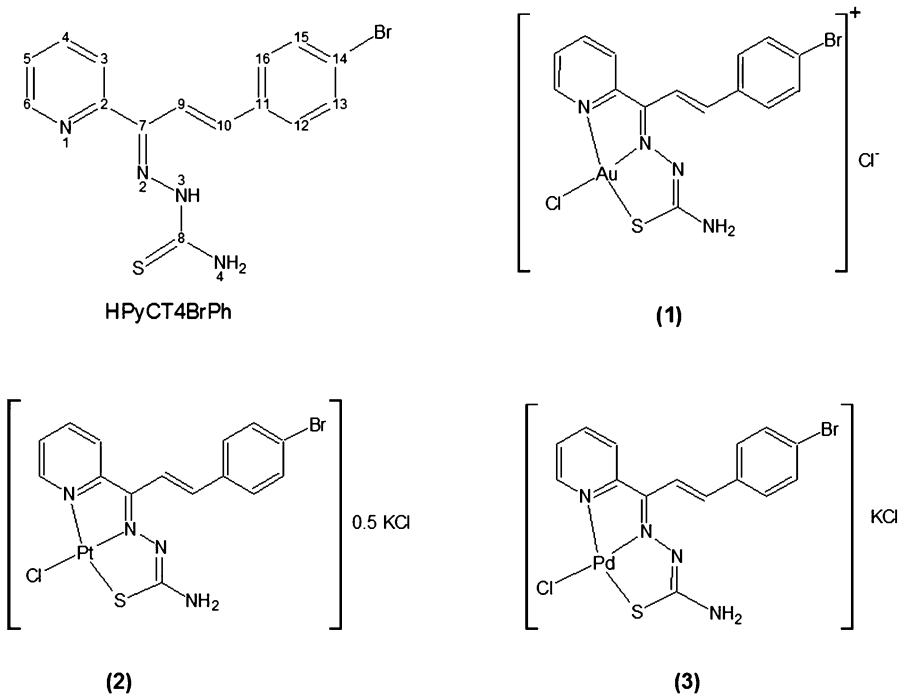


Fig. 1 Structure of 3-(4-bromophenyl)-1-pyridin-2-ylprop-2-en-1-one thiosemicarbazone (HPyCT4BrPh) and its complexes [Au(PyCT4BrPh)Cl]Cl (**1**), [Pt(PyCT4BrPh)Cl]0.5KCl (**2**), [Pd(PyCT4BrPh)Cl]KCl (**3**)

temperature with stirring for 24 h. The resulting solid was filtered off, washed with methanol and acetone, and dried in vacuo.

Black solid. Anal. Calc. for $C_{15}H_{12}AuBrCl_2N_4S$: C, 28.68; H, 1.93; N, 8.92. Found: C, 28.70; H, 2.07; N, 8.81 %. FW: 628.12 g mol^{-1}. IR (KBr, cm^{-1}): ν(C=N) 1600 m, ν(C–S) 722 w, ρ(py) 670 w. Molar conductivity (1×10^{-3} mol L^{-1} DMF): 71.8 Ω^{-1} cm^2 mol^{-1}. ^{1}H NMR [200 MHz, MeOD-d_4, ppm]: 10.64 (1H, d, H6), 10.18–10.05 (4H, m, H3, H10, H-N4H), 9.57–9.50 (1H, m, H4), 9.34–9.12 (5H, m, H5, H12, H13, H15, H16), 8.99 (1H, d, H9). Melting point: 174–175 °C. Yield: 72 %.

*[(3-(4-bromophenyl)-1-pyridin-2-ylprop-2-en-1-onethiosemicarbazonato)chloroplatinum(II)] hemi potassium chloride, [Pt(PyCT4BrPh)Cl]0.5KCl (**2**)*

The platinum(II) complex was obtained by stirring under reflux a methanol solution of HPyCT4BrPh (1.0 mmol) with an aqueous solution of K_2PtCl_4 in equimolar amounts for 2 h. The resulting solid was filtered off, washed with methanol and diethyl ether, and dried in vacuo.

Brown solid. Anal. Calc. for $C_{15}H_{12}BrCl_{1.5}N_4PtSK_{0.5}$: C, 28.69; H, 1.93; N, 8.92. Found: C, 28.23; H, 2.10; N, 8.72 %. FW: 628.06 g mol^{-1}. IR (KBr, cm^{-1}): ν(C=N) 1604 m, ν(C–S) 774 w, ρ(py) 668 w. Molar conductivity (1×10^{-3} mol L^{-1} DMF): 19.5 Ω^{-1} cm^2 mol^{-1}. ^{1}H NMR [400 MHz, DMSO-d_6, ppm]: 8.85 (1H, d, H6), 8.36–8.28 (3H, m, H-N4H, H10), 8.17 (1H, t, H4), 8.11 (1H, d, H3), 7.70 (1H, t, H5), 7.72–7.64 (2H, m, H12, H16), 7.62 (2H, d, H13, H15). ^{13}C NMR [50 MHz, DMSO-d_6, ppm]: 147.9 (C2), 125.4 (C3), 140.8 (C4), 126.3 (C5), 146.4 (C6), 159.5 (C7=N), 186.0 (C8=S), 117.1 (C9), 140.6 (C10), 135.9 (C11), 129.7 (C12, C16), 131.8 (C13, C15), 122.8 (C14). Melting point: 225–227 °C. Yield: 81 %.

*[(3-(4-bromophenyl)-1-pyridin-2-ylprop-2-en-1-onethiosemicarbazonato)chloropalladium(II)] potassium chloride, [Pd(PyCT4BrPh)Cl]KCl (**3**)*

The palladium(II) complex was prepared by mixing HPyCT4BrPh in methanol with an aqueous solution of K_2PdCl_4 in equimolar amounts (1.0 mmol). The reaction mixture was kept under reflux for 2 h. The resulting solid was filtered off, washed with methanol and diethyl ether, and dried *in vacuo*.

Maroon solid. Anal. Calc. for $C_{15}H_{12}BrCl_2N_4PdSK$: C, 31.24; H, 2.10; N, 9.72. Found: C, 30.88; H, 2.32; N, 9.64 %. FW: 576.68 g mol^{-1}. IR (KBr,

cm^{-1}): ν(C=N) 1610 m, ν(C–S) 776 w, ρ(py) 660 w. Molar conductivity (1×10^{-3} mol L^{-1} DMF): 16.0 Ω^{-1} cm^2 mol^{-1}. The main signals in ^1H NMR [400 MHz, DMSO-d_6, ppm]: 8.62 (1H, d, H6), 8.22 (2H, d, H-N4H), 8.16 (3H, d, H3, H4, H10), 7.71 (2H, d, H12, H16), 7.64 (3H, d, H5, H13, H15), 7.21 (1H, d, H9). ^{13}C NMR [50 MHz, DMSO-d_6, ppm]: 147.7 (C2), 124.9 (C3), 140.9 (C4), 125.6 (C5), 148.0 (C6), 158.2 (C7=N), 183.0 (C8=S), 116.0 (C9), 140.8 (C10), 135.6 (C11), 129.7 (C12, C16), 131.8 (C13, C15), 122.8 (C14). Melting point: dec. >300 °C. Yield: 72 %.

Cytotoxic activity

Cell lines

HL-60 (wild type human promyelocytic leukemia) and THP-1(human monocytic cells derived from an acute monocytic leukemia patient) cell lines were kindly given by Dr. Gustavo Amarante-Mendes (São Paulo University, Brazil). MCF-7 and MDA-MB 231 (human breast carcinoma) cells were kindly given by Dr. Alfredo Goes (Federal University of Minas Gerais, Brazil). Vero cells (derived from the kidney of an African green monkey) were donated by Erna Kroon (Federal University of Minas Gerais, Brazil).

All lineages were maintained in the logarithmic phase of growth in Roswell Park Memorial Institute (RPMI) 1640 or Dulbecco's Modified Eagle Medium (DMEM), supplemented with 100 U mL^{-1} penicillin and 100 mg mL^{-1} streptomycin (GIBCO BRL, Grand Island, NY) enriched with 2 mmol L^{-1} of L-glutamine and 10 % (for leukemic cells) or 5 % (for adherent cells) of fetal bovine serum. All cultures were maintained at 37 °C in a humidified incubator with 5 % CO_2 and 95 % air. The media were changed twice weekly and they were regularly examined.

Evaluation of the cytotoxic effect against human tumor cell lines

Cells were seeded at densities/well of 50,000 for HL-60 and THP-1 cells. Adherent (MCF-7, MDA-MB 231 and Vero) cells were inoculated at 10,000 cells/well. The plates were pre-incubated for 24 h at 37 °C to allow adaptation of cells prior to the addition of the test compounds. Freshly prepared solutions of the different compounds were screened at 1 μmol L^{-1}. Subsequently, the plates were inoculated for 48 h in an atmosphere of 5 % CO_2 and 100 % relative humidity. Control groups included treatment with 0.5 % DMSO (dimethyl sulfoxide, negative control) and 1 μmol L^{-1} of cisplatin or etoposide (positive control). Cell viability was estimated by measuring the rate of mitochondrial reduction of MTT (see below). All compounds were dissolved in DMSO prior to dilution. Compounds that inhibited the proliferation in more than 50 % were selected for determination of the half maximal inhibitory concentration (IC_{50}). IC_{50} values were determined over a range of ten-fold concentrations (100–0.001 μmol L^{-1}). All compounds were tested in triplicate, in three independent experiments.

In vitro cell viability MTT assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is a tetrazolium salt that is reduced to purple formazan crystals mainly by mitochondrial succinate dehydrogenase and the color intensity of the formazan dye is correlated to the number of viable cells (Mosmann 1983).

Briefly, after 4 h of the end of incubation of cells with the different compounds, 20 μL of MTT solution (5 mg mL^{-1} in phosphate-buffered saline) were added to each well, the supernatant was removed and 200 μL of 0.04 mol L^{-1} HCl in isopropyl alcohol were added to dissolve the formazan crystals. The optical densities (OD) were evaluated in a spectrophotometer at 595 nm. Controls included drug-containing medium (background) and drug-free complete medium. Drug-free complete medium was used as control (blank) and was treated in the same way as the drug-containing media. Results were expressed as percentage of cell proliferation, comparing with 0.5 % DMSO control and were calculated as follows: viability (%) = (mean OD treated—mean OD background)/(mean OD untreated cultured, i.e. 0.5 % DMSO—mean OD blank wells) ×100. Interactions of compounds and media were estimated on the basis of the variations between drug-containing medium and drug-free medium to escape from false-positive or false-negative in relation to the control (Ulukaya et al. 2004).

Inhibition of thioredoxin reductase (TrxR) and topoisomerase IB (Topo IB) enzymatic activities

Electronic spectra recorded with time (data not shown) indicated that complex (**1**) is stable in buffer/5 % DMF pH = 7.4 or buffer/5 % DMSO pH = 7.4 under the experimental conditions employed in the enzyme inhibition assays. The presence of absorptions in the 450–600 nm range, attributable to S → Au(III) and N → Au(III) ligand-to-metal charge transfer transitions (Lessa et al. 2012b), suggested that reduction of the metal did not take place.

Inhibition of thioredoxin reductase (TrxR) activity

Rat liver TrxR (Sigma) was used to determine TrxR inhibition by the compounds. The studies were carried out by means of the dithiobisnitrobenzoic acid (DTNB) reduction assay. This assay makes use of the fact that TrxR reduces the disulfide bonds of DTNB with formation of 5-thionitrobenzoic acid (TNB), which can be detected photometrically.

The assay was performed according to the manufacturer's instructions (Sigma product information sheet T9698) and to Ott et al. (2009) with appropriate modifications. First, the experiments were performed at 10 μmol L^{-1}. After, in order to determine the concentration of the compounds able to inhibit 50 % of the enzymatic activity (IC_{50}), new experiments were performed for each selected compound at 13 different concentrations in the 0.01–50 μmol L^{-1} range.

Initially, the TrxR rat liver solution was diluted with potassium phosphate buffer, pH 7.0. To 20 μL aliquots of this solution (each containing approximately 0.12 units of the enzyme), 20 μL of 5 % DMF in potassium phosphate buffer pH 7.0, containing the compounds in 13 different concentrations in the 0.01–50 μmol L^{-1} range or vehicle without compound (control) were added. The resulting solutions were incubated for 1 h at 37 °C with moderate shaking. The solutions were then transferred quantitatively to 96-well plates, and to each well 200 μL of reaction mixture (10 mL of reaction mixture consisted of 1.0 mL of 1.0 mol L^{-1} potassium phosphate buffer, pH 7.0; 0.20 mL of 500 mmol L^{-1} EDTA solution, pH 7.5; 0.80 mL of 63 mmol L^{-1} DTNB in ethanol; 0.10 mL of 20 mg mL^{-1} bovine serum albumin; 0.05 mL of 48 mmol L^{-1} NADPH and 7.85 mL of water) were added. To correct for non-enzymatic product formation, 40 μL of 1.0 mol L^{-1} potassium phosphate buffer, pH 7.0, and 200 μL of reaction mixture were processed simultaneously (blank value). After proper mixing, the formation of TNB was monitored in a microplate reader (Thermo Scientific Multiskan Go Spectrophotometer) at 412 nm for 10 min. The absorbance of the blank was subtracted from that of the control and treated wells. The enzymatic activities were calculated as the maximum absorbance produced in 10 min in each well. The experiments were performed in triplicate.

Inhibition of topoisomerase IB activity

Human topoisomerase IB was expressed and purified as described previously (Chillemi et al. 2005). Topo IB enzyme was incubated in 30 μL of reaction volume containing 0.5 μg of negatively supercoiled pBlue-Script KSII(+) DNA plasmid in Reaction Buffer (20 mmol L^{-1} Tris–HCl, 0.1 mmol L^{-1} Na_2EDTA, 10 mmol L^{-1} $MgCl_2$, 50 μg/mL acetylated BSA and 150 mmol L^{-1} KCl, pH 7.5). To assess the effects of the compounds under study on enzyme activity, different concentrations of the compounds were added. Reactions were stopped with a final concentration of 0.5 % SDS after 1 h at 37 °C. The samples were electrophoresed in a horizontal 1 % agarose gel in 50 mmol L^{-1} Tris, 45 mmol L^{-1} boric acid, 1 mmol L^{-1} EDTA). The gel was stained with ethidium bromide (5 μg mL^{-1}), de-stained with water and photographed under UV illumination. Where indicated, enzyme and inhibitor were pre-incubated at 37 °C for 1 min, prior to the addition of the substrate. The mixture was then incubated at 37 °C for 15 min.

Results and discussion

Formation of the complexes

Microanalyses and molar conductivity data were compatible with the formation of [Au(PyCT4BrPh)Cl]Cl (**1**), [Pt(PyCT4BrPh)Cl]0.5KCl (**2**), and [Pd(PyCT4BrPh)Cl]KCl (**3**). The presence of KCl in complexes (**2**) and (**3**) could have arisen from the precursor salts, contributing to the molar conductivity.

In fact, molar conductivity data suggest that **2** and **3** are weak electrolytes. Similar behavior was previously reported for platinum(II, IV) complexes with clioquinol (Ferraz et al. 2013a) and for a palladium(II) complex with D-penicillamine (Cervantes et al. 1998).

In complex (**1**) one anionic thiosemicarbazone ligand and one chloride ion are attached to the gold(III) center, and a chloride acts as counter-ion. In complexes [Pt(PyCT4BrPh)Cl]0.5KCl (**2**) and [Pd(PyCT4BrPh)Cl]KCl (**3**) one anionic thiosemicarbazone ligand is attached to the metal center together with a chloride ion. In addition, 0.5 KCl is present in complex (**2**) and 1.0 KCl in complex (**3**).

Spectroscopic characterization

The vibration attributed to ν(C=N) at 1560 cm^{-1} in the infrared spectrum of HPyCT4BrPh shifts to 1610–1600 cm^{-1} in the spectra of its complexes, in agreement with coordination via the azomethine nitrogen (Ferraz et al. 2011, 2013b).

The ν(C=S) absorption observed at 780 cm^{-1} in the spectrum of the free thiosemicarbazone shifts to 776–722 cm^{-1} in the spectra of the complexes, indicating coordination of the sulfur (Lessa et al. 2011; Ferraz et al. 2009). In addition, the vibration attributed to ν(N–H) in the free thiosemicarbazone disappears in the spectra of the complexes indicating deprotonation (Ferraz et al. 2012). The in-plane deformation mode of the pyridine ring at 554 cm^{-1} in the spectrum of the uncomplexed thiosemicarbazone shifts to 670–660 cm^{-1} in the complexes, suggesting coordination of the heteroaromatic nitrogen (Lessa et al. 2011; Ferraz et al. 2013b). Hence, the infrared data suggest that in the complexes the thiosemicarbazone is attached to the metal center through the N_{py}–N–S chelating system (see Fig. 1).

Although the thiosemicarbazone and its complexes were soluble in DMSO-d_6, at high concentration the gold(III) complex interacted with the solvent. Hence, the ^{1}H NMR spectrum of **1** was recorded in methanol-d_4. For complexes (**2**) and (**3**), the NMR spectra were recorded in DMSO-d_6. The ^{1}H resonances were assigned on the basis of chemical shifts and multiplicities. We did not obtain the ^{13}C NMR spectrum of **1** due to its low solubility in methanol-d_4. For **2** and **3**, the C-atom type (C, CH) was determined by using distortionless enhancement by polarization transfer (DEPT-135) experiments. The assignments of the protonated carbons were accomplished by 2D heteronuclear multiple quantum coherence experiments (HMQC).

Immediately after dissolution, the signals of all hydrogen and carbon atoms are duplicated in the ^{1}H and ^{13}C NMR spectra of the free thiosemicarbazone, indicating the presence of two isomers in DMSO-d_6. The signals at 13.07 and 11.44 ppm in the ^{1}H NMR spectrum were attributed to N3–H of the *Z* and *E* isomers, respectively. In the first, N3–H is hydrogen bonded to the heteroaromatic nitrogen while in the second N3–H is hydrogen bonded to the solvent (Da Silva et al. 2013a).

In the ^{1}H NMR spectra of complexes (**1**-**3**), only one signal was observed for each hydrogen, suggesting the presence of only the *E* configurational isomer in solution. The absence of the N3–H signal in the spectra of complexes (**2**) and (**3**) indicates coordination of an anionic thiosemicarbazone. In addition, the signals of all hydrogens undergo significant shifts upon coordination. Similarly, the signals of C7=N, C8=S and the pyridine carbons shift significantly, in agreement with coordination through the N_{py}-N-S system.

Cytotoxic activity

The cytotoxic activities of the compounds were assayed on HL-60 and THP-1 leukemia cells, and on MDA-MB 231 and MCF-7 solid tumor cells. In addition, cytotoxicity was evaluated against Vero, as a representative of non-malignant cells. Negative control was taken as 100 % survival.

First, the percentage of cell survival in the presence of the studied compounds at 1 μmol L^{-1} was assayed (data not shown). Results indicated that complex (**2**) and the metal precursors used in the syntheses of the complexes were not significantly active against the tested malignant cells. The obtained preliminary results of cell survival stimulated determining the concentration of HPyCT4BrPh and complexes (**1**) and (**3**) able to kill 50 % of the tumor cells (IC_{50}). IC_{50} values for the platinum complex (**2**) were not determined due to its low cytotoxic activity.

The determined IC_{50} values are shown in Table 1. Coordination to gold(III) resulted in enhanced cytotoxic activity against THP-1, MDA-MB 231 and MCF-7 cells. Complex (**1**) was 3-fold more active than HPyCT4BrPh against MCF-7 cells. In addition,

Table 1 Cytotoxic activity (IC_{50}, μmol L^{-1}) of HPyCT4BrPh and its gold(III) (**1**) and palladium(II) (**3**) complexes on HL-60, THP-1, MDA-MB 231, MCF-7 and Vero cell lines

Compound	IC_{50} values				
	HL60	THP-1	MDA-MB 231	MCF-7	Vero
HPyCT4BrPh	0.04 (±0.002)	1.27 (±0.32)	0.25 (±0.11)	1.23 (±0.25)	>10
1	0.26 (±0.20)	0.62 (±0.49)	0.09 (±0.05)	0.42 (±0.01)	>1
3	>1	>1	>1	>1	>1
Cisplatin	0.023 (±0.014)	0.015 (±0.011)	>100	>100	13.045 (±0.629)
Etoposide	0.855 (±0.389)	2.751 (±1.062)	>100	>100	>100

HPyCT4BrPh and its gold(III) complex (**1**) were less cytotoxic to Vero than to the assayed tumor cell lineages.

Studies on the mechanisms of action of complex (**1**)

As a preliminary investigation into the mode of action of the compounds under study, the effects of HPyCT4BrPh and its gold(III) complex (**1**) on the enzymatic activities of TrxR and Topo IB were evaluated.

Inhibition of thioredoxin reductase (TrxR) activity

Inhibition of TrxR activity was considered as a potential mechanism for the anti-proliferative activity of complex (**1**). The gold(I) containing anti-arthritic drug, triethylphosphinegold(I)tetraacetylthioglucose, "auranofin", was used as control due to its well known ability to inhibit TrxR (Andrade and Reed 2015). Thus, the in vitro inhibitory activities of HPyCT4BrPh, **1**, $HAuCl_4{\cdot}3H_2O$ and auranofin were evaluated using isolated rat liver TrxR by means of the DTNB reduction assay.

Initially, the ability of the selected compounds to inhibit TrxR activity was investigated at 10 μmol L^{-1}. HPyCT4BrPh was unable to inhibit the enzymatic activity in the assayed concentration. Thus, the free thiosemicarbazone, although being cytotoxic against the tumor cell lineages, did not show any inhibitory effect of TrxR activity. In fact, the main target of thiosemicarbazones is believed to be RDR (Beraldo and Gambino 2004). Complex (**1**) inhibited 77.9 %, while $HAuCl_4{\cdot}3H_2O$ and auranofin inhibited 97.5 and 95.2 % of TrxR activity, respectively. Similarly, in a previous work we demonstrated that [Au(H_{py}2-Ac4*m*T)Cl_2]Cl (H_{py}4Ac4*m*T stands for the thiosemicarbazone protonated at the pyridine nitrogen), a gold(III) complex with 2-acetylpyridine-4-*meta*-tolyl thiosemicarbazone inhibited 75 % of TrxR activity at 10 μmol L^{-1} (Ferraz et al. 2013b).

The obtained preliminary results on TrxR inhibition stimulated the determination of the concentration of **1** and $HAuCl_4{\cdot}3H_2O$ which decreases the enzymatic activity by 50 % in relation to the control (IC_{50}). Complex (**1**) inhibited the enzyme activity with $IC_{50} = 5.55$ μmol L^{-1} (95 % confidence interval = 5.11–6.03 μmol L^{-1}), suggesting TrxR as a possible biological target for this compound. In addition, auranofin ($IC_{50} = 0.30$ μmol L^{-1}; 95 % confidence interval = 0.27–0.32 μmol L^{-1}) and $HAuCl_4{\cdot}3H_2O$ ($IC_{50} = 0.62$ μmol L^{-1}; 95 % confidence interval = 0.59–0.65 μmol L^{-1}) also strongly inhibited the enzymatic activity, indicating that the inhibitory effect of **1** on TrxR may be related to the presence of gold.

In spite of the fact that $HAuCl_4{\cdot}3H_2O$ strongly inhibited TrxR activity, it proved to be devoid of any cytotoxic effect against the cell lines under study. The absence of cytotoxic activity of $HAuCl_4{\cdot}3H_2O$ may be due to its hydrophilic character, which probably hinders its passage through the cell membrane. In previous works we demonstrated that gold(I) and gold(III) complexes with α(*N*)-heterocyclic thiosemicarbazones (Lessa et al. 2011; Ferraz et al. 2013b) and with *bis*(thiosemicarbazones) (Lessa et al. 2012b) were potent inhibitors of TrxR activity. As previously suggested, the thiosemicarbazone, besides its own cytotoxic effect, probably acts as a carrier of gold into the cell.

The affinity of complex (**1**) for TrxR suggests metal binding to a selenol residue in the active site of the

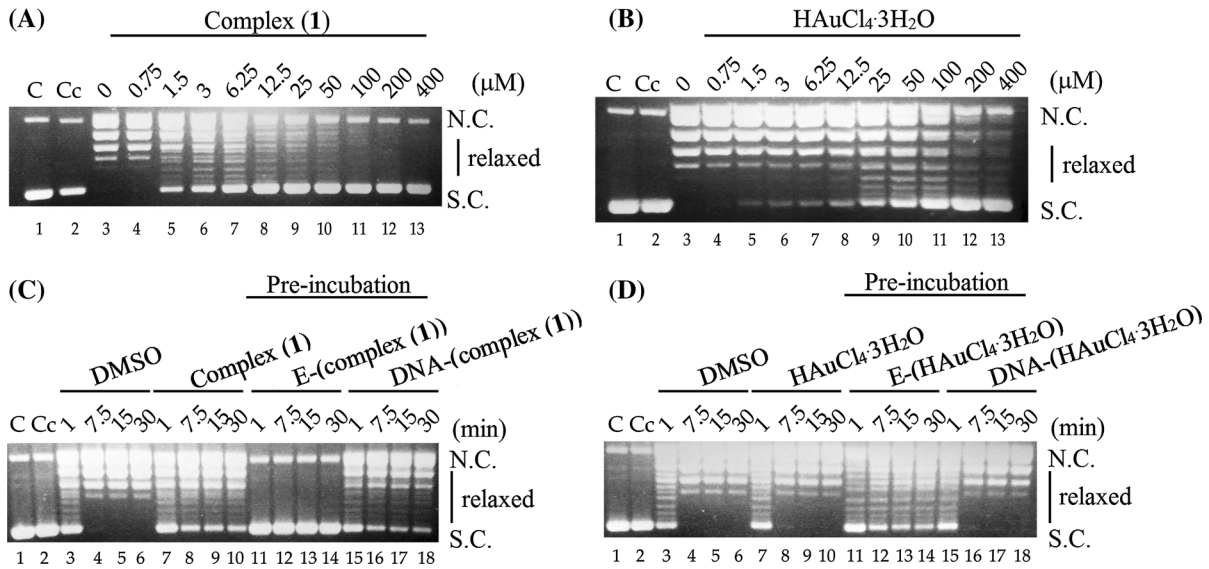


Fig. 2 Relaxation of supercoiled DNA by human topoisomerase IB in the presence of increasing concentrations of complex (**1**) (**A**) and $HAuCl_4{\cdot}3H_2O$ (**B**). *Lane 1*, supercoiled pBlueScript KSII(+) DNA plasmid. *Lane 2*, DNA plasmid in the presence of an excess of complex (**1**) or $HAuCl_4{\cdot}3H_2O$. (**C**) Relaxation activity of Topo IB in the presence of: DMSO (*lanes 3–6*), 1.5 μmol L^{-1} complex (**1**) (*lanes 7–10*), after 5 min enzyme-complex (**1**) pre-incubation, before substrate addition (*lanes 11–14*) and after 5 min DNA-complex (**1**), before enzyme addition (*lanes 15–18*). *Lane 1*, supercoiled pBlueScript KSII(+) DNA plasmid. *Lane 2*, DNA plasmid in the presence of 1.5 μmol L^{-1} complex (**1**). (**D**) Relaxation activity of Topo IB in the presence of: DMSO (*lanes 3–6*), 1.5 μmol L^{-1} $HAuCl_4{\cdot}3H_2O$ (*lanes 7–10*), after 5 min enzyme-$HAuCl_4{\cdot}3H_2O$ pre-incubation, before substrate addition (*lanes 11–14*) and after 5 min DNA-$HAuCl_4{\cdot}3H_2O$, before enzyme addition (*lanes 15–18*). *Lane 1*, supercoiled pBlueScript KSII(+) DNA plasmid. *Lane 2*, DNA plasmid in the presence of 1.5 μmol L^{-1} $HAuCl_4{\cdot}3H_2O$

enzyme. Hence, TrxR inhibition is possibly a contributing factor to its mode of action, as previously proposed for gold(III) complexes with 2-acetylpyridine-derived thiosemicarbazones (Parrilha et al. 2014).

Inhibition of topoisomerase IB (Topo IB) activity

Topo IB is a validated target for cancer chemotherapy. The effect of complex (**1**) on human topoisomerase IB was studied by a relaxation assay that permits to evaluate the potential inhibitory effect of the compounds following the differences in electrophoretic mobility of supercoiled DNA and its partially relaxed forms produced by the enzyme activity (Fig. 2**A** compare lanes 1 and 3).

Complex (**1**) inhibits Topo IB in a dose depended manner, starting from 1.5 μmol L^{-1} concentration. The effect is completed at around 50–100 μmol L^{-1}, as it can be appreciated in Fig. 2**A** from the intensity of the band corresponding to supercoiled DNA and from the absence of bands corresponding to the topoisomers. The inhibitory effect of **1** is improved when the complex is pre-incubated with the enzyme for 5 min before DNA addition. In this condition, a concentration of 1.5 μmol L^{-1} is enough to completely inhibit the enzymatic activity (Fig. 2**C**). Hence, Topo IB inhibition could take part in the mode of action of complex (**1**).

It is noteworthy that $HAuCl_4{\cdot}3H_2O$ alone was able to inhibit Topo IB activity but in this case inhibition was never completed (Fig. 2**B**). Pre-incubation of the enzyme with the gold precursor before DNA addition strengthens the effect, suggesting that gold interacts somewhere on the enzyme, reducing its ability to relax DNA (Fig. 2**D**).

The free thiosemicarbazone was unable to inhibit the enzyme (data not shown). Comparing the experiments of dose dependent Topo IB inhibition performed in the presence of complex (**1**) and in the presence of $HAuCl_4{\cdot}3H_2O$ we can state that gold(III) coordination to the thiosemicarbazone ligand improved the

inhibitory effect of gold about 8-16 times (Fig. 2 compare **A**, **B**).

Conclusions

Coordination of HPyCT4BrPh to gold(III) in complex (**1**) resulted in higher cytotoxic activity against all studied cell lineages except HL-60 cells.

Since complex (**1**), auranofin and $HAuCl_4 \cdot 3H_2O$ inhibited the enzymatic activity of TrxR, the inhibitory effect of **1** may be related to the presence of gold. However, unlike $HAuCl_4$, **1** not only inhibited TrxR activity but also showed strong cytotoxic effect, indicating that the thiosemicarbazone probably acts as a carrier of gold into the cell, as previously suggested by us.

While the free thiosemicarbazone was unable to inhibit the enzymatic activity of Topo IB, complex (**1**) inhibited Topo IB activity in a dose depended manner. $HAuCl_4 \cdot 3H_2O$ alone was also able to inhibit Topo IB activity. Pre-incubation of the enzyme with $HAuCl_4 \cdot 3H_2O$ before DNA addition strengthens the effect, suggesting that gold interacts somewhere on the enzyme, reducing its ability to relax DNA. Upon coordination of gold(III) to the thiosemicarbazone the inhibitory effect of gold significantly increased.

We demonstrated that TrxR and Topo IB inhibition could take part in the mechanism of cytotoxic action of **1** but not in that of the free thiosemicarbazone. Gold(III) probably binds to a selenol residue in the active site of TrxR and probably interacts somewhere on Topo IB. In both cases, inhibition of the enzymatic activity was due to the presence of gold(III) but the thiosemicarbazone plays an important role, probably as a carrier of the metal.

The main cellular target of platinum-based antitumor drugs is DNA. Although platinum(II) and gold(III) complexes are isoelectronic and isostructural, both presenting square planar geometry, their mechanisms of anti-proliferative activity are substantially distinct. The present work represents an important contribution to the understanding of the cellular targets and mode of cytotoxic action of gold(III) complexes.

Acknowledgments The authors are grateful to CNPq, INCT-INOFAR (Proc. CNPq 573.364/2008-6) and CAPES/PNPD.

References

Andrade RM, Reed SL (2015) New drug target in protozoan parasites: the role of thioredoxin reductase. Front Microbiol 6:1–7. doi:10.3389/fmicb.2015.00975

Baikar S, Malpathak N (2010) Secondary metabolites as DNA topoisomerase inhibitors: a new era towards designing of anticancer drugs. Pharmacogn Rev 4:12–26. doi:10.4103/0973-7847.65320

Beraldo H, Gambino D (2004) The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes. Mini Rev Med Chem 4:31–39. doi:10.2174/1389557043487484 (**and references therein**)

Bratsos I, Jedner S, Gianferrara T, Alessio E (2007) Ruthenium anticancer compounds: challenges and expectations. Chimia 61:692–697. doi:10.2533/chimia.2007.692

Castelli S, Vassallo O, Katkar P, Che CM, Sun RWY, Desideri A (2011) Inhibition of human DNA topoisomerase IB by a cyclometalated gold III compound: analysis on the different steps of the enzyme catalytic cycle. Arch Biochem Biophys 516:108–112. doi:10.1016/j.abb.2011.10.008

Castelli S, Katkar P, Vassallo O, Falconi M, Linder S, Desideri A (2013) A natural anticancer agent thaspine targets human topoisomerase IB. Anti-Cancer Agents Med Chem 13:356–363. doi:10.2174/187152013804711128

Cervantes G, Moreno V, Molins E, Quirós M (1998) Pd(II) and Pt(II) D-penicillamine complexes. Crystal structure of a tridentate D-penicillamine cluster of Pd(II). Polyhedron 17:3343–3350. doi:10.1016/S0277-5387(98)00114-4

Che CM, Siu FM (2010) Metal complexes in medicine with a focus on enzyme inhibition. Curr Opin Chem Biol 14:255–261. doi:10.1016/j.cbpa.2009.11.015

Chillemi G, Fiorani P, Castelli S, Bruselles A, Benedetti P, Desideri A (2005) Effect on DNA relaxation of the single Thr718Ala mutation in human topoisomerase I: a functional and molecular dynamics study. Nucleic Acids Res 33:3339–3350. doi:10.1093/nar/gki642

Colotti G, Ilari A, Boffi A, Morea V (2013) Metals and metal derivatives in medicine. Mini-Rev Med Chem 13:211–221. doi:10.2174/138955713804805238

Da Silva JG, Perdigão CCH, Speziali NL, Beraldo H (2013a) Chalcone-derived thiosemicarbazones and their zinc(II) and gallium(III) complexes: spectral studies and antimicrobial activity. J Coord Chem 66:385–401. doi:10.1080/00958972.2012.757762

Da Silva JG, Despaigne AAR, Louro SRW, Bandeira CC, Souza-Fagundes EM, Beraldo H (2013b) Cytotoxic activity, albumin and DNA binding of new copper(II) complexes with chalcone-derived thiosemicarbazones. Eur J Med Chem 65:415–426. doi:10.1016/j.ejmech.2013.04.036

De Oliveira KN, Andermark V, von Grafenstein S, Onambele LA, Dahl G, Rubbiani R, Wolber G, Gabbiani C, Messori L, Prokop A, Ott I (2013) Butyltin(IV) benzoates: inhibition of thioredoxin reductase, tumor cell growth inhibition, and interactions with proteins. ChemMedChem 8:256–264. doi:10.1002/cmdc.201200505

Dimmock JR, Elias DW, Beazely MA, Kandepu NM (1999) Bioactivities of chalcones. Curr Med Chem 6:1125–1149

Ferraz KSO, Ferandes L, Carrilho D, Pinto MCX, Leite MF, Souza-Fagundes EM, Speziali NL, Mendes IC, Beraldo H (2009) 2-Benzoylpyridine-*N*(4)-tolyl thiosemicarbazones and their palladium(II) complexes: cytotoxicity against leukemia cells. Bioorg Med Chem 17:7138–7144. doi:10.1016/j.bmc.2009.08.063

Ferraz KOS, Cardoso GMM, Bertollo CM, Souza-Fagundes EM, Speziali N, Zani CL, Mendes IC, Gomes MA, Beraldo H (2011) *N*(4)-tolyl-2-benzoylpyridine-derived thiosemicarbazones and their palladium(II) and platinum(II) complexes: cytotoxicity against human solid tumor cells. Polyhedron 30:315–321. doi:10.1016/j.poly.2010.10.014

Ferraz KSO, Silva NF, Da Silva JG, Speziali NL, Mendes IC, Beraldo H (2012) Structural studies on acetophenone- and benzophenone-derived thiosemicarbazones and their zinc(II) complexes. J Mol Struct 1008:102–107. doi:10.1016/j.molstruc.2011.11.035

Ferraz KSO, Reis DC, Da Silva JG, Souza-Fagundes EM, Baran EJ, Beraldo H (2013a) Investigation on the bioactivities of clioquinol and its bismuth(III) and platinum(II, IV) complexes. Polyhedron 63:28–35. doi:10.1016/j.poly.2013.07.008

Ferraz KSO, Da Silva JG, Costa FM, Mendes BM, Rodrigues BL, dos Santos RG, Beraldo H (2013b) *N*(4)-Tolyl-2-acetylpyridine thiosemicarbazones and their platinum(II, IV) and gold(III) complexes: cytotoxicity against human glioma cells and studies on the mode of action. Biometals 26:677–691. doi:10.1007/s10534-013-9639-x

Garbutcheon-Singh KB, Grant MP, Harper BW, Krause-Heuer AM, Manohar M, Orkey N, Aldrich-Wright JR (2011) Transition metal based anticancer drugs. Curr Top Med Chem 11:521–542. doi:10.2174/156802611794785226

Go ML, Wu X, Liu XL (2005) Chalcones: an update on cytotoxic and chemoprotective properties. Curr Med Chem 12:483–499. doi:10.2174/0929867053363153

Krajčiová D, Melník M, Havránek E, Forgácsová A, Mikuš P (2014) Copper compounds in nuclear medicine and oncology. J Coord Chem 67:1493–1519. doi:10.1080/00958972.2014.915966

Lessa JA, Mendes IC, da Silva PRO, Soares MA, dos Santos RG, Speziali NL, Romeiro NC, Barreiro EJ, Beraldo H (2010) 2-Acetylpyridine thiosemicarbazones: cytotoxic activity in nanomolar doses against malignant gliomas. Eur J Med Chem 45:5671–5677. doi:10.1016/j.ejmech.2010.09.021

Lessa JA, Guerra JC, de Miranda LF, Romeiro CFD, Da Silva JG, Mendes IC, Speziali NL, Souza-Fagundes EM, Beraldo H (2011) Gold(I) complexes with thiosemicarbazones: cytotoxicity against human tumor cell lines and inhibition of thioredoxin reductase activity. J Inorg Biochem 105:1729–1739. doi:10.1016/j.jinorgbio.2011.09.008

Lessa JA, Ferraz KSO, Guerra JC, de Miranda LF, Romeiro CFD, Souza-Fagundes EM, Barbeira PJS, Beraldo H (2012a) Spectroscopic and electrochemical characterization of gold(I) and gold(III) complexes with glyoxaldehyde *bis*(thiosemicarbazones): cytotoxicity against human tumor cell lines and inhibition of thioredoxin reductase activity. Biometals 25:587–598. doi:10.1007/s10534-012-9547-5

Lessa JA, Parrilha GL, Beraldo H (2012b) Gallium complexes as new promising metallodrug candidates. Inorg Chim Acta 393:53–63. doi:10.1016/j.ica.2012.06.003 (**and references therein**)

Lippert B (1999) Cisplatin. Chemistry and biochemistry of a leading anticancer drug. Wiley, Weinheim

Mendes IC, Moreira JP, Ardisson JD, dos Santos RG, da Silva PRO, Garcia I, Castiñeiras A, Beraldo H (2008) Organotin(IV) complexes of 2-pyridineformamide-derived thiosemicarbazones: antimicrobial and cytotoxic effects. Eur J Med Chem 43:1454–1461. doi:10.1016/j.ejmech.2007.09.016

Meng LH, Liao ZY, Pommier Y (2003) Non-camptothecin DNA topoisomerase I inhibitors in cancer therapy. Curr Top Med Chem 3:305–320. doi:10.2174/1568026033452546

Merlino A, Benitez D, Chavez S, da Cunha J, Hernández P, Tinoco LW, Campillo NE, Páez JA, Cerecetto H, González M (2010) Development of second generation amidinohydrazones, thio- and semicarbazones as *Trypanosoma cruzi*-inhibitors bearing benzofuroxan and benzimidazole 1,3-dioxide core scaffolds. Med Chem Commun 1:216–228. doi:10.1039/c0md00085

Mosmann Y (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63. doi:10.1016/0022-1759(83)90303-4

Mura P, Camalli M, Bindoli A, Sorrentino F, Casini A, Gabbiani C, Corsini M, Zanello P, Rigobello MP, Messori L (2007) Activity of rat cytosolic thioredoxin reductase is strongly decreased by *trans*-[bis(2-amino-5-methylthiazole)tetrachlororuthenate(III)]: first report of relevant thioredoxin reductase inhibition for a ruthenium compound. J Med Chem 50:5871–5874. doi:10.1021/jm0708578

Nobili S, Mini E, Landini I, Gabbiani C, Casini A, Messori L (2010) Gold compounds as anticancer agents: chemistry, cellular pharmacology, and preclinical studies. Med Res Rev 30:550–580. doi:10.1002/med.20168

Nowakowska Z (2007) A review of anti-infective and anti-inflammatory chalcones. Eur J Med Chem 42:125–137. doi:10.1016/j.ejmech.2006.09.019

Oehninger L, Stefanopoulou M, Alborzinia H, Schur J, Ludewig S, Namikawa K, Muñoz-Castro A, Köster RW, Baumann K, Wölfl S, Sheldrick WS, Ott I (2013) Evaluation of arene ruthenium(II) *N*-heterocyclic carbene complexes as organometallics interacting with thiol and selenol containing biomolecules. Dalton Trans 42:1657–1666. doi:10.1039/c2dt32319b

Ott I (2009) On the medicinal chemistry of gold complexes as anticancer drugs. Coord Chem Rev 253:1670–1681. doi:10.1016/j.ccr.2009.02.019

Ott I, Qian X, Xu Y, Vlecken DHW, Marques IJ, Kubutat D, Will J, Sheldrick WS, Jesse P, Prokop A, Bagowski CP (2009) A gold(I) phosphine complex containing a naphthalimide ligand functions as a TrxR inhibiting antiproliferative agent and angiogenesis inhibitor. J Med Chem 52:763–770. doi:10.1021/jm8012135

Parrilha GL, Ferraz KSO, Lessa JA, de Oliveira KN, Rodrigues BL, Ramos JP, Souza-Fagundes EM, Ott I, Beraldo H (2014) Metal complexes with 2-acetylpyridine-*N*(**4**)-orthochlorophenylthiosemicarbazone: cytotoxicity and effect on the enzymatic activity of thioredoxin reductase and glutathione reductase. Eur J Med Chem 84:537–544. doi:10.1016/j.ejmech.2014.07.055

Powis G, Wipf P, Lynch SM, Birmingham A, Kirkpatrick DL (2006) Molecular pharmacology and antitumor activity of palmarumycin-based inhibitors of thioredoxin reductase. Mol Cancer Ther 5:630–636. doi:10.1158/1535-7163.MCT-05-0487

Teitz Y, Ronen D, Vansover A, Stematsky T, Riggs JL (1994) Inhibition of human immunodeficiency virus by *N*-methylisatin-β4′:β4′-diethylthiosemicarbazone and *N*-allylisatin-β4′:4′-diallythiosemicarbazone. Antiviral Res 24:305–314. doi:10.1016/0166-3542(94)90077-9

Thapa P, Jun KY, Kadayat TM, Park C, Zheng Z, Magar TBT, Bist G, Shrestha A, Na Y, Kwon Y, Lee ES (2015) Design and synthesis of conformationally constrained hydroxylated 4-phenyl-2-aryl chromenopyridines as novel and selective topoisomerase II-targeted antiproliferative agents. Bioorg Med Chem 23:6454–6466. doi:10.1016/j.bmc.2015.08.018

Tiekink ERT (2002) Antimony and bismuth compounds in oncology. Crit Rev Oncol Hemat 42:217–224. doi:10.1016/S1040-8428(01)00217-7

Ulukaya E, Colakogullari M, Wood EJ (2004) Interference by anti-cancer chemotherapeutic agents in the MTT-tumor chemosensitivity assay. Chemotherapy 50:43–50. doi:10.1159/000077285

Vieira S, Castelli S, Desideri A (2015) Importance of a stable topoisomerase IB clamping for an efficient DNA processing: effect of the $Lys^{369}Glu$ mutation. Int J Biol Macromol 81:76–82. doi:10.1016/j.ijbiomac.2015.07.044

Zeglis BM, Divilov V, Lewis JS (2011) Role of metalation in the topoisomerase IIα inhibition and antiproliferation activity of a series of α-heterocyclic-N^4-substituted thiosemicarbazones and their Cu(II) complexes. J Med Chem 54:2391–2398. doi:10.1021/jm101532u