Inorganica Chimica Acta 414 (2014) 85–90

Contents lists available at ScienceDirect

Inorganica Chimica Acta

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

Synthesis and cytotoxic activity of gold(I) complexes containing phosphines and 3-benzyl-1,3-thiazolidine-2-thione or 5-phenyl-1,3,4-oxadiazole-2-thione as ligands

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ARTICLE INFO

Article history:
Received 8 October 2013
Received in revised form 14 January 2014
Accepted 22 January 2014
Available online 5 February 2014

Keywords:
Gold(I) complexes
Tertiary phosphines
Thioamides
Cancer

ABSTRACT

Heterocyclic compounds and their metal complexes display a broad spectrum of pharmacological properties. This work reports the preparation and characterization of four novel gold(I) complexes containing tertiary phosphine and 3-benzyl-1,3-thiazolidine-2-thione, 5-phenyl-1,3,4-oxadiazole-2-thione as ligands. The reaction of chloro(triphenylphosphine)gold(I) and chloro(triethylphosphine)gold(I) with thioamides, 3-benzyl-1,3-thiazolidine-2-thione and 5-phenyl-1,3,4-oxadiazole-2-thione in dichloromethane or dichloromethane/acetone resulted in the formation of the gold(I) complexes of general formula: $[SAuPR_3]Cl$, S = 3-benzyl-1,3-thiazolidine-2-thione, R = Ph or Et and $[SAuPR_3]$, S = 5-phenyl-1,3,4-oxadiazole-2-thione, R = Ph or Et. Spectroscopic evidence suggested that gold is coordinated to the exocyclic sulfur atom in all cases and this was confirmed by X-ray crystallographic data obtained for complex (**4**). The cytotoxicity of the compounds has been evaluated in comparison to cisplatin in two different tumor cell lines, colon cancer (CT26WT) and metastatic skin melanoma (B16F10), and also in a kidney normal cell (BHK-21). The gold complexes showed a better activity than cisplatin and presented a high selectivity index.

1. Introduction

The discovery of the antitumor properties of cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) by Rosenberg represents a landmark in medicinal chemistry [1]. The use of cisplatin in chemotherapy resulted in a reduction of about 80% of the death rate among men suffering from testicular tumor in 1978 [2]. Since then, interest in Medicinal Inorganic Chemistry has continued to grow with the emergence of new targets and novel opportunities for intervention of Coordination Chemistry in Medicinal Chemistry. Gold(I) complexes have attracted great attention as potential antitumor agents due to the fact that many of them have been shown to inhibit the growth of tumor cells and to have potential for treating cisplatin-resistant tumors [3].

Gold compounds have been used in medicine for a long time. However, it was only after Forestier described the anti-arthritic properties of gold(I) compounds in 1935 that the scientific community began a more methodical investigation of the toxic and beneficial effects of gold compounds in rheumatoid arthritis [4]. Nowadays gold compounds such as auranofin (triethylphosphine-(2,3,4,6-tetra-*O*-acetyl-β-1-D-thiopyranosato-S)gold(I)), solganol (gold(I) thioglucose), and myochrysin (sodium gold(I) thiomalate), are used clinically in the treatment of rheumatoid arthritis.

The antineoplastic activity of auranofin has been evaluated on a variety of animal and human tumor cell lines *in vitro* and *in vivo* [5,6]. These studies have shown that this drug presents potent cytotoxic activity against several cell lines, specially for P338 leukemia.

The mechanism of action of gold compounds is still not well understood and has been continually investigated by the scientific community. Some investigations have suggested that DNA is not

Abbreviations: NMR, nuclear magnetic resonance; DMSO, dimethysulfoxide; HRMS(ESI), high-resolution mass spectra (eletrospray ionization); TMS, tetramethylsilane; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RPMI, Roswell Park Memorial Institute Medium; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic.

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http://dx.doi.org/10.1016/j.ica.2014.01.042
0020-1693/

the primary target for these compounds although some gold complexes such as *Bis*[1,2-bis(diphenylphosphino)ethane]gold(I) chloride produce DNA protein cross-links and DNA strand breaks in cells [7]. The biological effects of gold complexes could be mediated by an anti-mitochondrial mechanism. Studies indicate that the mechanism of action of these compounds involves the enzyme thioredoxin reductase, which is involved in the mechanism of proliferation of tumor tissues [8,9]. Previous work on the chemical reactivity of auranofin and other gold(I) complexes containing phosphine also demonstrated that these compounds may react with serum proteins, cellular proteins, glutathione and other small molecular weight thiols [7].

Advances in chemotherapy of gold compounds have been pursued by several research groups [10]. A variety of auranofin analogues and phosphine gold(I) compounds containing S-donor ligands has been developed and shown to possess potent cytotoxic activities [11].

Considering that auranofin has shown similar or greater *in vitro* activity than cisplatin and has also exhibited potent cytotoxic activity against melanoma and leukemia cell lines and anti-tumor activity against leukemia, several complexes of gold(I) have been evaluated for cytotoxic and anti-tumor activity [8].

The use of nanotechnology in cancer treatment is also an attractive research area. Gold nanoparticles have been investigated as drug delivery systems and also in phothermal therapy due to their unique properties. The field referred to as nanomedicine still faces many challenges but gold nanoparticles are one of the more promising areas of research [12,13].

The chemical structure of the ligands present in gold(I) complexes is an important parameter for biological activity. For instance, Yeo et al. [14] have investigated the influence of the R substituints (R = Me, Et and iPr) in triphenylphosphinegold(I) carbonimidothioates upon *in vitro* cytotoxicity against HT-29 colon cancer cell line and have found that R = Me results in the most active compound. Several gold(I) and gold(III) complexes derived from 2-(2′-pyridyl)benzimidazole including mononuclear and binuclear species have shown relevant antiproliferative activities *in vitro* against A2780 human ovarian carcinoma cells, resistant or sensitive to cisplatin [15].

Previous structure–activity relationship studies of auranofin and other gold(I) compounds have shown the importance of the phosphine ligand for biological potency [16]. Gold compounds containing tertiary phosphines with a linear S–Au–P arrangement have been found to be more active than similar compounds with no phosphinic substituents. The lipophilicity introduced in the compounds due to coordination of tertiary phosphines seems to be responsible for their enhanced cytotoxicity since it facilitates transport across cell membranes [10,16,17].

3-Benzyl-1,3-thiazolidine-2-thione and 5-phenyl-1,3,4-oxazadiazole were chosen as ligands since they are members of the heterocyclic class of compounds which exhibit interesting biological properties such as anti-inflammatory and analgesic activities. This class of compounds also exhibits insecticide, herbicide and fungicide activities [18]. Compounds containing the thiazolidine ring are able to interact with a variety of biological targets and have been extensively reported in the literature [19]. The thiazole skeleton is a constituent of many biomolecules including β-lactam antibiotics such as the penicillins and natural products such as thiamin [20]. Compounds containing the 1,3,4-oxadiazole moiety exhibit antimicrobial, anti-HIV [21], antitubercular [22], antimalarial [23], anti-inflammatory [24], anticonvulsant [25], and anticancer [26] properties.

In this work we report the preparation, characterization and cytotoxic activity against two tumor cell lines of four new gold(I) complexes containing 3-benzyl-1,3-thiazolidine-2-thione and 5-phenyl-1,3,4-oxadiazol-2-thione and tertiary phosphine (PPh_3 or PEt_3) as ligands.

2. Experimental

2.1. Materials and methods

All reagents and solvents were reagent grade and were used without prior purification. The progress of all reactions was monitored by thin-layer chromatography which was performed on 2.0 × 6.0 cm aluminium sheets precoated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm. Infrared (IR) spectra were recorded in a Bomem FTIR MB-102 spectrometer in the region 4000–360 cm^{-1} of the sample supported as a KBr pellet, with 4 cm^{-1} of spectral resolution, and an average of 64 scans. Only significant peaks were recorded. ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded as solutions in $CDCl_3$ and DMSO-d_6 on a Bruker spectrometer. The chemical shifts were expressed as δ (in ppm) with respect to a standard internal TMS reference (^1H NMR). Raman spectra were obtained using a Bruker RFS 100 FT-Raman instrument equipped with a germanium detector refrigerated by liquid nitrogen, with excitation at 1064 nm from a Nd:YAG laser, power between 103 mW for sample in solid phase, in the range between 4000 and 50 cm^{-1}, and spectral resolution of 4 cm^{-1}, with an average of 500 scans. The high-resolution mass spectra were recorded on a Micromass LCT spectrometer with electrospray ionization, at the Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France. Elemental analyses were performed at Central Analítica, USP-Brazil. Diffraction data for single crystals of $C_{14}H_{20}N_2OPSAu$ (**4**) were collected using a Oxford GEMINI A Ultra diffractometer with Mo Kα (λ = 0.71073 Å) and temperature of 120 K. Data collection, reduction and cell refinement were carried out by CRYSALIS RED, Oxford diffraction Ltda – Version 1.171.32.38 software [27]. The structures were solved and refined using SHELXL-97 [28]. An empirical isotropic extinction parameter *x* was refined according to the method described by Larson [29]. A Multiscan absorption correction was applied [30]. The structures were drawn by ORTEP-3 for Windows [31] and MERCURY softwares [32].

2.2. Synthesis of ligands

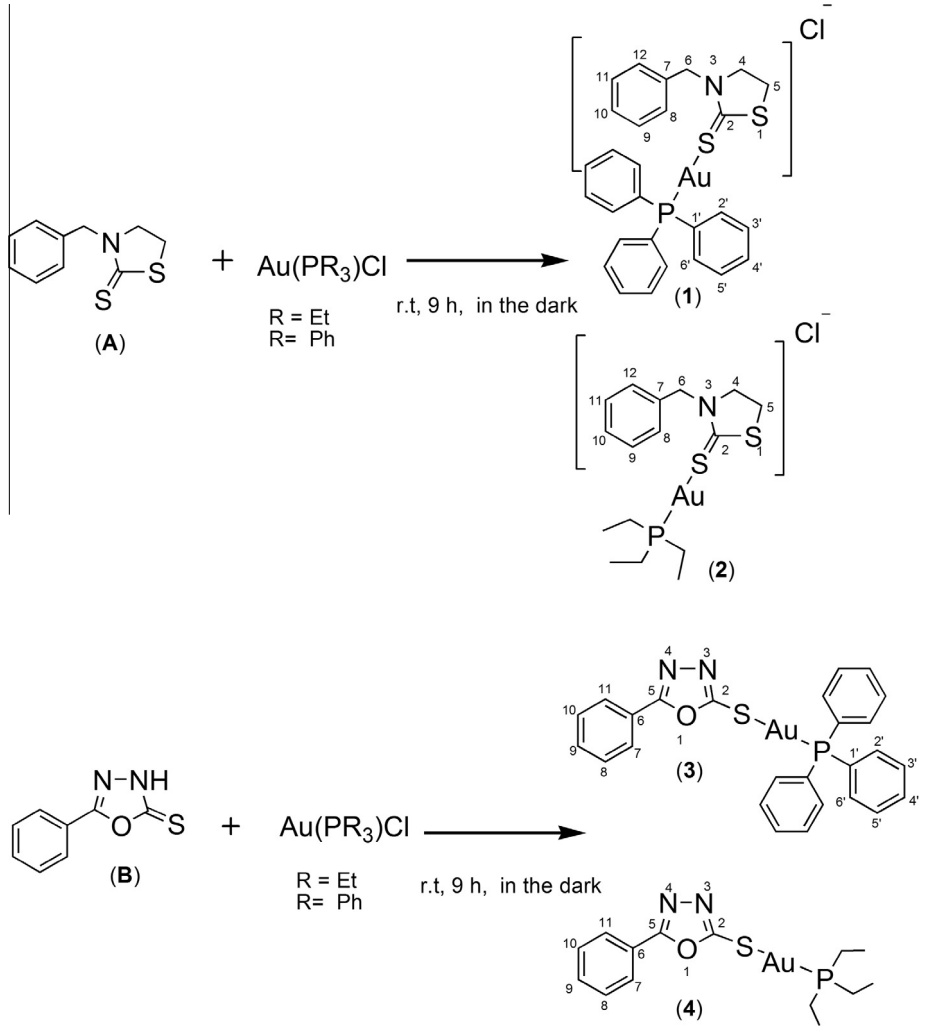
Ligand (**A**) was prepared from benzyl chloride according to the experimental procedure described in ref [33] and ligand (**B**) was prepared from benzoyl chloride according to the experimental procedure described in ref [34].

(***A***)*:* as a white solid, m.p. 131 °C, lit [33] m.p. 132–133 °C. IR ν_{max} KBr (cm^{-1}): 3024; 2942; 1488; 1178; 983; 731; 398; Raman ν_{max} (cm^{-1}): 1157; 1001; 707; ^1H NMR (300 MHz, $CDCl_3$) δ: 3.23 (t, 2H, H5, $J_{5,4}$ = 7.8 Hz); 3.94 (t, 2H, H4, $J_{4,5}$ = 7.8 Hz); 4.97 (s, 2H, H6); 7.23–7.34 (m, 5H, H–Ar). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 27.2 (C5); 52.8 (C6); 56.0 (C4); 128.2 (C8, C12); 128.3 (C10); 129.1 (C9, C11); 135.1 (C7); 197.2 (C2).

(***B***)*:* as a white solid, m.p. 217–223 °C, lit [34] m.p. 219 °C. IR ν_{max} KBr (cm^{-1}): 3142; 3099; 2952; 1501; 1487; 967; 696; 684; Raman ν_{max} (cm^{-1}): 3072; 1488; 1359; 970; 698; ^1H NMR (300 MHz, DMSO-d_6) δ: 7.53–7.61 (m, 4H, H8, H9, H10, NH); 7.86 (dd, 2H, $J_{7,9} = J_{11,9}$ = 1.5 Hz, $J_{7,8} = J_{11,10}$ = 8.1 Hz, H7, H11); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 122.4 (C6); 125.9 (C8, C10); 129.3 (C7, C11); 132.1 (C9); 160.4 (C-5); 177.5 (C2); HRMS(ESI): *m/z* calc. for $[C_8H_6N_2OS]$ $[M-H]^-$ 177.0123, found 177.0128.

2.3. Synthesis of complexes (Scheme 1)

To a solution of $Au(PPh_3)Cl$ (0.198 g, 0.4 mmol) or $Au(PEt_3)Cl$ (0.140 g, 0.4 mmol) in dichloromethane (3 mL), ligand (**A**) (0.4 mmol) dissolved in dichloromethane (4 mL) or ligand (**B**) (0.4 mmol) dissolved in acetone (3 mL) was slowly added during 3 h. After stirring for 9 h at room temperature in the dark, the



Scheme 1. Synthesis of complexes (**1**), (**2**), (**3**) and (**4**).

solvent was removed under reduced pressure to furnish a white residue which was purified by preparative chromatography (eluant:dichloromethane) to give the desired compounds (**1**), (**2**), (**3**) and (**4**) in 46%, 54%, 62% and 68% yields, respectively.

Compound $Au(PEt_3)Cl$ is commercially available and $Au(PPh_3)Cl$ was synthesized from $K[AuCl_4]$ according to the literature [35].

(**1**): as a white solid, m.p. 125–128 °C. IR ν_{max} KBr (cm^{-1}): 3032; 2919; 1488, 1179; 999; 749; 384; Raman ν_{max} (cm^{-1}): 1155, 1002; 696; 391; ^1H NMR (300 MHz, $CDCl_3$) δ: 3.24 (t, 2H, H5, $J_{5,4}$ = 7.8 Hz); 3.95 (t, 2H, H4, $J_{4,5}$ = 7.8 Hz); 4.99 (s, 2H, H6); 7.24–7.32 (m, 5H, H–Ar); 7.35–7.56 (m, 15H, Ar–PPh_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 27.2 (C5); 52.8 (C6); 55.9 (C4); 128.3 (C8, C12); 128.4 (C10); 128.5 (C1′); 129.1 (C9, C11); 129.4 (d, C3′, C5′, $J_{3'\text{-}P} = J_{5'\text{-}P}$ = 11.7 Hz); 131.1 (d, C4′, $J_{4'\text{-}P}$ = 2.3 Hz); 134.3 (d, C2′, C6′, $J_{2'\text{-}P} = J_{6'\text{-}P}$ = 14.0 Hz); 135.2 (C7); 197.4 (C2); HRMS(ESI): *m*/*z* calc. for $[C_{28}H_{26}NPS_2Au]Cl$ $[M]^+$ 668.0910, found 668.0911; $[M+H]^+$ Calc. 669.0988, found 669.0962.

Anal. Calc. for $[C_{28}H_{26}NS_2PAu]Cl$: C, 47.77; H, 3.72; N, 1.99. Found: C, 47.79; H, 3.74; N, 2.28%.

(***2***): as a white solid, m.p. 62–63 °C. IR ν_{max} KBr (cm^{-1}): 3025; 2964; 2924; 2872; 1489; 982; 774; 383; Raman ν_{max} (cm^{-1}): 1155, 1003; 686; 392; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.12–1.24 (m, 9H, CH_3); 1.78–1.89 (m, 6H, CH_2); 3.23 (t, 2H, H5, $J_{5,4}$ = 7.8 Hz); 3.94 (t, 2H, H4, $J_{5,4}$ = 7.8 Hz); 4.97 (s, 2H, H6); 7.32–7.45(m, 5H, HAr); ^{13}C NMR (75 MHz, $CDCl_3$) δ: (9.2 CH_3); 18.3 (d, CH_2, $J_{CH2\text{-}P}$ = 36.2 Hz); 27.2 (C5); 52.8 (C6); 56.0 (C4); 128.3 (C8, C12); 128.5 (C10); 129.1 (C9, C11); 135.2 (C7); 197.4 (C2); HRMS(ESI): *m*/*z* calc. for $[C_{16}H_{26}NPS_2Au]Cl$ $[M]^+$ 524.0910, found 524.0905; $[M+H]^+$ calc. 525.0988, found 525.0956.

Anal. Calc. for $[C_{16}H_{26}NS_2PAu]Cl$: C, 34.32; H, 4.68; N, 2.50. Found: C, 34.49; H, 4.70; N, 2.53%.

(***3***): as a white solid, m.p. 65–69 °C. IR ν_{max} KBr (cm^{-1}): 3054; 2926; 1437; 996; 747; 708; 691; 380 Raman ν_{max} (cm^{-1}): 3058; 1443; 958; 715; 378; ^1H NMR (300 MHz, DMSO-d_6) δ: 7.53–7.65 (m, 18H, H8, H9, H10, Ar-PPh_3); 7.81–7.85 (m, 2H, H7, H11); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 123.7 (C6); 125.7 (C8, C10); 128,4 (d, C1′, $J_{1'\text{-}P}$ = 58.4 Hz) 129.2 (C7, C11); 129.6 (d, C3′, C5′, $J_{3'\text{-}P} = J_{5'\text{-}P}$ = 11.5 Hz); 131.2 (C9); 132.3 (C4′); 133.8 (d, C2′, C6′ $J_{2'\text{-}P} = J_{6'\text{-}P}$ = 13.9 Hz); 163.8 (C5); 168.1 (C2); HRMS(ESI): *m*/*z* calc. for $[C_{26}H_{20}N_2OPSAu]$ $[M+H]^+$ (637.0778), found 637.0784.

Anal. Calc. for $C_{26}H_{20}N_2OSPAu$: C, 49.07; H, 3.17; N, 4.40. Found: C, 49.32; H, 3.22; N, 4.39%.

(***4***): as a white solid, m.p. 87–88 °C. IR ν_{max} KBr (cm^{-1}): 3076; 2963; 1438; 995; 757; 709; 693; 391 Raman ν_{max} (cm^{-1}): 3064; 1440; 957; 700; 366; ^1H NMR (300 MHz, DMSO-d_6) δ: 1.10–1.21 (m, 9H, CH_3); 1.92–2.04 (m, 6H, CH_2) 7.54–7.56 (m, 3H, H8, H9, H10); 7.85–7.88 (m, 2H, H7, H11); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 9.6 (CH_3); 17.5 (d, $J_{CH2\text{-}P}$ = 34.7 Hz); 124.3 (C6); 126.2 (C8);

126.4 (C10); 129.8 (C7); 129.9 (C11); 131.7 (C9); 164.2 (C5); 169.1 (C2); HRMS(ESI): *m/z* calc. for $[C_{14}H_{20}N_2OPSAu]$ $[M+H]^+$ (493.0778), found 493.0769.

Anal. Calc. for $C_{14}H_{20}N_2OSPAu$: C, 34.15; H, 4.09; N, 5.69. Found: C, 34.08; H, 4.07; N, 5.70%.

2.4. Cytotoxicity assay

Cytotoxic activity was investigated against the following tumor cell lines: CT26WT – colon cancer cells, B16-F10 – mouse metastatic skin melanoma, (Ludwig Institute of Cancer Research - São – Paulo Brazil) and non-tumor cell, BHK-21 – Baby Hamster Kidney.

All the cell lines were propagated in RPMI 1640 culture medium pH 7.4, supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), Hepes (4.0 mM), $NaHCO_3$ (14.0 mM), ampicillin (0.27 mM), and streptomycin (0.06 mM).

Cells were harvested by trypsinization and seeded in 96-well tissue culture plates (100 μL/well) at defined density (1×10^3 viable cells/well) and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. Stock solutions of the test substances in DMSO were serially diluted in cell culture medium (<1% DMSO). After drug exposure for 72 h at 37 °C and 5% CO_2, cells were incubated with MTT (0.01 M in water solution – 10 μL/well) for 4 h at 37 °C and 5% CO_2. MTT is metabolized by viable cells resulting in a violet complex product that, after being solubilized in 100 μL of DMSO, can be quantified through colorimetric assay using a plate reader (absorbance at 570 nm).

The negative control (100% value of viability) was obtained with cells exposure in RPMI 1640 medium supplemented with 10% FBS. Cisplatin was used as positive control against these cell lines.

The raw data were normalized to the untreated control cells and compared to the metabolic activity of the viable treated cells. IC_{50} values were calculated by four parametric nonlinear regressions using GRAPHPAD Prism 5.0 software.

3. Results and discussions

The complexes (Scheme 1) were synthesized by adding the corresponding ligands, previously dissolved in dichloromethane or dichloromethane/acetone 1:1, to a solution of $Au(PPh_3)Cl$ or $Au(PEt_3)Cl$ in dichloromethane at room temperature, and were isolated by preparative plate chromatography.

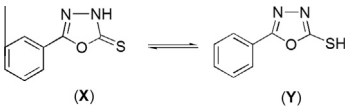
The complexes were characterized by ^1H NMR, ^{13}C NMR, infrared, Raman, high-resolution mass spectra and complex (**4**) by X-ray crystallography. The integrations of the signals in the NMR spectra showed that the stoichiometric ratio for the complexes formed was 1:1 ligand:complex.

Ligand (**A**) presents three bonding sites: the thiocarbonyl sulfur atom, the nitrogen atom and the endocyclic sulfur atom. The isolated pairs of electrons from the endocyclic sulfur atom and nitrogen should be in resonance with the thiocarbonyl group, thus contributing to greater delocalization of electrons and consequently decreasing the ability of coordination.

Ligand (**B**) can display two tautomeric forms (**X**) and (**Y**) as shown in Fig. 1.

3.1. Vibrational spectra

The infrared and Raman spectra of complexes (**1**), (**2**), (**3**) and (**4**) were analyzed in comparison to the IR and Raman spectra of 3-benzyl-1,3-thiazolidine-2-thione and 5-phenyl-1,3,4-oxadiazole-2-thione. A shift of some absorptions representing the contribution of the C═S vibration can be observed. They are produced by vibrational coupling of N—C═S due to the mixed vibrations [36]. For complexes (**1**) and (**2**) it has been found that the most



(**X**) (**Y**)

Fig. 1. Thione-thiol tautomerization of 5-phenyl-1,3,4-oxadiazole-2-thione.

significant change was observed in thioamide group IV, indicating that after the coordination, C═S presents some C–S character. The displacement of the thioamide IV band suggests that the metal is bound to the sulfur of the thione group.

The presence of a new absorption band around 380 cm^{-1}, attributed to *ν*Au–S, was observed in the IR spectra of complexes (**1**), (**2**), (**3**), and (**4**). For these complexes significant shifts of thioamide bands were also observed (Fig. S1 and S2 and Table S1).

According to Rao and Venkataraghavan [37], the extreme variations in the assignment of the C═S stretching frequency in nitrogen-containing thiocarbonyl derivatives is undoubtedly due to vibrational coupling effects. In most of these systems, the *ν*C═S stretching vibration is not localized.

The absorption corresponding to *ν*SH was not observed in the infrared and Raman spectra of ligand (**B**), complexes (**3**) and (**4**). We can observe an absorption band in the spectrum of the free ligand corresponding to *ν*NH at ~3140 cm^{-1} and characteristic thioamide bands [38] indicating that it is present in the tautomeric form (**X**).

The thione form that predominates for ligand (**B**) can coordinate to the gold(I) through N3 or S2. However, the sulfur atom tends to be the most likely binding site for $AuPPh_3$ and $AuPEt_3$ since gold tends to bind soft, polarizable atoms such as sulfur.

The infrared spectra of the complexes (**3**) and (**4**) have shown the absence of *ν*SH, *ν*NH and thioamide I, II, III bands and the appearance of a new band at 747–757 cm^{-1} attributed to *ν*CS indicating that the ligand assumes the tautomeric form (**Y**) after the formation of the complexes.

The assignments of the absorptions in the Raman spectra have also shown the absence of the NH tautomer in the solid state for the two complexes. The most relevant infrared and Raman spectra absorptions are summarized in Table S1.

3.2. X-ray structural determination

A crystal of $C_{14}H_{20}N_2OPSAu$ complex (**4**) (Fig. 2) was obtained by recrystallization from a mixture of dimethylsulfoxide and

Table 1
Crystal data, data collection and structure refinement for $C_{14}H_{20}N_2OPSAu$ (**4**).

Empirical formula	$C_{14}H_{20}AuN_2OPS$
Formula weight (g mol^{-1})	492.32
T (K)	120(2)
λ (Å)	0.71073
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a (Å)	7.3012 (1)
b (Å)	13.4183 (2)
c (Å)	17.1808 (2)
α (°)	90.00
β (°)	90.00
γ (°)	90.00
V ($Å^3$)	1683.20 (4)
Z	4
D_{calc} (g cm^{-3})	1.943
Crystal size (mm^3)	0.43 × 0.26 × 0.19
Extinction coefficient	None
Collected reflections	120111
Independent reflections	4559
S	1.120
R [$I > 2\sigma(I)$]	0.034
wR	0.083

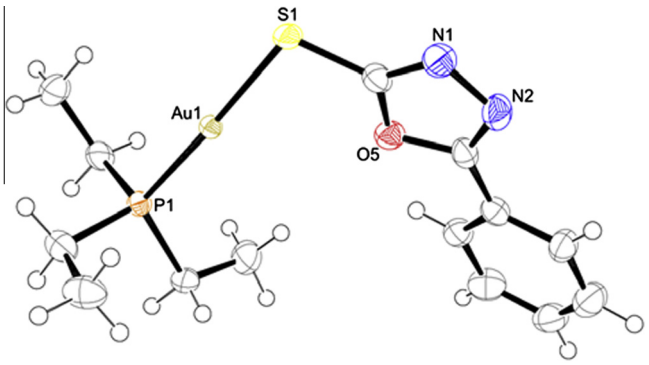


Fig. 2. Perspective view of complex (**4**), ellipsoids are drawn at the 50% probability level and hydrogen atoms are shown as spheres of arbitrary radii.

dichloromethane and isolated by filtration. The structure of (**4**) was determined using single-crystal X-ray diffraction. Crystal data, data collection and structure refinementdetails are summarized in Table 1. Complex (**4**) crystallizes in the orthorhombic crystal system and space group $P2_12_12_1$. The central metal ion, gold(I), is coordinated by the sulfur atom of the 5-phenyl-1,3,4-oxadiazole-2-thiol ligand and with a tertiary phosphine (PEt_3) in a linear geometry, with the bond angle P1–Au1–S1 = 175.85° (8), as shown in Table 2 which lists some other relevant geometrical features of the complex. The coordination environment of Au(I) in complex (**4**) can be seen in Fig. 2. The 5-phenyl-1,3,4-oxadiazole-2-thiol ligand is twisted relative to the angle of 180° formed between P1–Au1–S1. The overall twist measured as the dihedral angle between the mean plane of the phenyl-thiol rings and the plane formed between P1–Au1–S1 is ca. 68.38° (9). The crystal structure of complex (**4**) clearly indicates the dominance of the thiol form of the ligand (**B**), as evidenced by the absence of the H atom at the N1 atom and the C2–S1 distance (1.709 (7) Å) which is intermediate between a single and a double bond (1.820 and 1.600 Å, respectively). One hydrogen atom from a CH_3 group of the phosphine is involved in an intermolecular C–H···N hydrogen bond forming a two-dimensional array along the *bc* plane. Fig. 3 shows the crystal packing of the complex.

3.3. NMR studies

In the ^{1}H NMR spectra of complexes (**1**) and (**3**) signals were observed in the δ 7.35–7.65 ppm region corresponding to aromatic hydrogens of the PPh_3 group. For complexes (**2**) and (**4**) two signals were observed in the following regions: δ 1.12–1.24 ppm (CH_3); 1.78–1.89 ppm (CH_2) and δ 1.10–1.21 ppm (CH_3); 1.92–2.04 ppm (CH_2) corresponding to hydrogens of the PEt_3 group.

No changes were observed in the ^{1}H NMR spectra of compounds (**1**) and (**2**) after coordination. In the ^{13}C NMR spectra, the signal corresponding to the carbon atom of the thione group showed a small chemical shift, on the order of δ 0.2 ppm.

In the ^{13}C NMR spectra of the 5-phenyl-1,3,4-oxadiazol-2-thione ligand signals were observed at δ 177.5 (C2) and δ 160.4 (C5) ppm, whereas in the spectra of complexes (**3**) and (**4**) the corresponding signals appear at δ 168.1 (C2), δ 163.8 (C5) and δ 169.1 (C2), δ 164.2 (C5) ppm, respectively, showing a chemical shift to lower frequency for carbon (C2) due to the presence of the metal and a chemical shift to higher frequency for (C5).

Table 2
Selected geometrical parameters (Å, °).

Au1–P1	2.2607 (15)
Au1–S1	2.3270 (15)
S1–C2	1.709 (7)
S1–Au1A	2.3356 (19)
P1–C31	1.800 (7)
P1–C30	1.816 (7)
P1–C32	1.827 (8)
P1–Au1A	2.2777 (19)
O5–C2	1.370 (8)
O5–C5	1.382 (9)
P1–Au1–S1	175.85 (8)
C2–S1–Au1	104.1 (2)
C2–S1–Au1A	100.7 (2)
C31–P1–Au1	111.8 (2)
C30–P1–Au1	115.6 (2)
C32–P1–Au1	112.5 (3)
C31–P1–Au1A	107.9 (2)
C30–P1–Au1A	111.7 (2)
C32–P1–Au1A	120.2 (3)
N1–C2–S1	126.8 (6)
O5–C2–S1	120.2 (5)

3.4. Biological tests

The antitumor activity of the four gold(I) complexes and two ligands was evaluated in comparison with cisplatin in two different cell lines: colon cancer (CT26WT) and metastatic skin melanoma (B16F10). They were also examined for their cytotoxic properties in a normal kidney cell (BHK-21). The tumor cells were chosen to compose two different tumor types, namely, melanoma and carcinoma and to also assess the activity in cell lines of different embryonic origin such as epithelial and fibroblast. This was done in an effort to overcome and control any differences. The third cell line is a normal cell, which was used to evaluate the selectivity index, allowing comparison of the cytotoxicity of the compounds in tumor and normal cells.

IC_{50} values, calculated from the cell viability dose response curves obtained after 72 h drug treatment in the MTT test, are shown in Table 3. Complexes (**1**) and (**3**) were more cytotoxic in colon cells (CT26WT) exhibiting smaller IC_{50} < 0.10 μM while complex (**3**) was more cytotoxic in melanoma cells (B16F10) exhibiting IC_{50} = 0.12 - μM. All the compounds were more cytotoxic than cisplatin in both tumor cell lines. Although the ligands in the free form showed significant activity, complexation favors the biological activity in all cases. Complex (**3**) was about 82 and 54 times more cytotoxic than ligand (**B**) in CT26WT and B16F10 cells respectively, while complex (**1**) showed a cytotoxicity about 72 times greater than ligand (**A**) in colon cells (CT26WT). Complex (**1**) displayed greater activity in colon

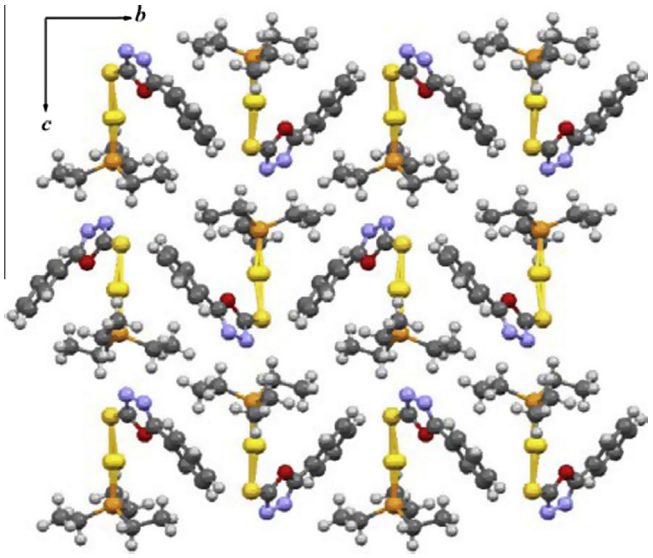


Fig. 3. Crystal packing of (**4**) as seen along *bc* plane. Color codes: C: gray, H: white, N: blue, O: red, P: yellow, Au: orange. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Cytotoxic activities against cell lines. IC_{50} (μM ± SD) and SI.

Compounds	Tumor cells IC_{50} (μM ± SD)[a]			Non-tumor cells IC_{50} (μM ± SD)[a]	
	B16F10	SI[b]	CT26WT	SI[b]	BHK-21
(A)ligand	2.28 ± 1.4	36.7	17.23 ± 1.0	4.9	83.70 ± 1.4
(1)	0.40 ± 0.10	7.8	<0.10	31.0	3.10 ± 0.30
(2)	0.13 ± 0.05	70.0	0.20 ± 0.03	45.5	9.10 ± 2.40
(B)ligand	6.53 ± 0.6	12.8	8.28 ± 0.4	10.1	83.60 ± 1.0
(3)	0.12 ± 0.03	27.9	<0.10	33.5	3.35 ± 0.50
(4)	0.18 ± 0.05	34.2	0.40 ± 0.04	15.4	6.15 ± 0.20
Cisplatin	6.40 ± 2.20	1.3	0.70 ± 0.20	12.0	8.40 ± 1.90

[a] SD – standard deviation of triplicate of two independent experiments.
[b] SI – selectivity index.

cells (CT26WT) (IC_{50} < 0.10 μM) than in B16F10 cells (IC_{50} = 0.40 - μM). The ligands and complexes exhibited a high selectivity index in colon cells (CT26WT) and are more selective than cisplatin.

Complexes (**2**) and (**4**) were also much more cytotoxic than their respective free ligands. The highest SI was found for complex (**2**) that presents a combination of alkyl phosphine and thiazolidine ligands.

In general, aryl phosphine complexes (**1**) and (**3**) were more toxic on colon cells (CT26WT) compared to alkyl phosphine (**2**) and (**4**). The same trend was not observed on melanoma cell line (B16F10). A correlation between the molecular structure of the complexes and the biological activity could not be clearly observed.

4. Conclusion

This work describes the synthesis and characterization of four novel gold(I) complexes. The structural characterization of the complexes was established on the basis of high-resolution mass, NMR, IR spectra and through an X-ray structure obtained for complex (**4**). The FT-IR spectra showed that the S-atom of thione group is coordinated to the metal in all cases. Compound (**3**), which showed cytotoxic activity against B16F10 and CT26WT cancer cells with IC_{50} < 0.12 μM and IC_{50} < 0.10 μM, was more potent than its triethylphosphine analogue complex (**4**). The ligands showed significant cytotoxic activity. All complexes were more active than cisplatin.

Acknowledgments

The authors thank FAPEMIG, CAPES and CNPq for financial support. This work is a collaboration research project of members of the Rede Mineira de Química (RQ-MG) supported by FAPEMIG (Project: REDE-113/10)

Appendix A. Supplementary material

CCDC 949615 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2014.01.042.

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