Journal of Inorganic and General Chemistry
ZAAC
Zeitschrift für anorganische und allgemeine Chemie
www.zaac.wiley-vch.de
ARTICLE

DOI: 10.1002/zaac.201600278

Synthesis, Characterization, and *in vitro* Cytotoxicity of Gold(I) Complexes of 2-(Diphenylphosphanyl)ethylamine and Dithiocarbamates

Adam A. A. Sulaiman,[a] Muhammad Altaf,[b] Anvarhusein A. Isab,*[a] Abdullah Alawad,[c] Saleh Altuwaijri,[d] and Saeed Ahmad[e]

Keywords: Dithiocarbamate; Gold; Anticancer activities; Spectroscopy

Abstract. Gold(I) complexes of 2-(diphenylphosphanyl)ethylamine or (2-aminoethyl)diphenylphosphine (AEP), and dithiocaarbamates (R_2NCS_2) were prepared by the reaction of these ligands with $(CH_3)_2S$-AuCl in dichloromethane. The synthesized complexes [Au(AEP)Cl] (**1**), $[Au(AEP)_2]Cl$ (**2**), and $[Au_2(R_2NCS_2)_2]_n$ (R_2 = dimethyl (**3**), diethyl (**4**), and dibenzyl (**5**)) were characterized by elemental analysis, IR, ^1H, ^{13}C and ^{31}P NMR spectroscopy. The complexes were evaluated for anticancer activity against three cancer cells, A549 (human lung carcinoma), HCT15 (human colon cancer), and MCF7 (human breast cancer) cell lines. Three of the five tested complexes showed significant *in vitro* cytotoxicity and for A549, the inhibition effect of three compounds is greater than *cisplatin*.

Introduction

The success of platinum compounds against different types of cancers[1–4] triggered a great deal of interest in the field of anti-tumor metallodrugs.[4–10] In this regard, gold(I) and gold(III) complexes have attracted special attention[7–19] because some gold(I) complexes have already been used over many years for the treatment of rheumatoid arthritis.[7,20] The antirheumatic gold(I)-phosphine drug, auranofin emerged as the lead compound for the antiproliferatively active gold species.[21–23] A number of auranofin analogues were also evaluated and it was demonstrated that the presence of a P–Au–S motif in these compounds enhanced their anticancer activity.[22] *Sadler*, *Berners-Price* and co-workers carried out the cytotoxic studies of gold(I) complexes with bisphosphine ligands, $R_2P(CH_2)_nPR_2$, against a range of tumor cells, with the highest activity observed when R = phenyl, n = 2, 3 or the analogous $Ph_2PCH{=}CHPPh_2$ (dppey) ligand.[8,24,25] In recent years, an increasing number of gold(I)-phosphine complexes have shown to display potent anti-cancer activities.[10,26–37] However, the reports on cytotoxic properties of gold(I) complexes containing mixed P,N type ligands are limited.[38,39] Several mixed ligand gold(I) complexes of phosphines and dithiocarbamates have also been evaluated for the antitumor activity against a number of cells.[40–42] But, there are only few reports in the literature on the anticancer potential of dinuclear gold(I)-dithiocarbamates[18] although analogous gold(III) complexes have received a considerable attention as potential anticancer agents.[19]

Mechanistic studies suggest that, in contrast to *cisplatin*,[1–4] DNA is not the primary target for the gold complexes. Rather, their cytotoxicity is mediated by their ability to alter mitochondrial function and inhibit protein synthesis.[8,28–32,43–45] The enzyme thioredoxin reductase (TrxRa) is considered as the most relevant target for bioactive gold coordination compounds.[8,30,43–45] Auranofin is found to be a potent inhibitor of mitochondrial thioredoxin reductase *in vitro* and *in vivo*, since it is able to block the active site of the enzyme.[46,47] *Wedlock* et al. used nano-scale secondary ion mass spectroscopy and energy filtered transmission electron microscopy as imaging techniques to visualize gold in situ in human breast cancer cells treated by anticancer gold(I)-bisphosphine compound. Their investigations provided the evidence of biomechanism of gold compounds based on inhibition thioredoxin system.[48]

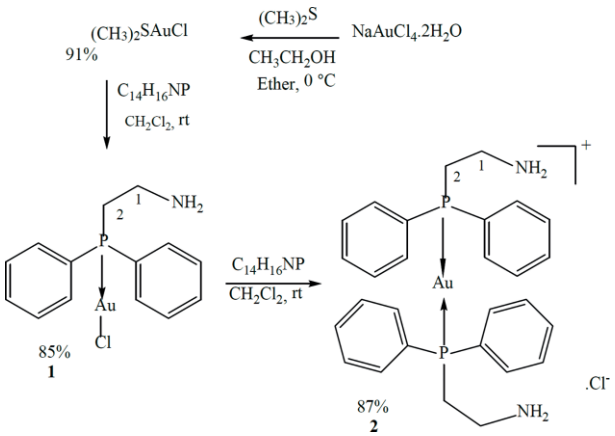
Motivated by the promising results of our recent studies on the antitumor properties of gold(I) complexes containing phosphine and dithiocarbamate ligands,[17,41,42] we prepared in the current study, two new gold(I) complexes of 2-(diphenylphosphanyl)ethylamine or (2-aminoethyl)diphenylphosphine (**1**, **2**) and three gold(I)-dithiocarbamates (**3**–**5**). The complexes were characterized by elemental analysis, IR, ^1H, ^{13}C, and ^{31}P NMR spectroscopy and their antitumor activity was examined

* Dr. A. A. Isab
E-Mail: aisab@kfupm.edu.sa
[a] Department of Chemistry
King Fahd University of Petroleum and Minerals
Dhahran 31261, Saudi Arabia
[b] Center of Excellence in Nanotechnology (CENT)
King Fahd University of Petroleum and Minerals
Dhahran 31261, Saudi Arabia
[c] National Center for Stem Cell Technology (NCSCT)
Life Sciences and Environmental Research Institute
King Abdulaziz City for Science and Technology (KACST)
Riyadh 11442, Saudi Arabia
[d] SAAD Research & Development Center
Clinical Research Lab.
SAAD Specialist Hospital
P.O.Box 30353, Al-Khobar 31952, Saudi Arabia
[e] Department of Chemistry
College of Sciences and Humanities
Prince Sattam bin Abdulaziz University
Al-Kharj 11942, Saudi Arabia

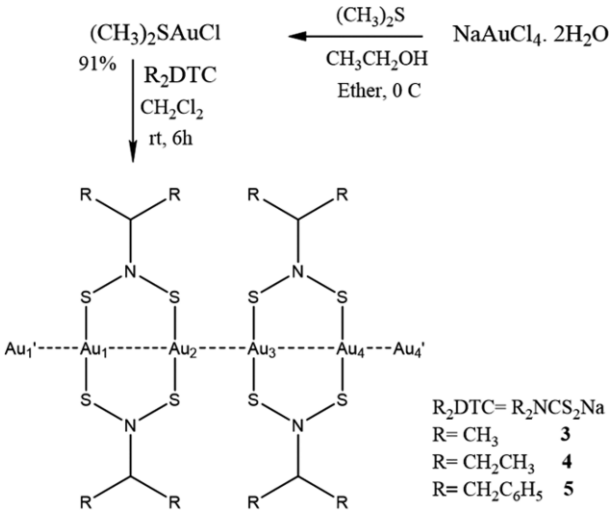
against A549 (human lung carcinoma), HCT15 (human colon cancer), and MCF7 (human breast cancer) cell lines by MTT assay. The crystal structure of one of the synthesized complexes, $[Au_2(diethyldithiocarbamate)_2]_n$ was determined by X-ray crystallography, but it was found to be the same as the already reported one.[49]

Results and Discussion

The mono- and bisphosphine-gold(I) complexes of 2-(diphenylphosphanyl)ethylamine or (2-aminoethyl)diphenylphosphine (**1**, **2**) were prepared by mixing $(CH_3)_2S$-AuCl and AEP in the molar ratios of 1:1 and 1:2, respectively. The reaction of dithiocarbamates with $(CH_3)_2S$-AuCl yielded the complexes with the empirical formula, Au(dithiocarbamate) as indicated by the elemental analysis. The procedures for synthesis of complexes are explained in Scheme 1 and Scheme 2 respectively. Previous structural studies on gold(I) complexes with amino/imino-phosphines reveal that the central gold atoms in these complexes are usually coordinated only by phosphorus atoms adopting a linear environment. The nitrogen atom does not bind to the metal atom.[38,39,50] Therefore, it is expected that the most probable structures of complex **1** and **2** are those shown in Scheme 1. The X-ray diffraction measurement of Au-



Scheme 1. Synthesis and expected structures of gold(I) complexes **1** and **2**.



Scheme 2. Synthesis of gold(I)-dithiocarbamate polymers **3**–**5**.

(diethyldithiocarbamate) crystal revealed that it existed as a polymer consisting of dimeric units ($[Au_2(C_4H_{10}NCS_2)_2]_n \cdot x CH_2Cl_2$) as reported previously.[49] Based on this measurement and earlier investigations,[49,51] it is suggested that the gold(I)-dithiocarbamates would exist in the form of polymers having the formula, $[Au_2(diethyldithiocarbamate)_2]_n$ with gold:dithiocarbamate ratio of 1:1. The central Au^I atom in **3** adopts a nearly linear arrangement and the neighboring units are associated to each other through gold-gold interactions.

Spectroscopic Analysis

The IR spectra for the free ligands and complexes **2**–**5** are summarized in Table 1. The formation of gold(I)-dithiocarbamate complexes was confirmed by the presence of the ν(C–N) and ν(C–SS) absorption bands. The ν(C–N) band represents a carbon–nitrogen bond order intermediate between a single bond (ν = 1360–1250 cm^{-1}) and a double bond (ν = 1690–1640 cm^{-1}).[52,53] The strong ν(C–N) vibration of complexes **3**–**5** were assigned at 1483, 1480, and 1433 cm^{-1} respectively. Hence, these frequency modes suggest a partial double bond character due to partial delocalization of electron density. For

Table 1. Mid FT-IR frequencies /cm^{-1} for free ligands and complexes **1**–**5**.

Free ligand[a] / complex	Stretch NH	Stretch C–H	Stretch =C–H	Stretch C–H(CH_2)	Bend C–H(CH_2)	Stretch Ar(C=C)	Stretch N–C	Stretch C=S
DMDTC	–	2952	–	–	–	–	1488	926
DEDTC	–	2948	–	2979 asym	1379	–	1466	986
DBDTC	–	–	3099	2922 asym	1347	1600	1445	985
1	3431, 3354	–	3087	2917 asym, 2857 sym	1310	1603	1432	–
2	3421, 3332	–	3085	2909asym, 2852 sym	1275	1599	1496	–
3	–	2990	–	2990 asym	1485	–	1483	1100, 993
4	–	2970		2925 asym, 2856 sym	1264	–	1480	1098, 1067
5		2923	3082	2923 asym, 2850 sym	1265	1602	1433	1100, 1076

a) DMDTC = dimethyldithiocarbamate, DEDTC = diethyldithiocarbamate, DBDTC = dibenzyldithiocarbamate.

Table 2. ^{1}H NMR chemical shifts /ppm for free ligands and gold(I) complexes **1**–**5**.

Free ligand / complex	H-1, H-1′	H-2, H-2′	NH	Aromatic Hs
DMDTC	–	–	–	–
DEDTC	–		–	–
DBDTC	–	–	–	7.39, 7.32, 7.24
AEP	2.78, 2.77	2.19, 2.16	1.3	7.40, 7.38,7.25
1	3.31, 3.17	2.97, 2.73	4.03	7.65, 7.45
2	3.02, 2.85	2.56, 1.26	4.04, 3.13	–
3	–	–	–	–
4	–	–	–	–
5	–	–	–	7.77, 7.43, 7.32, 7.17

Table 3. ^{13}C and ^{31}P NMR chemical shifts /ppm for free ligands and gold(I) complexes **1**–**5**.

Free ligand / complex	C-1[a)]	C-2	C-SS(3)	Aromatic C	^{31}P
DMDTC	46.7	–	208.3	–	–
DEDTC	49.5	12.1	206.4	–	–
DBDTC	56.9	–	213.1	127.3–136.7	–
AEP	38.9	32.6	–	127.5–137.2	–23.46
1	37.7	31.5	–	128.0–138.4	21.39
2	36.6	28.6	–	129.2–133.1	26.55
3	46.6	–	196.1	–	–
4	49.4	12.2	205.7	–	–
5	54.3	–	210.8	–	–

a) C-1 represents carbon atom next to nitrogen.

the (–C–SS) stretching two bands of medium intensity were observed at 1100, 993; 1098, 1072; 1100, and 1072 cm^{-1} for **3**, **4**, and **5** respectively. The presence of these two bands is an indication of asymmetrical bidentate binding of dithiocarbamate ligands.[54]

The ν(C–H) stretching vibrations of **3** and **4** were observed at 2990 and 2970 cm^{-1} respectively, whereas the ν(=C–H) mode of **5** for dibenzyldithiocarbamate was detected at 3082 cm^{-1}. The spectra of phosphine complexes **1** and **2** displayed two bands in the region of 1602–1599 cm^{-1}, which are assigned to ν(C=C) bands of the aromatic ring.

In the ^{1}H NMR spectra of free ligands and their gold(I) complexes the NC–H resonances of **3**, **4**, and **5** appeared around 3.55, 3.92, and 4.85 ppm, respectively, as given in Table 2. The CH_2 protons of benzyl group are diastereotopic and therefore give two signals (4.85 and 4.87 ppm). The aromatic protons of **1**, **2**, and **5** are observed between 7–8 ppm. The aminoethyl group gives resonances between 2–3 ppm. The ^{13}C NMR spectra of gold(I) dithiocarbamate polymers showed significant upfield shifts with respect to free dithiocarbamate ligands. The CS_2 resonances of uncoordinated dithiocarbamate were observed in the range of 206.4–213.1 ppm, whereas in complexes, they appeared in the range of 196.1–210.8 ppm. Similarly, in phosphine complexes, upfield shifts were also observed in aromatic and aminoethyl resonances. The ^{31}P NMR chemical shifts for complexes **1** and **2** showed significant shifts towards upfield region 21.39 and 26.55 as shown in Table 3.

In vitro Cytotoxic Activities of Gold(I) Complexes 1–5

Herein, the synthesized gold(I)-phosphine complexes **1** and **2**, as well as the gold(I)-dithiocarbamates **3**–**5**, and *cisplatin* (standard classical anticancer drug) were tested for *in vitro* cytotoxicity against A549, MCF7, and HCT15 human cancer cell lines using MTT assay. The dose-dependent inhibition of cell proliferation was obtained by specific increase in concentration of tested compounds against fixed number of three human cancer cell lines. The graphical representation of results is given Figure 1, Figure 2, and Figure 3. The IC_{50} values obtained from the curve of the concentration vs. percentage of cell viability are given in Table 4.

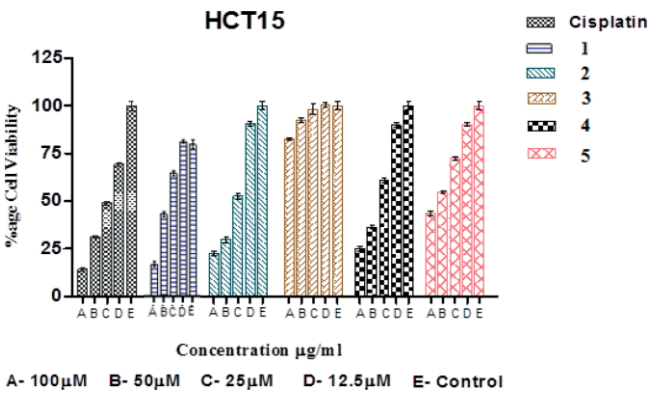


Figure 1. Effect of concentration of complexes **1**–**5** on cell viability of HCT15 cell line.

It can be seen that the IC_{50} values of complexes **2**, **4**, and **5** (34.42 ± 1.02, 19.56 ± 0.85, 29.25 ± 1.81 µM, respectively) for A549 cells are lower than that of *cisplatin* (42.2 ± 2.01 µM). These results suggest that the anticancer effect of these compounds is better than that exhibited by *cisplatin*. The antiproliferative potential of **2** and **4** for HCT15 cells is also comparable to *cisplatin*, whereas for MCF7 cells it is about half with respect to *cisplatin*. Complex **3** is much less potent than that of

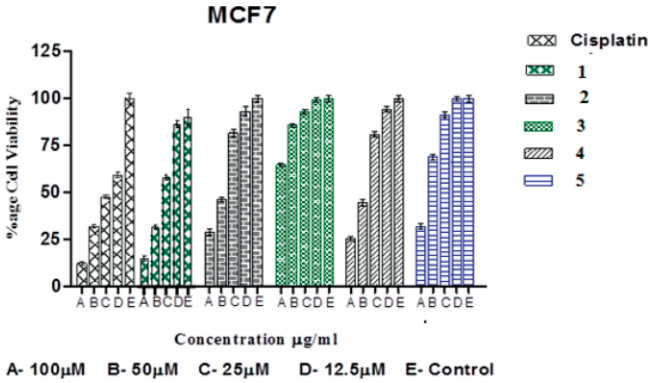


Figure 2. Effect of concentration of complexes **1–5** on cell viability of MCF7 cell line.

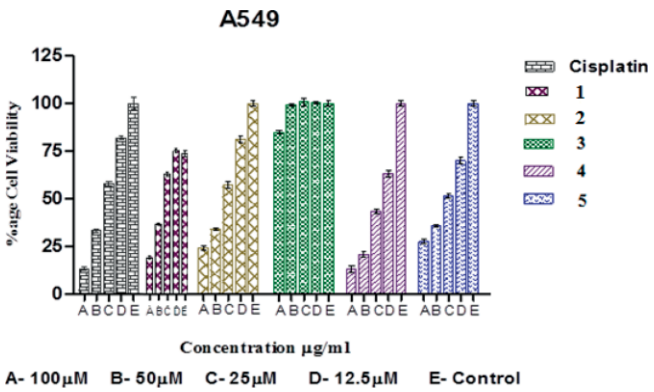


Figure 3. Effect of concentration of complexes **1–5** on cell viability of A549 cell line.

Table 4. IC_{50}values /µM of gold(I) complexes **1–5** against HCT15, A549, and MCF7 cancer cell lines.

Complex	HCT15	A549	MCF7
cisplatin	32.00 ± 2.12	42.20 ± 2.01	23.25 ± 3.79
1	51.21 ± 0.83	53.97 ± 0.94	38.34 ± 0.22
2	34.19 ± 2.49	34.42 ± 1.02	51.73 ± 2.25
3	256.63 ± 3.39	304.17 ± 5.9	138.82 ± 4.51
4	38.92 ± 2.38	19.56 ± 0.85	48.75 ± 1.96
5	69.07 ± 3.35	29.25 ± 1.81	76.32 ± 2.52

cisplatin. Its IC_{50} values are the highest of the investigated complexes showing that it is the least effective for all the studied cells. Complex **4** is the most active among the series against all three cell lines. The high activity of **4** may be attributed to the strong binding of dithiocarbamate sulfur to gold(I) that would prevent it to interact with plasma proteins in tissues, unlike *cisplatin*, which is strongly bound to plasma proteins through sulfur atoms of thiols.[3] It was also observed that the gold-dithiocarbamates are more effective for A549 cells, whereas gold-phosphine complexes exhibit greater cytotoxicity against MCF7 cells. These findings suggest that the activity of the complexes depend on the types of cancer cells along with the structure and composition of the complexes.

Conclusions

In this paper, the synthesis, and characterization of gold(I) complexes of 2-(diphenylphosphanyl)ethylamine or (2-aminoethyl)diphenylphosphine (AEP) and dithiocarbamates as well as their anticancer activity against three human cancer cell lines; MCF7 (human breast cancer), HCT 15 (human colon cell line), and A549 (human lung carcinoma) are reported. Three of the five studied gold(I) compounds exhibited better cytotoxicity against A549 cell line than the standard drug, *cisplatin*. Moreover, they showed moderate *in vitro* cytotoxicity against HCT15 and MCF7 cancer cell lines. The significant cytotoxicity of gold(I) compounds against A549 human cancer cells has made them strong candidates as potential anticancer agents for further exploration against lung cancer.

Experimental Section

Chemicals: Sodium tetrachloroaurate(III), sodium salts of di-alkyl/aryl dithiocarbamates (dimethyl compound as monohydrate) and dimethylsulfide were purchased from Sigma-Adrich Co. St. Louis, MO, USA. 2-(Diphenylphosphanyl)ethylamine was obtained from Strem Chemicals Inc., Massachusetts, United States. All solvents ethanol, diethyl ether, and dichloromethane (Fluka AG) were used without further purification.

Measurements: Elemental analyses were obtained on Perkin-Elmer Series 11 (CHNS/O), Analyzer 2400. The solid state FT-IR spectra of the ligands and their gold(I) complexes were recorded with a Perkin-Elmer FT-IR 180 spectrophotometer using KBr pellets over the range 4000–400 cm^{-1}. The ^{1}H and ^{13}C NMR spectra were recorded with a JEOL-LA 500 NMR spectrophotometer, operating at 500.0 and 125.65 MHz respectively using TMS as an internal reference. The ^{13}C NMR spectra were measured with ^{1}H broadband decoupling and spectral conditions: 32 k point data, 1 s acquisition time, 2.5 s pulse delay, and 5.12 µs pulse width. ^{31}P NMR spectra were obtained at 200.0 MHz using phosphoric acid as external standard. All spectra were recorded at 297 K in $CDCl_3$.

Synthesis of Gold(I) Complexes: The precursor complex, $(CH_3)_2SAuCl$ was synthesized by a procedure already described in the literature.[55] Yield 0.268 g, 90 %. C_2H_6SAuCl (294.55 g·mol^{-1}): calcd. C 8.35; H 2.13 %; found C 8.12; H 1.83 %. **^{1}H NMR** ($CDCl_3$, ppm): δ = 2.75 (s, 6 H). **^{13}C NMR** ($CDCl_3$, ppm): δ = 25.3 ppm.

Synthesis of [Au(AEP)Cl] (1): A solution of $(CH_3)_2SAuCl$ (0.147 g, 0.5 mmol) in dichloromethane (5 mL) was cooled to 5 °C and added dropwise to a solution of 2-(diphenylphosphanyl)ethylamine (0.115 g, 0.5 mmol) in dichloromethane (5 mL). A clear colorless solution appeared that was stirred for 1 h and filtered. The solution was concentrated by low evaporation of solvent at room temperature. The product was obtained as a white to cream-colored solid. It was recrystallized from acetone/dichloromethane mixture and dried overnight in vacuo. Yield 0.2 g (85 %). $C_{14}H_{16}AuClNP$ (461.68 g·mol^{-1}): calcd. C 36.42; H 3.39; N 3.03 %; found: C 35.90; H 2.84; N 3.36 %. **IR** (KBr): $\tilde{\nu}$ = ν(N–H) 3431, 3354; $\nu(CH_2)$ 2917_{asym}, 2857_{sym}, ν(C–H) 1310_{bend}; ν(Ar–C=C) 1603; ν(N–C) 1432 cm^{-1}. **^{1}H NMR** ($CDCl_3$, ppm): δ = 4.03 (s, NH); 3.31, 3.17[m, C(1)*H*, *H*']; 2.97, 2.73 [m, C(2)*H*, *H*']; 7.45–7.65 (m, 10 H, C_5H_5). **^{13}C NMR** ($CDCl_3$, ppm): δ = 37.65 C(1), 31.50 C(2), 129.28–133.26 C(C_5H_5). **^{31}P NMR** ($CDCl_3$, ppm): δ = 21.39.

Synthesis of [Au(AEP)$_2$]Cl (2): The bis complex (**2**) was prepared by adding 0.115 g (0.5 mmol) 2-(diphenylphosphanyl)ethylamine to [Au(AEP)Cl] (**1**) (0.231 g, 0.5 mmol) (Au:AEP = 1:2) in dichloromethane (5 mL). A yellow solution appeared that was stirred for 3 h and filtered. The solution was concentrated by low evaporation of solvent at room temperature. A yellow solid (**2**) was obtained that was recrystallized from acetone/dichloromethane and dried overnight in vacuo. Yield 0.3 g (87 %). $C_{28}H_{32}AuClN_2P_2$ (690.68 g·mol^{-1}): calcd. C 48.67; H 4.67; N 4.05 %; found: C 47.89; H 5.52; N 4.04 %. **IR** (KBr): $\tilde{\nu}$ = ν(N–H) 3421, 3332; ν(CH$_2$) 2909$_{asym}$, 2852$_{sym}$, ν(C–H) 1275$_{bend}$; ν(Ar–C=C) 1566; ν(N–C) 1496 cm^{-1}. **^{1}H NMR** ($CDCl_3$, ppm): δ = 4.04, 3.13 (s, 2 H, NH); 3.02, 2.85 [m, C(1)*H*, *H*']; 2.56, 1.26 [m, C(2)*H*, *H*']; 7.33–7.74 (m, 20 H, C_5H_5). **^{13}C NMR** ($CDCl_3$, ppm): δ = 36.63 C(1), 28.57 C(2), 129.17–133.08 C(C_6H_5). **^{31}P NMR** ($CDCl_3$, ppm): δ = 26.55.

Synthesis of Gold(I)-Dithiocarbamate Complexes, [Au$_2$(*R*$_2$NCS$_2$)$_2$]$_n$ (3–5): To a solution of $(CH_3)_2SAuCl$ (0.147g, 0.5 mmol) in dichloromethane (10 mL) was added 1 equiv. of the respective dithiocarbamate in ethanol (10 mL) at room temperature with continuous stirring for 6 h. The reaction mixture was filtered off and the clear yellow solution was kept for slow evaporation. A yellow solid was obtained for **3** and **5**, whereas for **4** orange needle-like crystals were obtained.

3: $Au_2(C_2H_6NCS_2)_2$ (634.37 g·mol^{-1}): calcd. C 11.37; H 2.06; N 3.85 %; found: C 11.36; H 1.91; N 4.42 %. **IR** (KBr): $\tilde{\nu}$ = ν(C–H) 2990, ν(C–H) 1375$_{bend}$; ν(N–C) 1483; ν(C=S) 1100, 993 cm^{-1}. **^{1}H NMR** ($CDCl_3$, ppm): δ = 3.55 (s, 6 H, CH_3). **^{13}C NMR** ($CDCl_3$, ppm): δ = 196.12 –N*C*S$_2$(1), 46.64 C(2).

4: $Au_2(C_4H_{10}NCS_2)_2$ (690.47 g·mol^{-1}): calcd. C 17.72; H 2.56; N 3.88 %; found: C 17.39; H 2.92; N 4.06 %. **IR** (KBr): $\tilde{\nu}$ = ν(C–H) 2970, ν(C–H) 1377$_{bend}$; ν(–CH$_2$)2925$_{asym}$, 2856$_{sym}$; ν(–CH$_2$)$_{bend}$ 1264; ν(N–C) 1480; ν(C=S) 1098, 1067 cm^{-1}. **^{1}H NMR** ($CDCl_3$, ppm): δ = 1.32 (t, 6 H, CH_3), 3.92 (t, 4 H, C*H*$_2$CH$_3$). **^{13}C NMR** ($CDCl_3$, ppm): δ = 205.58 –N*C*S$_2$(1), 49.35 C(2), 12.20 C(3).

5: $Au_2(C_{14}H_{14}NCS_2)_2$ (926.74 g·mol^{-1}): calcd. C 36.33; H 2.56; N 2.88 %; found: C 37.58; H 3.05; N 3.02 %. **IR** (KBr): $\tilde{\nu}$ = ν(CH$_2$) 2923$_{asym}$, 2850$_{sym}$, ν(C–H) 1265$_{bend}$; ν(Ar–C=C) 1602; ν(N–C) 1433; ν(C=S) 1100, 1072 cm^{-1}. **^{1}H NMR** ($CDCl_3$, ppm): δ = 4.85, 4.78 (d, 4 H, CH_2), 7.17–7.77(m, 10 H, C_5H_5). **^{13}C NMR** ($CDCl_3$, ppm): δ = 210.80 –N*C*S$_2$ (1), 54.27 C(2), 128.58–140.93 C(C_6H_5)

MTT Assay for *in vitro* Cytotoxicity of Complexes: The anticancer activity of complexes **1–5** was measured as reported earlier[41] against a panel of representative human tumor cell lines, which include, MCF7 (human breast cancer), HCT15 (human colon adenocarcinoma), and A549 (human lung carcinoma) cell lines. The cells were seeded at 3×10^3 cells/well in 100 µL DMEM containing 10 % Fetal Bovine Serum (FBS) in 96-well tissue culture plate and incubated for 72 h at 37 °C, 5 % CO_2 in air and 90 % relative humidity in CO_2 incubator. After incubation, 100 µL of 50, 25, and 12.5 µM solutions of *cisplatin* and complexes **1–5** prepared in Dulbecco's Modified Eagle's Medium (DMEM), were added to the cells and the cultures were incubated for 24 h. The medium of wells was discarded and 100 µL DMEM containing MTT (0.5 mg·mL^{-1}) was added to the wells and incubated in CO_2 incubator at 37 °C in dark for 4 h. After incubation, a purple colored formazan produced in the cells appeared as dark crystals in the bottom of the wells. The culture medium was discarded from each well carefully to prevent disruption of monolayer and 100 µL of dimethyl sulfoxide (DMSO) was added in each well. The solution in the wells was thoroughly mixed to dissolve the formazan crystals which produce a purple solution. The absorbance of the 96 well-plates was taken at 570 nm with Lab systems Multiskan EX ELISA reader against a reagent blank. The experimental results are presented as micro-mole concentration of 50 % cell growth inhibition (IC_{50}) of each compound. The MTT assay was performed in three independent experiments.

Acknowledgements

This project was funded by the National Plan for Science and Innovation (MARIFAH)–King Abdulaziz City for Science and Technology (KACST) through the Science and Technology Unit at King Fahd University of Petroleum and Minerals (KFUPM) of Saudi Arabia, award Numbers 14-MED64–04 and 11-MED1670–04.

References

[1] T. C. Johnstone, K. Suntharalingam, S. J. Lippard, *Chem. Rev.* **2016**, *116*, 3436–3486.
[2] K. S. Lovejoy, S. J. Lippard, *Dalton Trans.* **2009**, 10651–10659.
[3] S. Ahmad, *Chem. Biodiv.* **2010**, *7*, 543–566.
[4] S. Medici, M. Peana, V. M. Nurchi, J. I. Lachowicz, G. Crisponi, M. A. Zoroddu, *Coord. Chem. Rev.* **2015**, *284*, 329–350.
[5] M. Hanif, M. V. Babak, C. G. Hartinger, *Drug Discov. Today* **2014**, *19*, 1640–1648.
[6] R. S. Wai-Yin, D. L. Ma, E. L. Wong, C. M. Che, *Dalton Trans.* **2007**, 4884–4892.
[7] S. Ahmed, A. A. Isab, S. Ali, A. R. Al-Arfaj, *Polyhedron* **2006**, *25*, 1633.
[8] S. J. Berners-Price, A. Filipovska, *Metallomics* **2011**, *3*, 863–873.
[9] C. Nardon, G. Boscutti, D. Fregona, *Anticancer Res.* **2014**, *34*, 487–92.
[10] M. Altaf, S. Ahmed, A.-N. Kawde, N. Baig, A. Alawad, S. Aluwairji, H. Stoeckli-Evans, A. A. Isab, *New J. Chem.* **2016**, *40*, 8288–8295.
[11] B. Bertrand, A. Casini, *Dalton Trans.* **2014**, *43*, 4209–4219.
[12] E. R. T. Tiekink, *Crit. Rev. Oncol. Hematol.* **2002**, *42*, 225–248.
[13] C. Gabbiani, A. Casini, M. L. Messori, *Gold Bull.* **2007**, *40*, 73–81.
[14] N. Cutillas, G. S. Yellol, C. de Haro, C. Vicente, V. Rodriguez, J. Ruiz, *Coord. Chem. Rev.* **2013**, *257*, 2784–2797.
[15] T. Zou, C. T. Lum, C. N. Lok, J. J. Zhang, C. M. Che, *Chem. Soc. Rev.* **2015**, *44*, 8786−8801.
[16] A. Gautier, F. Cisnetti, *Metallomics* **2012**, *4*, 23–32.
[17] M. Altaf, M. Monim-ul-mehboob, A. A. Seliman, A. A. Isab, V. Dhuna, G. Bhatia, K. Dhuna, *J. Organomet. Chem.* **2014**, *765*, 68–79.
[18] L. Ronconi, L. Giovagnini, C. Marzano, F. Bettio, R. Graziani, G. Pilloni, D. Fregona, *Inorg. Chem.* **2005**, *44*, 1867–1881.
[19] a) L. Cattaruzza, D. Fregona, M. M. Maurizio, L. Ronconi, F. A. Ambrogio, A. Colombatti, A. D. Aldinucci, *Int. J. Cancer* **2011**, *128*, 206–215; b) C. Marzano, L. Ronconi, F. Chiara, M. Cecilia, F. I. Giron, P. Cristofori, A. Trevisan, D. Fregona, *Int. J. Cancer* **2011**, *129*, 487–496.
[20] C. F. Shaw III, *Chem. Rev.* **1999**, *99*, 2589–2600.
[21] C. K. Mirabelli, R. K. Johnson, C. M. Sung, L. Faucette, K. Muirhead, S. T. Crooke, *Cancer Res.* **1985**, *45*, 32–39.
[22] C. K. Mirabelli, R. K. Johnson, D. T. Hill, L. F. Faucette, G. R. Girard, G. Y. Kuo, C. M. Sung, S. T. Crooke, *J. Med. Chem.* **1986**, *29*,218–223.
[23] S. H. Park, J. H. Lee, J. S. Berek, M. C. Hu, *Int. J. Oncol.* **2014**, *45*, 1691–1698.
[24] S. J. Berners-Price, G. R. Girard, D. T. Hill, B. M. Sutton, P. S. Jarrett, L. F. Faucette, R. K. Johnson, C. K. Mirabelli, P. J. Sadler, *J. Med. Chem.* **1990**, *33*, 1386–1392.
[25] S. J. Berners-Price, C. K. Mirabelli, R. K. Johnson, M. R. Mattern, F. L. McCabe, L. F. Faucette, C. M. Sung, S. M. Mong, P. J. Sadler, S. T. Crooke, *Cancer Res.* **1986**, *46*, 5486–5493.

[26] P. C. Healy, B. T. Loughrey, M. L. Williams, P. G. Parsons, *J. Inorg. Biochem.* **2010**, *104*, 625–631.
[27] F. Caruso, M. Ross, J. Tanski, C. Pettinari, F. Marchetti, *J. Med. Chem.* **2003**, *46*, 1737.
[28] F. Caruso, R. Villa, M. Rossi, C. Pettinari, F. Paduano, M. Pennati, M. G. Daidone, N. Zaffaroni, *Biochem. Pharmacol.* **2007**, *73*, 773–781.
[29] V. Gandin, A. P. Fernandes, M. P. Rigobello, B. Dani, F. Sorrentino, F. Tisato, M. Bjornstedt, A. Bindoli, A. Sturaro, R. Rella, C. Marzano, *Biochem. Pharmacol.* **2010**, *79*, 90–101.
[30] K. K. Ooi, C. I. Yeo, K. P. Ang, A. M. Akim, Y. K. Cheah, S. N. A. Halim, H. L. Seng, E. R. T. Tiekink, *J. Biol. Inorg. Chem.* **2015**, *20*, 855–873.
[31] O. Rackham, S. J. Nichols, P. J. Leedman, S. J. Berners-Price, *Biochem. Pharmacol.* **2007**, *74*, 992–1002.
[32] G. Lupidi, L. Avenali, M. Bramucci, L. Quassinti, R. Pettinari, H. K. Khalife, H. Gali-Muhtasib, F. Marchetti, C. Pettinari, *J. Inorg. Biochem.* **2013**, *124*, 78–87.
[33] J. D. Chaves, F. Neumann, T. M. Francisco, C. C. Correa, M. T. P. Lopes, H. Silva, A. P. S. Fontes, M. V. Almeida, *Inorg. Chim. Acta* **2014**, *414*, 85–90.
[34] H. Scheffler, Y. You, I. Ott, *Polyhedron* **2010**, *29*, 66–69.
[35] M. Ali, L. Dondaine, A. Adolle, C. Sampaio, F. Chotard, P. Richard, F. Denat, A. Bettaieb, P. Le Gendre, V. Laurens, C. Goze, C. Paul, E. Bodio, *J. Med. Chem.* **2015**, *58*, 4521–4528.
[36] Y. Wang, M. Liu, R. Cao, W. Zhang, M. Yin, X. Xiao, Q. Liu, N. Huang, *J. Med. Chem.* **2013**, *56*, 1455–1466.
[37] R. Hayashi, K. Nakatsui, D. Sugiyama, T. Kitajima, N. Oohara, M. Sugiya, S. Osada, H. Kodama, *J. Inorg. Biochem.* **2014**, *137*, 109–114.
[38] T. Traut-Johnstone, S. Kanyanda, F. H. Kriel, T. Viljoen, P. D. R. Kotze, W. E. van Zyl, J. Coates, D. J. G. Rees, M. Meyer, R. Hewer, D. B. G. Williams, *J. Inorg. Biochem.* **2015**, *145*, 108–120.
[39] H. Chiririwa, J. R. Moss, D. Hendricks, G. S. Smith, R. Meijboom, *Polyhedron* **2013**, *49*, 29–35.
[40] F. K. Keter, I. A. Guzei, M. Nell, W. E. Van Zyl, J. Darkwa, *Inorg. Chem.* **2014**, *53*, 2058–2067.
[41] M. Altaf, M. Monim-ul-Mehboob, A. A. Isab, V. Dhuna, G. Bhatia, K. Dhuna, S. Altuwaijri, *New J. Chem.* **2015**, *39*, 377–385.
[42] M. Altaf, M. Monim-ul-Mehboob, A. A. A. Seliman, M. Sohail, M. I. M. Wazeer, A. A. Isab, L. Li, V. Dhuna, G. Bhatia, K. Dhuna, *Eur. J. Med. Chem.* **2015**, *95*, 464–472.
[43] P. J. Barnard, S. J. Berners-Price, *Coord. Chem. Rev.* **2007**, *251*, 1889–1902.
[44] A. Bindoli, M. P. Rigobello, G. Scutari, C. Gabbiani, A. Casini, L. Messori, *Coord. Chem. Rev.* **2009**, *253*, 1692–1707.
[45] F. Magherini, A. Modesti, L. Bini, M. Puglia, I. Landini, S. Nobili, E. Mini, M. A. Cinellu, C. Gabbiani, L. Messori, *J. Biol. Inorg. Chem.* **2010**, *15*, 573–582.
[46] M. P. Rigobello, G. Scutari, R. Boscolo, A. Bindoli, *Br. J. Pharmacol.* **2002**, *136*, 1162–1168.
[47] C. Marzano, V. Gandin, A. Folda, G. Scutari, A. Bindoli, M. P. Rigobello, *Free Radic. Biol. Med.* **2007**, *42*, 872–881.
[48] L. E. Wedlock, M. R. Kilburn, J. B. Cliff, L. Filgueira, M. Saunder, S. J. Berners-Price, *Metallomics* **2011**, *3*, 917–925.
[49] D. Paliwoda, P. Wawrzyniak, A. Katrusiak, *J. Phys. Chem. Lett.* **2014**, *5*, 2182–2188.
[50] M. F. Fillat, M. C. Gimeno, A. Laguna, E. Latorre, L. Ortego, M. D. Villacampa, *Eur. J. Inorg. Chem.* **2011**, 1487–1495.
[51] R. J. Roberts, N. Bélanger-Desmarais, C. Reber, D. B. Leznoff, *Chem. Commun.* **2014**, *50*, 3148–3150.
[52] M. Shahid, T. Rüffer, H. Lang, S. A. Awan, S. Ahmad, *J. Coord. Chem.* **2009**, *62*, 440–445.
[53] M. Altaf, H. Stoeckli-Evans, S. S. Batool, A. A. Isab, S. Ahmad, M. Saleem, S. A. Awan, M. A. Shaheen, *J. Coord. Chem.* **2010**, *63*, 1176–1185.
[54] R. Kellner, G. S. Nikolov, N. Trendafilova, *Inorg. Chim. Acta* **1984**, *84*, 233.
[55] S. Ahmad, M. N. Akhtar, A. A. Isab, A. R. Al-Arfaj, M. S. Hussain, *J. Coord. Chem.* **2000**, *51*, 225–234.

Received: August 1, 2016
Published Online: ■