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Synthesis, Spectroscopic Characterization and Anticancer Activity of New Mono and Binuclear Phosphanegold(I) Dithiocarbamate Complexes

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Abstract

A new series of mononuclear [t-$Bu_3PAuS_2CN(C_7H_7)_2$] (**1**), and binuclear [$(DPPM)Au_2(S_2CN(CH_3)_2)_2$] (**2**), [$(DPPM)Au_2(S_2CN(C_2H_5)_2)_2$] (**3**) and [$(DPPM)Au_2(S_2CN(C_7H_7)_2)_2$] (**4**) [where DPPM = 1,1-bis(diphenylphosphino)methane, $S_2CN(CH_3)_2$=dimethyldithiocarbamate, $S_2CN(C_2H_5)_2$= diethyldithiocarbamate and $S_2CN(C_7H_7)_2$ = dibenzyldithiocarbamate] gold(I) complexes have been prepared by reacting gold(I) precursors and dialkyl/diaryl dithiocarbamate ligands. The complexes were characterized by analytical technique and spectroscopic methods such as CHNS analysis, FTIR spectroscopy; ^{1}H, ^{13}C and ^{31}P NMR measurements. The molecular

structure of [t-$Bu_3PAuS_2CN(C_7H_7)_2$] (**1**) complex was determined by X-ray diffraction. The gold(I) complexes (**2** and **3**) were found particularly better potent *in vitro* cytotoxic agents in comparison to cisplatin against HeLa, HCT15 and A549 cancer cell lines. These metal complexes could serve as attractive anticancer agents for the developments of novel therapeutic strategies and to treat cervix, lung and colon cancers.

Key words: Mononuclear and Binuclear Dithiocarbamato Gold(I) complexes; 1,1-Bis(diphenylphosphino)methane (DPPM), *in vitro* anticancer activity, cervix, lung and colon cancers

1. Introduction

The triumphant depiction of cisplatin, oxaliplatin and carboplatin as metal-based anticancer drugs is well acknowledged in the field of chemotherapy [1-6]. Indeed, such drugs have been used for the treatment of cancer patients world-wide. Cisplatin and its analogues have serious side effects, such as oto-, neuro-, and nephrotoxicity, which decrease its effectiveness in cancer therapy [7-10]. Resultantly, gold(I) and gold(III) complexes had been investigated as non-platinum based anticancer candidates [11-14].

The study of gold complexes, bearing different functional ligands exhibiting physical, chemical, biological and pharmacological properties, has gained much attention [11-14]. The gold(I) complexes have long been studied as anti-arthritic and anti-microbial agents [15-19]. For instance, the drugs like Auranofin, Solganol and Myocrisin have frequently been used for the treatment of rheumatoid arthritis [20-24]. Interestingly, the extensive cell-based (*in vitro*) and animal (*in vivo*) studies have revealed the potent anti-cancer activities of diverse classes of gold(I) and gold(III) complexes with a wide range of ligands against a panel of human cancer cell lines [25-27].

An enormous number of bridged di-gold(I) complexes, preferably existed in a linear 2-coordinate configuration like [ClAu(P-P)AuCl] (where P-P is a bisphosphine), are more effective than free ligands and exhibit a broad range of anticancer activity [28, 29]. This has inspired the synthesis of stable 4-coordinate digold(I) diphosphine complexes [30-32]. The effect of structural variation in chelated bis(diphosphine) gold(I) complexes $[Au(R_2P(CH_2)_nPR_2)]X$ on their cytotoxicity and activity against P388 leukaemia, B16 melanoma and M5076 reticulum cell sarcoma has been studied [33]. J. W. Faamaua *et al.*

reported compounds of general formula [($Ph_2P(CH_2)nPPh_2$)($AuS_2CNR_2)_2$], n = 1, 2 or 3 and R = Et or c-hexyl [34].

Since the first decade of 21st century, a new class of gold complexes with dithiocarbamate ligands has favorably been emerged as anticancer agents. In this regards, Fregona and coworkers synthesized and characterized some novel gold(III) compounds containing N,N-dimethyldithiocarbamate and ethyl sarcosine dithiocarbamate exhibiting potential chemical and biological profile [35]. Dibromo(N,N-dimethyldithiocarbamato)gold(III) also showed a noteworthy inhibition of *in-vivo* MDA-MB-231 breast cancer growth [36]. Zhang *et al.* reported that gold(I)-dithiocarbamato species, namely [Au(ESDT)](2) could hamper the chymotrypsin-like activity of purified 20S proteasome and 26S proteasome in human breast cancer MDA-MB-231 cells, resulting in accumulation of ubiquitinated proteins and proteasome target proteins, and induction of cell death [37]. Recently, the modern research has progressively targeted in search of new gold(I) complexes as potential anticancer drugs [38-42].

Worldwide, lung and colorectal cancers are frequent causes of cancer-related death regardless of males and females while a cervix cancer is still overwhelmingly reason of cancer deaths in females exclusively. Therefore, there is dire need of designing new drugs in order to treat such lethal diseases through chemotherapy. In the wake of chrysotherapeutic agents, gold(I) complexes could be developed with a new combination of P and S donor ligands which may have better selectivity and activity with least side effects for cancer treatment.

Gold(I) complexes based on monophosphine/bisphosphine and dithiocarbamate mixed ligands have been addressed. Rationally, we are planning to design the new dithiocarbamato gold(I) with monophosphine / bisphosphine complexes which would potentially lead to the development of new anticancer agents and the treatment of a variety of cancer effectively through chrysotherapy.

In the current study, we are presenting the synthesis of gold(I) complexes of phosphine and dialkyl/diaryldithiocarbamate mixed ligands, their structure analysis by mid-IR spectroscopy and NMR measurements; and molecular structure determination by single crystal X-ray diffraction. Finally, the characterized gold(I) complexes have systematically been examined for *in vitro* cytotoxic activity against various human cancer cell lines e.g. A549 (human lung carcinoma), HCT15 (human colon carcinoma), and HeLa (human cervix cancer).

2. Experimental

2.1. Materials and Methods

Chemicals and solvents used in the synthesis were of analytical grade and were used without further purification. All the reactions were carried under normal ambient conditions. All chemicals were obtained from Sigma-Aldrich St. Louis, Missouri United States and Strem Chemicals, Massachusetts, United States.

Elemental analyses were performed on Perkin Elmer Series 11 (CHNS/O), Analyzer 2400. The solid state FTIR spectra of free ligands and their corresponding gold(I)

complexes were recorded on a Perkin–Elmer FTIR 180 spectrophotometer or NICOLET 6700 FTIR using KBr pellets over the range 4000–400 cm^{-1}.

^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a LAMBDA 500 spectrophotometer operating at 500.01, 125.65 and 200.0 MHz respectively; corresponding to a magnetic field of 11.74 T. Tetramethylsilane (TMS) was used as an internal standard for ^{1}H and ^{13}C NMR measurements. Triphenylphosphine (TPP) was used as an external standard for ^{31}P NMR measurement. The ^{13}C NMR spectra were obtained with ^{1}H broadband decoupling. The spectral conditions were: 32 k data points, 0.967 s acquisition time, 1.00 s pulse delay and 45° pulse angle.

Structures of gold(I) precursor and free ligands used in this study is as shown in Scheme I. The ^{1}H, ^{13}C and ^{31}P NMR chemical shifts of metal precursors and free ligands are given in Tables 1 and 2 (see supplementary materials). Purposed structures of the synthesized complexes (**1-4**) are given in Scheme II.

2.2. Synthesis of gold(I) complexes

2.2.1. [t-$Bu_3PAuS_2CN(C_7H_7)_2$] (1)

[t-Bu_3PAuCl] (0.217 g, 0.05 mmol) in 10 mL of dichloromethane was added in sodium dibenzyldithiocarbamate (0.136 g,0.05 mmol) in 15 mL of ethanol at room temperature. Upon continuous stirring the reaction mixture for 3 h, the transparent light yellow solution was obtained, filtered to avoid any impurity and kept undisturbed for crystallization by slow evaporation at room temperature. The colorless block like crystals

was obtained after seven days. A suitable quality crystal was chosen for X-ray diffraction analysis. Yield: 0.312 g, (93 %). Anal. Calc. for $C_{27}H_{41}AuNPS_2$: C, 48.28; H, 6.15; N, 2.09; S, 9.54; Found: C, 48.17; H, 6.33; N, 2.02; S, 9.43. IR cm^{-1}: 3035 (w), 2995 (m), 2905 (m), 1491 (s), 1456 (s), 1378 (m), 1213 (s), 1170 (m), 1022 (m), 972 (s), 806 (m), 521 (s), 478 (m). NMR ($CDCl_3$-d_1): ^{1}H, δ 1.57 (27H, C(2)H), 5.17 (4H, C(4)H), 7.32–7.34 (10H, H(Ph));^{13}C, δ 32.29 C(2), 39.40C(1),55.79 C(4), 127.35–136.30 C(Ph), and 210.15 C(3); ^{31}P: δ -7.83.

2.2.2. [(DPPM)$Au_2(S_2CN(CH_3)_2)_2$] (2)

[μ-Bis(diphenylphosphino)methane]dichlorodigold(I), [(DPPM)$(AuCl)_2$] (0.425 g, 0.05 mmol) in 10 mL CH_2Cl_2 was added in Sodium dimethyldithiocarbamate monohydrate (0.144 g,0.10 mmol) in 15 mL C_2H_5OH at room temperature. Upon continuous stirring the reaction mixture for 3 h, the transparent yellow solution was obtained, filtered to avoid any impurity and kept undisturbed for crystallization by slow evaporation at room temperature. The yellow very small crystals were obtained after five days. Anal. Calc. for $C_{31}H_{34}Au_2N_2P_2S_4$: C, 36.55; H, 3.36; N, 2.75; S, 12.59; Found: C, 36.45; H, 3.53; N, 2.87; S, 12.68. Yield: 0.397 g, (78%). IR cm^{-1}: 3038 (w), 2980 (w), 2917 (w), 1481 (m), 1432 (s), 1370 (m), 1270 (m), 1147 (w), 1099 (m), 970 (m), 918 (w), 550 (s), 479 (m).NMR (DMSO-d_6): ^{1}H, δ 2.49 (2H, C(1)H), 4.47 (12H, C(3)H), 7.31–7.79 (20H, H(Ph));^{13}C, δ 30.68 C(1), 44.76 C(3), 128.78–133.25 C(Ph), and 208.15 C(2); ^{31}P: δ 39.66.

2.2.3. [(DPPM)$Au_2(S_2CN(C_2H_5)_2)_2$] (3)

[μ-Bis(diphenylphosphino)methane]dichlorodigold(I), [(DPPM)$(AuCl)_2$] (0.425 g, 0.05 mmol) in 10 mL CH_2Cl_2 was added in Sodium diethyldithiocarbamatetrihydrate (0.226 g,

0.10 mmol) in 15 mL of C_2H_5OH at room temperature. Upon continuous stirring the reaction mixture for 3 h, the transparent yellow solution was obtained on the addition of 3 mL water was for clarity, filtered to avoid any impurity and kept undisturbed for slow evaporation at room temperature. After three days yellow semi-crystalline product was obtained. Anal. Calc. for $C_{35}H_{42}Au_2N_2P_2S_4$: C, 39.11; H, 3.94; N, 2.61; S, 11.93; Found: C, 39.05; H, 3.83; N, 2.57; S, 11.68. Yield: 0.392 g, (73%). IR cm^{-1}: 3043 (w), 2970 (w), 2921 (w), 1486 (m), 1432 (s), 1374 (m), 1265 (m), 1137 (w), 1087 (m), 982 (m), 908 (w), 560 (s), 478 (m). NMR (DMSO-d_6): ^{1}H, δ 1.22 (12H, C(4)H), 2.49 (2H, C(1)H),4.49 (8H, C(3)H), 7.33–7.79 (20H, H(Ph));^{13}C, δ 12.17 C(4), 30.66C(1), 49.06 C(3), 128.83–133.29 C(Ph), and 206.62 C(2); ^{31}P: δ 40.69.

2.2.4. [(DPPM)$Au_2(S_2CN(C_7H_7)_2)_2$] (4)

[μ-Bis(diphenylphosphino)methane]dichlorodigold(I), [(DPPM)$(AuCl)_2$] (0.425 g, 0.05 mmol) in 10 mL CH_2Cl_2 was added in sodium diebenzyldithiocarbamatetrihydrate (0.272 g, 0.10 mmol) in 15 mL of C_2H_5OH at room temperature. Upon continuous stirring the reaction mixture for 3 h, a turbid solution was obtained initially. The transparent pale yellow solution was obtained on addition of 3 mL of water for the removal of turbidity, filtered to avoid any impurity and kept in dark for slow evaporation. The bright yellow crystalline product was obtained after seven days. Anal. Calc. for $C_{55}H_{50}Au_2N_2P_2S_4$: C, 49.93; H, 3.81; N, 2.12; S, 9.69; Found: C, 49.85; H, 3.85; N, 2.15; S, 9.58. Yield: 0.549 g, (83%).IR cm^{-1}: 3025 (w), 2919 (w), 1489 (s), 1432 (s), 1351 (m), 1209 (s), 1147 (m), 1025 (m), 970 (s), 810 (w), 518 (m), 479 (m). NMR (DMSO-d_6): ^{1}H, δ 2.49 (2H, C(1)H), 5.00 (8H, C(3)H), 7.23–7.83 (40H, H(Ph));^{13}C, δ 30.99 C(1), 56.10C(3), 126.88–135.96 C(Ph), and 208.54 C(2); ^{31}P: δ 40.20.

2.3 Stability test of gold(I) complexes in DMSO-d_6

Compounds (**1** and **2**) were dissolved in DMSO-d_6 and analyzed by ^{1}H and ^{13}C {1H} NMR measurements. The extent of decomposition over time was determined by comparing the NMR spectra collected after 1, 6, 12, 24, 48 and 72 h. No significant change in the chemical shifts and the splitting patterns of compounds (**1** and **2**) was observed in their time dependent ^{1}H NMR spectra.

2.4. UV-visible measurements

UV-vis spectroscopy was used to determine the stability of the complexes (**1-4**) in 1% DMSO solution. Electronic spectra were recorded on freshly prepared solutions and after 72 h of each complex at room temperature. Electronic spectra were obtained for complexes (**1-4**) using a Lambda 200, Perkin-Elmer UV-vis spectrometer.

2.5. X-ray Diffraction studies

Pale yellow plates like crystals of compound (**1**) were obtained by recrystallization of the final product using a mixture of solvents i.e. C_2H_5OH and H_2O in 4:1 v/v ratio under slow evaporation at room temperature. The intensity data were collected at 173K (-100°C) on a Stoe Mark II-Image Plate Diffraction System [43] equipped with a two-circle goniometer using MoKα graphite mono chromated radiation (λ = 0.71073 Å). The structure was solved by Direct methods with SHELXS-97 [44]. The refinement and all further calculations were carried out with SHELXL-2013 [44]. The C-bound H-atoms were included in the calculated positions and treated as riding atoms: C-H = 0.95, 0.99 and 0.98 Å for CH (aromatic), CH_2 and CH_3, respectively, with $U_{iso}(H) = 1.5U_{eq}$(C-methyl) and $= 1.2U_{eq}$ (C) for other H-atoms. The non-H atoms were refined anisotropically, using

weighted full-matrix least-squares on F^2. A semi-empirical absorption correction was applied using the MULscanABS routine in PLATON [45]. Figure 1 was drawn using the programs MERCURY [46]. A summary of crystal data and refinement details for gold(I) complex **(1)** are given in Table 1. Selected bond lengths and bond angles are given in Table 2.

2.6. Cell cultures

A549, HeLa and HCT15 human cancer cells were seeded and maintained in triplicate at 4 x 10^3 cells/well in 100 µL DMEM (Dulbecco's Modified Eagle's Medium) containing 10%.FBS (Fetal Bovine Serum) in 96-wells tissue culture plate and incubated for 72 h at 37^{o}C, 5 % CO_2 in air and 90 % relative humidity in CO_2 incubator.

2.7. MTT assays for anticancer activity of gold (I) complexes (1-4)

100 µL of cisplatin and complexes (**0-4**) in 50, 25, 12.5 and 6.25 µg/mL concentrations, prepared in DMEM, were added to 5000 cancer cells after incubation. The resultant cultures were incubated for 24 h. The medium of wells was discarded. 100 µL DMEM containing MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) (5 mg/mL) was added to the wells and incubated in CO_2 incubator at 37^{o}C in dark for 4 h. After incubation, a purple colored formazan (artificial chromogenic dye, product of the reduction of water insoluble tetrazolium salts e.g., MMT by dehydrogenases and reductases) in the cells is produced and appeared as dark crystals in the bottom of the wells. The medium of culture was discarded from each well carefully to avoid disruption of monolayer. 100 µL of Dimethylsulphoxide (DMSO) was added in each well. The solution was thoroughly mixed in the wells to dissolve the formazan crystals which

ultimately result into a purple solution. The absorbance of the 96-wells plate was taken at 570 nm with Lab systems Multiskan EX-Enzyme-linked immunesorbent assay (EX-ELISA) reader against a reagent blank. All data presented are mean ± standard deviation.

3. Results and Discussion

3.1. Chemistry

Addition of dibenzyl dithiocarbamate to tri-*tert* butylphosphine gold(I) chloride afforded the formation of mononuclear gold(I) crystalline complex (**1**). Moreover, addition of dimethyl dithiocarbamate, diethyl dithiocarbamate, dibenzyl dithiocarbamate to [μ-Bis(diphenylphosphino)methane]dichlorodigold(I) afforded the formation of three binuclear gold(I) complexes (**2-4)** respectively in good yields. The mononuclear monophosphine gold(I) complex (**1)** and binuclear bisphosphine gold(I) complexes (**2-4**) containing methyl, ethyl and benzyl groups in dialkyl/diaryl dithiocarbamate ligand have been evaluated to know the steric effects on *in vitro* cytotoxicity. Complexes (**1-4**) were completely soluble in polar organic solvents i.e. DMSO and DMF.

3.2. Spectroscopic Characterization

Dithiocarbamate compounds can be identified *via* the presence of certain absorbance peaks primarily ν(C–N) and ν(C–S). The region 1480–1550 cm^{-1} is primarily associated with the R_2N–CSS 'thioureide' band in the infrared spectra of dithiocarbamate compounds which defines the carbon-nitrogen bond order between a single bond at 1250–1350 cm^{-1} and a double bond at 1640–1690 cm^{-1} [47].

The distinctive thioureide band, ν(C–N) was detected at 1456 cm^{-1}, 1481cm^{-1}, 1486 cm^{-1} and 1489 cm^{-1} in complexes (**1-4**) respectively. Since these frequency modes lie in between those associated with single C–N and double C=N bonds, hence the partial double bond character of 'thioureide' bond was confirmed for all gold(I) complexes [48]. The presence of the 'thioureide' band between 1545-1430 cm^{-1} suggest a considerable double bond character in the C…N bond vibration of the $S_2C–NR_2$ group [49]. A strong absorption in this region of the FTIR spectrum results into a strong signal of dithiocarbamato gold(I) complexes [50].

The C=S thiocarbonyl stretching splits into two peaks (doublet) with medium intensity at 1022 cm^{-1} and 972 cm^{-1}; 1099 cm^{-1} and 995 cm^{-1}; 1087 cm^{-1} and 982 cm^{-1}; and 1025 cm^{-1} and 970 cm^{-1} for complexes (**1-4**) respectively. The spectroscopic data suggests monodentate modes of coordination for the dithiocarbamate ligands in complexes (**1-4**) in analogy of compound $[(Ph_2P(CH_2)_2PPh_2)(AuS_2CNEt_2)_2$ [34].

In addition to the polar thioureide ion $S_2C=N^+R_2$ band, the common bands for sp^3 and sp^2 hybridized C–H stretches are observed within 2995–22917 cm^{-1} and above 3000 cm^{-1} respectively which are very comparable to those of sodium salt of diethyldithiocarbamate. In complexes (**1-4**), the stretch bands of aromatic (phenyl) and the saturated aliphatic C-H methyl group of coordinated dialkyl/diaryldithiocarbamate correspond above and below 3000 cm^{-1} [51].

The ^1H NMR chemical shifts of metal precursors [t-Bu_3PAuCl], $[(DPPM)(AuCl)_2]$ and free dialkyl/diaryldithiocarbamate ligands are given (Table 1S; supplementary materials). Small upfield and downfield shifts for the mono and bisphosphine coordinated ligands

protons have been observed for complexes (**1-4**); with respect to the chemical shifts of free metal precursor as given in synthesis part of experimental section for these complexes. In all four complexes slight downfield and upfield shifts for proton(s) of the coordinated dimethyl dithiocarbamate, diethyl dithiocarbamate and dibenzyldithiocarbamate have also been seen in gold(I) complexes **(1-4)** respectively in comparison to free dialkyl/diaryldithiocarbamate ligands (See Table 1; supplementary materials).

The ^{13}C and ^{31}P NMR chemical shifts of metal precursors [t-Bu_3PAuCl], [$(DPPM)(AuCl)_2$] and free dialkyl/diaryldithiocarbamate ligands (Table 2S; supplementary materials). The ^{13}C NMR spectra of complexes (**1-4**) showed many resonances as given in synthesis part of experimental section for these complexes. There is an up-field chemical shift of C=S carbon of coordinated dialkyldithiocarbamate with respect to free dialkyl/diaryldithiocarbamate ligands. The ^{13}C chemical shifts of C=S carbon of dimethyl thiocarbamate, diethyl thiocarbamate and dibenzyl thiocarbamate are observed in the range 206-210 ppm. The upfield shifts of C=S carbon are additional confirmations for the coordination of dialkyl/diaryl dithiocarbamates ligands in our synthesized complexes **(1-4)** [52].

UV-vis spectra of complexes (**1-4**) were monitored at room temperature for 3 days. The spectra were recorded just after mixing; and after 3 days are illustrated in Figure 1S (see supplementary). It is observed that the transitions remain relatively unmodified over a period of 3 days. These observations show substantial evidence for the stability of these gold(I) complexes (**1-4**) under the experimental conditions. However, slight changes in

the intensity of characteristic bands were noticed with time; without significant shift in absorption peak of spectra.

3.3. Crystal structure of complex [t-$Bu_3PAuS_2CN(C_7H_7)_2$] (1)

X-ray structure of [t-$Bu_3PAuS_2CN(C_7H_7)_2$] (**1**) is shown in Figure 1. In this structure, gold(I) is coordinated with one P donor atom of tri-tert-butylphosphine and S donor atom of dibenzyldithiocarbamate ligand molecules.

The Au–S and Au–P bond distances are 2.3365 (13) and 2.2824 (13) Å respectively. The Au–P and Au–S bond distances are comparable with [$Et_3PAu(S_2CNEt_2)$] complex [53]. The geometry around Au(I) metal atom is linear and similar to other analogous Au(I) complexes [54-57]. S–Au–P bond angle is 178.33 (5)° in the molecular structure of [t-$Bu_3PAuS_2CN(C_7H_7)_2$] (**1**) complex which is very close to angle of 180° for ideal linear geometry. Hence, the complex (**1**) shows s mall deviation from ideal linear geometry around gold(I) atom (Table 2) and confirms the presence of distorted linear geometry in this molecule.

3.4. *in vitro* Cytotoxicity of gold(I) complexes (1-4) in human colon, cervix and lung cancer cells

Human A549 lung cancer cells, human HeLa cervix cancer cells and human HCT15 colon cancer cells have been used to examine the *in vitro* cytotoxic activity of cisplatin, metal precursor (**0**) and the synthesized gold(I) complexes (**1-4**).

The concentration (dose) dependent *in vitro* cytotoxic effect was obtained by the specific increase in concentrations of cisplatin, gold(I) precursor (**0**) and gold(I) complexes **(1-4)**

against a panel of human cancer cells. The viability of cancer cells vs. concentrations of gold(I) complex is graphically presented in Figures 2-4**.** Gold(I) precursor (**0**) and synthesized complexes (**1-4**) invariably inhibited the proliferation of all cancer cells in a concentration dependent manner. Generally, the growth inhibition of cancer cells is higher for the synthesized complexes (**1-4**) in comparison to that of gold(I) precursor (**0**). Particularly, the degree of anti-proliferation of gold(I) of the synthesized complexes (**2** and **3**) is significantly greater than those of the synthesized complexes (**1** and **4**) as illustrated in Figures 2-4.

The IC_{50} values for cisplatin, gold(I) precursor (**0**) and complexes (**1-4**) against three cancer lines are given in Table 3. The IC_{50} data for the synthesized gold(I) complexes (**1-4**) against selected human cancer cell lines i.e. A549, HeLa and HCT15 are in the range of 1.43(0.42) to 133.10(3.62) μM.

It is clearly inferred from the IC_{50} values against A549 cell line that *in vitro* cytotoxicity of complexes **2** and **3** is significantly greater 15-25 times than gold(I) precursor (**0**) and 5-8 times than cisplatin respectively. A similar trend has been observed in HeLa cell line that *in vitro* cytotoxicity of complexes **2** and **3** in terms of IC_{50} is improved almost 75 folds than gold(I) precursor; and 12-15 folds than cisplatin respectively. In short, the order of *in vitro* cytotoxicity is (**2**) > (**3**) > cisplatin > (**1**) > (**4**) > precursor (**0**) against A549, HeLa and HCT15 cancer cell lines. It is pertinent to mention that the effectiveness trend of complexes (**2** and **3**) cytotoxicity against three cell lines is HeLa > A549 > HCT15. It can be concluded from this studies that complexes (**2** and **3**) are the most effective cytotoxic agents against HeLa cancer cell line. Against HeLa cell line

cytotoxicity of complexes (**2** and **3**) is better than the equivalent $Au(PEt_3)Cl$, as the following IC_{50}(μM) value against the same line show: Au(PEt3)Cl: 1.7(0.06) μM [58].

As far as, *in vitro* cytotoxicity against A549, HeLa and HCT15 cell lines is concerned, two out of four synthesized complexes (**2** and **3**) show much better anticancer activity than classical and well known anticancer drug cisplatin. The much better inhibition of growth of cancer cells by synthesized complexes than gold(I) precursor complex can be attributed to dithiocarbamate as labile co-ligands bonded with central gold(I) ions in synthesized complexes (**1-4**) by replacing chloride ions in these mononuclear and binuclear complexes.

As we know in drug design and discovery; selectivity and inhibition of target biomolecules is very important. In this regard our results are fruitful and very encouraging for further exploration of anticancer activity of gold(I) complexes. In short the IC_{50} values of gold(I) complexes (**2** and **3**) having dialkyldithiocarbamate ligands show much better cytotoxicity than gold(I) complexes (**1** and **4**) having diaryldithiocarbamate ligand. The lower cytotoxic activity of gold(I) complexes (**1** and **4**) is due to bulky size of dithiocarbamate ligand, this fact is well understood. The steric hindrance of bulky ligand makes the approach of gold(I) ions difficult to biomolecules in these complexes. Overall the anticancer activity of synthesized complexes against A549, HeLa and HCT15 human cancer cell lines are interesting and in μM range as found in previous anticancer studies of gold complexes [59-62].

4. Conclusions

In this study, the anticancer properties of four thiolate Au(I) derivatives with phosphine

ligands against three human cancer cell lines, HCT15, HeLa and A549 cell lines have been evaluated. Two complexes exhibit very strong cytotoxic effects *in vitro* against HCT15, HeLa and A549 cell lines especially complexes with the dimethyldithiocarbamate and diethyldithiocarbamate ligands which showed excellent cytotoxic activity against all tumorous cell lines. These gold(1) complexes are interesting examples of a group of Au(I) thiolate compounds, which contain the S–Au–P arrangement and have also attracted interest as potential anticancer agents. Structural changes like dithiocarbamate ligand are useful to increase the activity of the compounds, reaching the maximum value for complex (**2**). From this accumulated experience, this dinuclear complex seems to be the most promising compounds.

Gold(I) complexes (**1-4**) illustrates the inhibitory effect on the growth of all cancer cells in concentration dependent mode. The screening of the cytotoxic activity based on IC_{50} data against the HCT15 (human colon cancer cells) HeLa (human cervical cancer cells) and A549 (human lung carcinoma cells) lines based IC_{50} data shows that the compounds (**2** and **3**) are highly effective, particularly against HeLa and HCT15 cell lines. It shows an ability to circumvent the cellular resistance to cisplatin. When this ability is compared to the equivalent amount of cisplatin and gold(I) precursor complexes, we observe a much better behavior of the binuclear gold(I) complexes (**2** and **3**). The higher anticancer activity of gold(I) complexes than cisplatin is very encouraging and could be very useful impetus for anticancer drug discovery.

Supplementary material

Supplementary crystallographic data of CCDC deposit number is 994019 for the complex (**1)** and can be obtained free of charge *via* www.ccdc.cam.ac.uk/data_request/cif, by e-mailing data_request@ccdc.cam.ac.uk, or by contacting the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033.

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References

[1] B. Rosenberg, L. Van Camp and T. Krigas, Nature, 205 (1965) 698.

[2] N. Cutillas, G. S. Yellol, C. de Haro, C. Vicente, V. Rodriguez and J. Ruiz, Coord. Chem. Rev., 257 (2013) 2784.

[3] Ž. D. Bugarčić, J. Bogojeski, B. Petrović, S. Hochreuther and R. Van Eldik, *Dalton. Trans.*, 41 (2012) 12329.

[4] C. Vetter, C. Wagner, J. Schmidt and D. Steinborn, Inorg. Chim. Acta, 359 (2006) 4326.

[5] A. Casini and L. Messori, Curr. Top. Med. Chem. 11 (2011) 2647.

[6] E. Márta Nagy, L. Ronconi, C. Nardon and D. Fregona, Mini-Reviews in Med. Chem., 12 (2012) 1216.

[7] S. Ahmad, A. A. Isab and S. Ali, Transition Met. Chem., 31 (2006) 1003.

[8] S. R. McWhinney, R. M. Goldberg and H. L. McLeod, Mol. Cancer Ther., 8 (2009) 10.

[9] W. Liu and R. Gust, Chem. Soc. Rev. 42 (2013) 755.

[10] X. Yao, K. Panichpisal, N. Kurtzman, and K. Nugent, Am. J. Med. Sci., 334 (2007) 115.

[11] S. S. Al-Jaroudi, M. Monim-ul-Mehboob, M. Altaf, M. Fettouhi, M. I. M. Wazeer, S. Altuwaijri and A. A. Isab, New J. Chem., 38 (2014) 3199.

389 [12] S. M. Janković, A. Djeković, Ž. D. Bugarčić, S. V. Janković, G. Lukić, M. Folic
390 and D. Čanović, Biometals, 25 (2012) 919.

391 [13] S. S. Al-Jaroudi, M. I. M. Wazeer, A. A. Isab and S. Altuwaijri, Polyhedron, 50
392 (2013) 434.

393 [14] R. B. Bostancioglu, K. Isik, H. Genc, K. Benkli and A. T. Koparal, J. Med.
394 Chem., 27 (2012) 458.

395 [15] O. Crespo, V. V. Brusko, M. C. Giameno, M. L. Tornil, A. Laguna and N. G.
396 Zabirov, Eur. J. Inorg. Chem., (2004) 423.

397 [16] K. Nomiya, R. Noghuchi and M. Oda, Inorg. Chim. Acta, 298 (2000) 24.

398 [17] H.-Q. Liu, T.-C. Cheung, S.-M. Peng and C.-M. Che, J. Chem. Soc., Chem.
399 Comm., (1995) 1787.

400 [18] C. J. O'Connor and E. Sinn, Inorg. Chem., 17 (1978) 2067.

401 [19] M. A. Cinellu, G. Minghetti, M. V. Pinna, S. Stoccoro, A. Zucca, and M.
402 Manassero, J. Chem. Soc., Dalton Trans., (1998) 1735.

403 [20] S. H. van Rijt and P. J. Sadler, Drug Discovery Today, 14 (2009) 1089.

404 [21] R. Noghuchi, A. Hara, A. Sugie and K. Nomiya, Inorg. Chem. Comm., 9 (2006)
405 355.

406 [22] K. Nomiya, R. Noghuchi, K. Ohsawa, K. Tsuda and M. Oda, J. Inorg. Biochem.,
407 78 (2000) 363.

[23] B. P. Howe, Met.-Based Drugs., 4 (1997) 273.

[24] V. J. Ctalano and A. O. Etogo, J. Organomet. Chem., 690 (2005) 6041.

[25] J. C. Lima and L. Rodríguez, J. Med. Chem., 11 (2011) 921.

[26] C-M. Che and R. W-Y. Sun, Chem. Commun., 47 (2011) 9554.

[27] P. Calami, A. Carotti, T. Guerri, L. Messori, E. Mini, P. Orioli and G. P. Speroni, J. Inorg. Biochem., 66 (1997) 103.

[28] R. K. Johnson, C. K. Mirabelli, L. F. Faucette, F. L. McCabe, B. M. Sutton, D. L. Bryan, G. R. Girard and D. T. Hill, Proc. Amer. Assoc. Cancer Res., 26 (1985) 254.

[29] C. K. Mirabelli, L. F. Faucette, F. L. McCabe, B. M. Sutton, D. L. Bryan, G. R. Girard, D. T. Hill, J. O. Bartus, S. T. Crooke and R. K. Johnson, J. Med. Chem., 30 (1987) 2181.

[30] S. J. Berners-Price, M. A. Mazid and P. J. Sadler, J. Chem. Soc., Dalton Trans., (1984) 969.

[31] S. J. Berners-Price and P. J. Sadler, Inorg. Chem., 25 (1986) 3822.

[32] D. T. Hill, G. R. Girard, U. S. Patent 4755611, July 1988.

[33] G. F. Rush, D. W. Albers, P. Meunies, K. Leffler, P. F. Smith, Toxicologist, 7 (1987) 59.

[34] J. W. Faamaua and E. R.T. Tiekinka, J. Coord. Chem., 31(2) (1994) 93.

[35] L. Ronconi, L. Giovagnini, C. Marzano, F. Bettio, R. Graziani, G. Pilloni, and D. Fregona, Inorg. Chem., 44 (2005) 1867.

[36] V. Milacic, D. Chen, L. Ronconi, K. R. Landis-Piwowar, D. Fregona and Q. P. Dou, Cancer Res., 66 (2006) 10478.

[37] X. Zhang, M. Frezza, V. Milacic, L. Ronconi, Y. Fan, C. Bi, D. Fregona and Q. P. Dou, J. Cell Biochem., 109 (2010) 162.

[38] S. Ahmad, A. A. Isab, S. Ali and A. R. Al-Arfaj, Polyhedron, 25 (2006) 1633.

[39] D. V. Partyka, T. J. Robilotto, M. Zeller, A. D. Hunter, T. G. Gray, Proc. Natl. Acad. Sci., (U S A) 105 (2008) 14293.

[40] Y. Wang, Q.-Y. He and C.-M. Che, J.-F. Chiu, Proteomics, 6 (2006) 131.

[41] Y. Shi, W. Chu, Y. Wang, S. Wang, J. Du, J. Zhang, S. Li, G. Zhou, X. Qin and C. Zhang, Inorg. Chem. Comm., 30 (2013) 178.

[42] M. Monim-ul-Mehboob, M. Altaf, M. Fettouhi, A. A. Isab, M. I. M. Wazeer, M. N. Shaikh and S. Altuwaijri, Polyhedron, 61 (2013) 225.

[43] Stoe & Cie, X-Area & X-RED32, GmbH, Darmstadt, Germany, (2009).

[44] G. M. Sheldrick, Acta Cryst., A64 (2008) 112.

[45] A. L. Spek, Acta Cryst., D65 (2009) 148.

[46] C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J.van de Streek and P. A. Wood, J. Appl. Cryst., 41 (2008) 466.

[47] A. J. Odola and J. A. O. Woods, J. Chem. Pharm. Res., 3 (2011) 865.

[48] F. Jian, Z. Wang, Z. Bai, X. You, H. Fun, K. Chinnakali and L. A. Razak, Polyhedron, 18 (1999) 3401.

[49] A. Jayaraju, M. M. Ahamad, R. M. Rao, J. Sreeramulu, Der Pharma Chemica, 4 (2012) 1191.

[50] J. Chatt, L. A. Duncanson and L. M. Venanzi, Nature, 177 (1956)1042.

[51] C. J. Pouchert, Aldrich Library of FT-IR Spectra, 2nd ed., Aldrich Chemical Company, Milwaukee, 1 (1997).

[52] M. Altaf, M. Monim-ul-Mehboob, A. A. A. Seliman, A. A. Isab, V. Dhuna, G. Bhatia and K. Dhuna, J. Organomet. Chem., 765 (2014) 68.

[53] S. Y. Ho and E. R. T. Tiekink, Z. Kristallogr, 220 (2005) 342.

[54] I. Sänger, H.-W. Lerner, T. Sinke and M. Bolte, Acta Cryst., E68 (2012) m708.

[55] P. Lu, T. C. Boorman, A. M. Z. Slawin and I. Larrosa, J. Am. Chem. Soc., 132 (2010) 5580.

[56] R. E. Marsh, Acta Cryst., B58 (2002) 893.

[57] H. Schmidbaur, B. Brachthiuser, O. Steigelmann, and H. Beruda, Chem. Ber., 125 (1992) 2705.

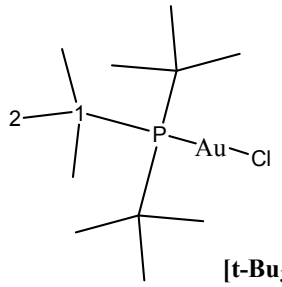
[58] E. Barreiro, J. S. Casas, M. D. Couce, A. Sánchez, A. Sánchez-Gonzalez, J. Sordo, and E. M. Vázquez-López, J. Inorg. Biochem., 138 (2014) 89.

[59] E. Barreiro, J. S. Casas, M. D. Couce, A. Sánchez, J. Sordo and E. M. Vázquez-López, J. Inorg. Biochem., 131 (2014) 68.

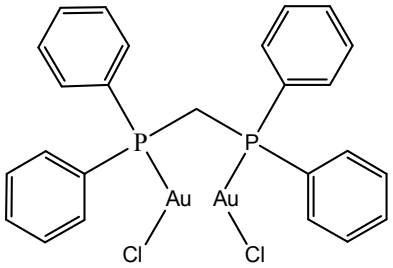
[60] R. Kivekäs, E. Colacio, J. Ruiz, J. D. López-González and P. León, Inorg. Chim. Acta., 159 (1989) 103.

[61] L. Ortego, F. Cardoso, S. Martins, M. F. Fillat, A. Laguna, M. Meireles, M. D. Villacampa and M. C. Gimeno, J. Inorg. Biochem., 130 (2014) 32.

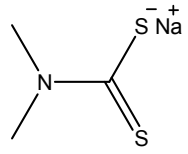
[62] I. Ott, T. Koch, H. Shorafa, Z. Bai, D. Poeckel, D. Steinhilber and R. Gust, Org. Biomol. Chem., 3 (2005) 2282.



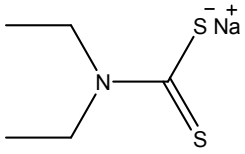
[t-Bu$_3$PAuCl]



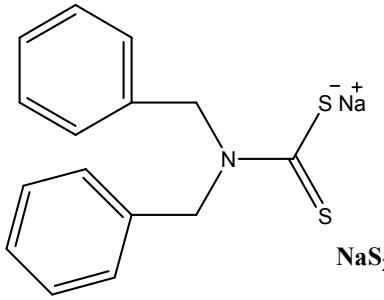
[(DPPM)(AuCl)$_2$]



NaS$_2$CN(CH$_3$)$_2$



NaS$_2$CN(C$_2$H$_5$)$_2$



NaS$_2$CN(C$_7$H$_7$)$_2$

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478 **Scheme 1** Skeletal structures and condensed formulae of precursor and dithiocarbamate
479 ligands for ^{13}C and ^{1}H NMR data.

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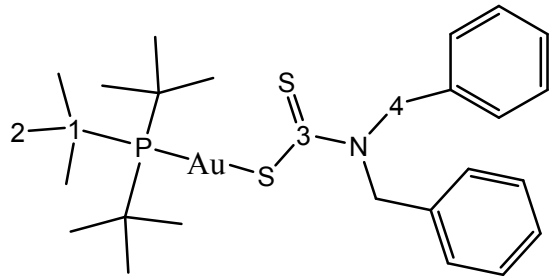
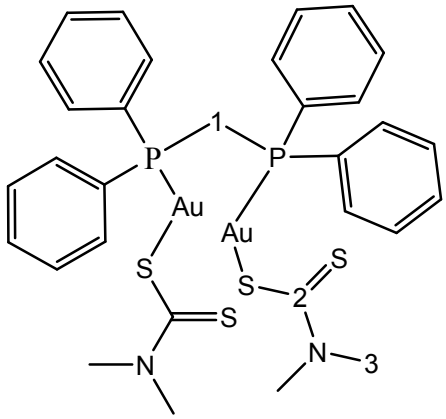
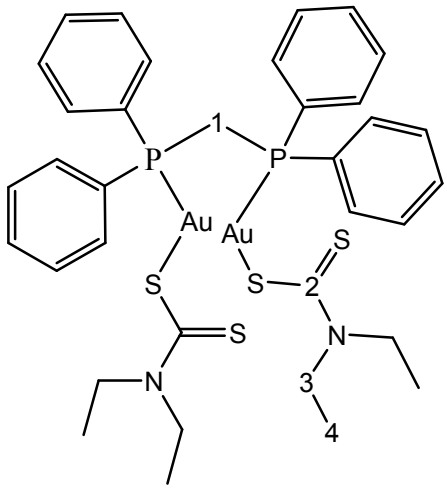
Complex [t-$Bu_3PAuS_2CN(C_7H_7)_2$]



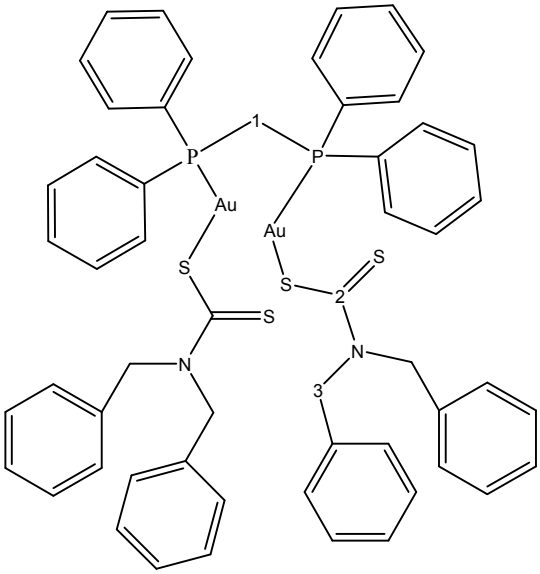
Complex [(DPPM)$Au_2(S_2CN(CH_3)_2)_2$]



Complex [(DPPM)$Au_2(S_2CN(C_2H_5)_2)_2$]



Complex $[(DPPM)Au_2(S_2CN(C_7H_7)_2)_2]$



Scheme 2 Skeletal structures and condensed formulae of complexes (**1-4**); representing non-equivalent carbons and protons for ^{1}H and ^{13}C NMR data.

509 **Table 1 Crystallographic characteristics, experimental and structure refinement**
510 **details for crystal structure of complex (1).**

Parameters	[t-$Bu_3PAuS_2CN(C_7H_7)_2$]
Empirical formula	$C_{27}H_{41}AuNPS_2$
Empirical formula weight	671.66
Crystal size/mm	0.45 × 0.30 × 0.07
Wavelength/Å	0.71073
Temperature/K	173
Crystal symmetry	Orthorhombic
Space group	Pbca
a/Å	12.3157 (12)
b/Å	19.6569 (19)
c/Å	22.945 (2)
V/ $Å^3$	6375.6 (4)
Z	8
D_c/Mg m^{-3}	1.606
μ(Mo-Kα)/mm^{-1}	5.52
F(000)	2688
θ Limits/°	1.8–26.2
Collected reflections	17654
Unique reflections	3454
Observed reflections	5296
Goodness of fit on F^2	0.79
$R_1[F^2 > 2\sigma(F^2)]$	0.028
w$R_2(F^2)$	0.062
Largest diff. peak, hole/e $Å^{-3}$	1.08, −0.79

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518 **Table 2 Selected bond distances (Å) and bond angles (°) for complex (1).**
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Bond Length (Å)		Bond Angles (°)	
Au1—P1	2.2824 (13)	P1—Au1—S1	178.33 (5)
Au1—S1	2.3365 (13)	C13—S1—Au1	100.26 (16)
S1—C13	1.749 (5)	C5—P1—C1	110.4 (2)
S2—C13	1.701 (5)	C5—P1—C9	110.4 (2)
		C1—P1—C9	109.7 (2)
		C5—P1—Au1	110.40 (16)
		C1—P1—Au1	106.82 (18)
		C9—P1—Au1	109.03 (19)

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Table 3 IC_{50} data (μM) of cispaltin and gold(I) complexes (0-4) against A549, HeLa and HCT15 cancer cell lines.

	IC_{50}(μM)		
Complex	**A549**	**HeLa**	**HCT15**
Cisplatin	41.67(1.17)	19.20(1.81)	29.67(2.35)
$[(DPPM)(AuCl)_2]$(**0**)	136.33(3.17)	108.73(3.06)	148.90(3.54)
(**1**)	96.53(3.25)	25.90(1.77)	93.67(2.34)
(**2**)	5.80(1.93)	1.43(0.42)	9.53(1.35)
(**3**)	9.10(1.61)	1.63(0.45)	11.97(1.66)
(**4**)	105.30(3.68)	93.87(3.15)	133.10(3.62)

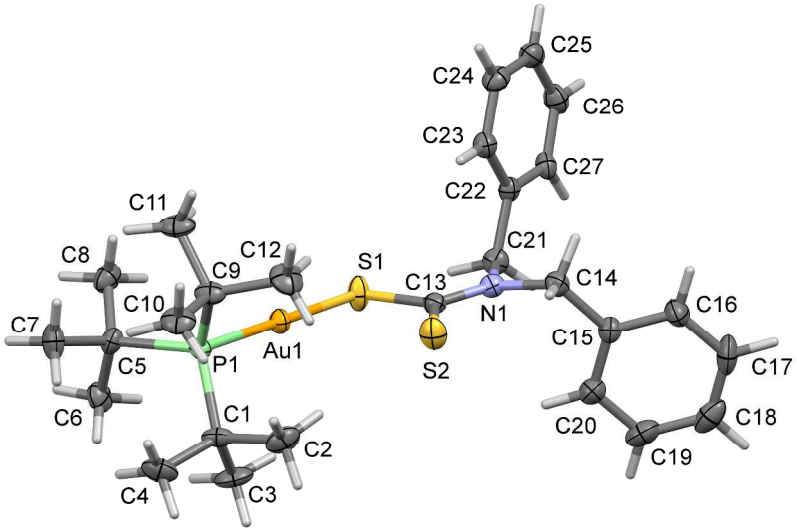


Figure 1 A view of the molecular structure of complex (**1**) with atom labeling. The displacement ellipsoids are drawn at the 50% probability level.

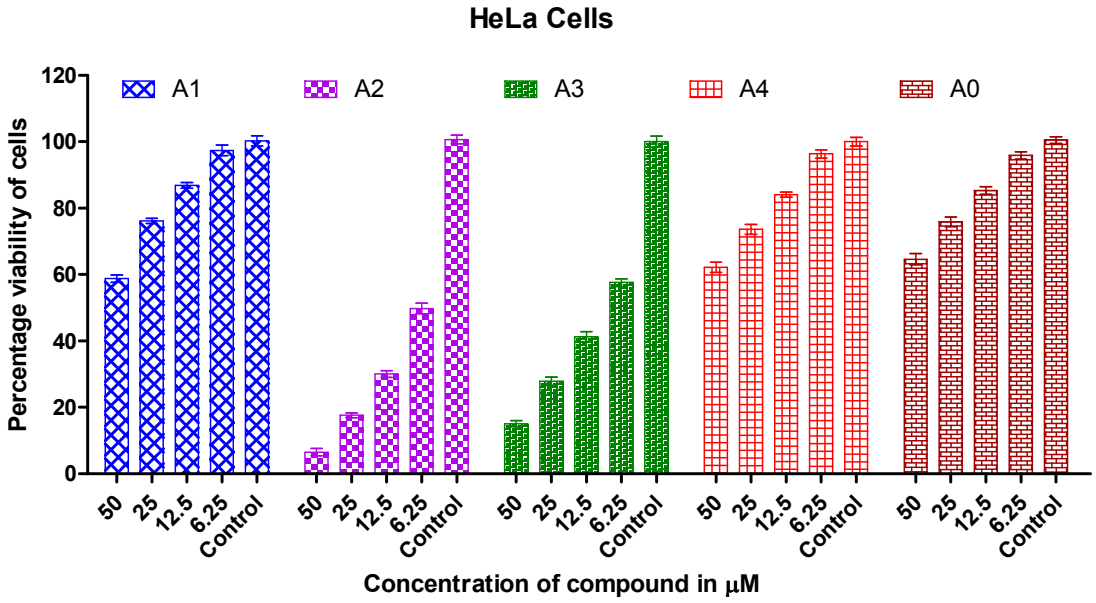
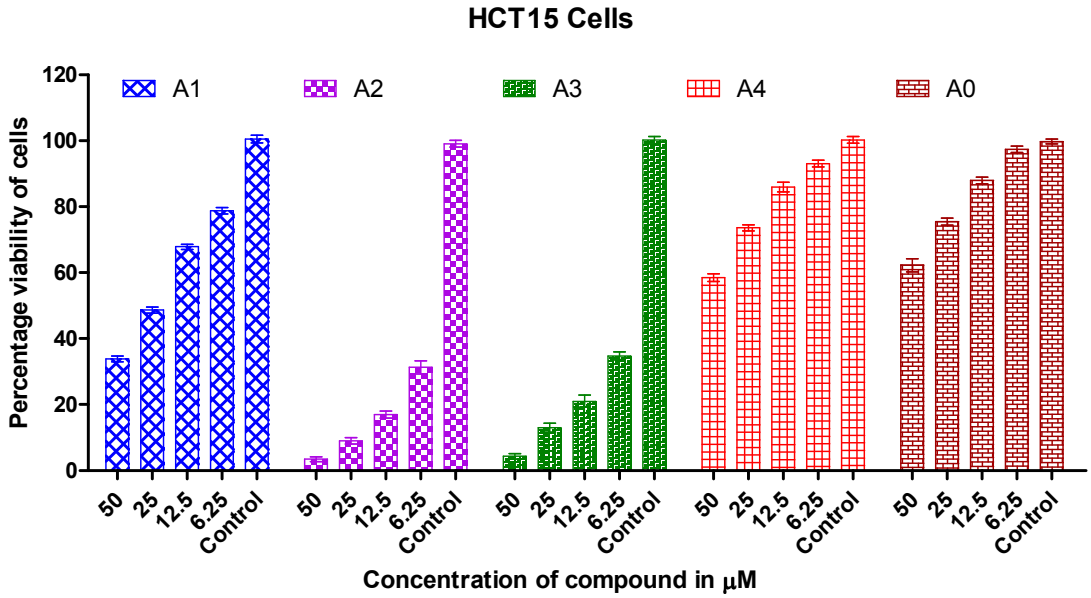


Figure 2 Graph showing the concentration dependent *in vitro* cytotoxic effect of complexes (**0-4**) on viability of HeLa cancer cells.



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553 **Figure 3** Graph showing the concentration dependent *in vitro* cytotoxic effect of
554 complexes (**0-4**) on viability of HCT15 cancer cells.

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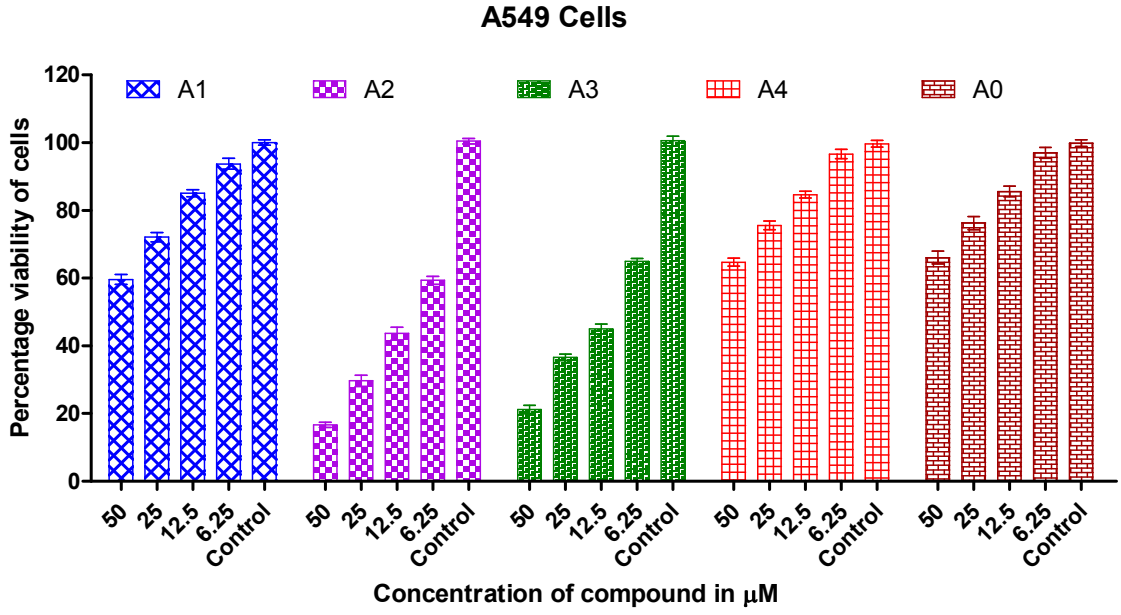

556

557 **Figure 4** Graph showing the concentration dependent *in vitro* cytotoxic effect of
558 complexes (**0-4**) on viability of A549 cancer cells.

559