Synthesis, crystal structure and spectral studies of silver(I) cyclohexyldiphenylphosphine complexes: towards the biological evaluation on malignant and non-malignant cells

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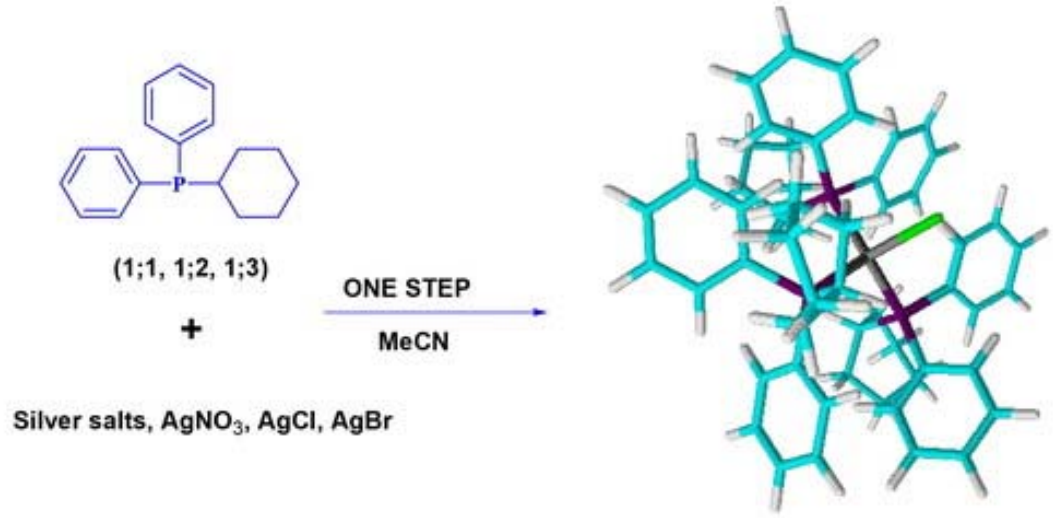
Abstract

Cancer is a fast-growing disease responsible for many deaths worldwide. Due to its aggressive and forever-changing nature, it is imperative to find alternative anti-cancer agents that could possibly treat this disease. Silver(I) phosphine complexes were synthesized by reaction of $AgNO_3$, AgCl, and AgBr with cyclohexyldiphenylphosphine to produce 1:1, 1:2, or 1:3 molar ratios of new silver complexes with the formulas $[Ag(PPh_2Cy)NO_3]$ (**1**), $[Ag(PPh_2Cy)_2NO_3]$ (**2**), $[Ag(PPh_2Cy)_3NO_3]$ (**3**), $[Ag(PPh_2Cy)_2Cl]$ (**4**), $[Ag(PPh_2Cy)_3Cl]$ (**5**), and $[Ag(PPh_2Cy)_3Br]$ (**6**), respectively. The complexes were characterized by elemental analyses, FT-IR and ^1H, ^{13}C, ^{31}P NMR spectroscopic techniques. The crystal structures of **5** (CCDC 1480482) and **6** (CCDC 2183297) were determined by single-crystal X-ray diffractometry. All six complexes were evaluated as potential anti-cancer agents in four different human malignant (SNO, MCF-7, A375, and A549) cell lines and one human non-malignant (HEK293) cell line. Overall, these complexes were significantly cytotoxic to both cancerous and non-cancerous cells and are therefore not considered suitable anti-cancer agents in their current form.



Keywords: Silver(I) complexes; crystal structure; cyclohexyldiphenylphosphine; anti-cancer activity; cytotoxicity

1. Introduction

Cancer is the second leading cause of death worldwide [1]. It was estimated that in 2020 around 19 million new cases and 10 million deaths were collectively reported globally. These numbers are projected to be more than double by 2040 [2]. Therefore, it is imperative to find new novel anti-cancer agents for the treatment thereof. The medicinal uses and applications of metals and metal complexes are of increasing clinical and commercial importance [3,4]. Metals and metal complexes are extensively used in cancer therapy as diagnostic tools or treatment strategies for cancer [5,6]. Since the discovery of cisplatin as an anti-cancer agent, several other metal complexes have been screened for anti-cancer activity including platinum analogues of cisplatin [7–11], zinc(II) [12–14], copper(II) [15,16], gold [17–21], ruthenium [22–25], iron [26], cobalt [27], and silver(I) complexes [28–35]. However, cisplatin and some potent anti-cancer agents cause severe side effects emanating from dose-dependent toxicity and resistance as well as having a narrow spectrum of activity [36–39]. Therefore, it is crucial to find an anti-cancer agent that is effective enough to destroy cancer cells without causing any side effects.

The search for anti-cancer agents shifted focus toward other metal complexes such as silver(I) phosphine complexes [40]. It is known that silver(I) complexes can form many stable geometries. The geometry of these complexes depends on the metal-to-ligand ratios, the counter ion present, and the solvent being used [41–45]. Tertiary phosphine complexes of silver(I) of the type $[AgXL_n]$, where L = tertiary phosphine, n = 1–4, and X = coordinating or non-coordinating anion, were first prepared as early as 1937, and several publications have resulted on this topic [46–50]. The reaction of silver(I) salts with monodentate tertiary phosphines in a 1:2 stoichiometric ratio generally results in the formation of either monomeric $[AgX(PR_3)_2]$/$[Ag(PR_3)_2]X$ [51–61] or dimeric complexes $[AgX(PR_3)_2]_2$ [61–63]

depending on the donor properties of the phosphine ligand, the bulkiness of the ligand, and the donor properties of the anion. The metal centers in the majority of the neutral $[AgX(PR_3)_2]$ and $[AgX(PR_3)_2]_2$ complexes are predominantly four coordinate, with the anion acting as either a bidentate chelating ligand or as a bridging ligand. Two or three coordination have been found only when the anion is a weak donor or the substituents on the phosphine ligand are bulky [48, 55, 58–61].

Such silver(I) complexes exhibit a wide range of applications in medicine and the chemical industry. Some silver-containing agents, including silver phosphine complexes [28–35, 64,65], silver carbene complexes [66], bidentate silver compounds [67], and silver(I) imidazole [68] complexes, have all been reported as potential anti-cancer agents following *in vitro* assessments.

To date, there are no reported cases of complexes synthesized using $AgNO_3$, AgCl, AgBr, and cyclohexyldiphenylphosphine complexes, which have been studied as effective anti-cancer agents. Herein is reported the synthesis, crystallography, and spectroscopy characterization of six new silver(I) cyclohexyldiphenylphosphine complexes, $[Ag(PPh_2Cy)NO_3]$ (**1**), $[Ag(PPh_2Cy)_2NO_3]$ (**2**), $[Ag(PPh_2Cy)_3NO_3]$ (**3**), $[Ag(PPh_2Cy)_2Cl]$ (**4**), $[Ag(PPh_2Cy)_3Cl]$ (**5**), and $[Ag(PPh_2Cy)_3Br]$ (**6**). All six complexes were tested for their anti-cancer activity in malignant and non-malignant cell models.

2. Experimental

2.1. Materials and instrumentation

All chemicals used to synthesize the silver(I) phosphine complexes were purchased from Sigma–Aldrich and used as received. The chemicals include cyclohexyldiphenylphosphine (95%), silver salts (99%), acetonitrile (HPLC grade, 99.9%), deuterated chloroform ($CDCl_3$) (99.8%) and deuterated dimethyl sulfoxide $[(CD_3)_2SO]$ (99.9%). The purity of the solvent and chemicals were determined by gas chromatography (GC), and nuclear magnetic resonance spectroscopy (NMR) or Fourier transformed infrared spectroscopy (FTIR), respectively.

All NMR measurements (^{1}H-, ^{13}C{H}- and ^{31}P{H}-NMR) were carried out using a Bruker Ultrashield Avance III 500 MHz spectrometer with a B-ACS 60 auto-sampler. The NMR spectra were recorded at room temperature, operating at 500 MHz for ^{1}H-NMR, 125.8 MHz for ^{13}C{H}-NMR and 202.5 MHz for ^{31}P{H}-NMR. Deuterated solvents used include $CDCl_3$ and $(CD_3)_2SO$. The signals are recorded in parts per million (ppm). The peaks were referenced to the internal residual *protio* impurities or solvent reference signals in $CDCl_3$ and $(CD_3)_2SO$; this includes δ = 7.24 and 2.49 ppm for ^{1}H, and δ = 77.0 and 39.5 ppm for ^{13}C, respectively. The data are reported as follows: the chemical shift in ppm (δ) and multiplicity: singlet (s); doublet (d); triplet (t); quartet (q); multiplet (m); doublet of doublets (dd) and double of triplets (dt), *J*-coupling (where necessary), integration of protons and functional group.

Infrared spectra were recorded on a Shimadzu IRAffinity-1S with wavelength 7800–350 cm^{-1} (0.5–16 cm^{-1} resolution). A scanning region between 4000 and 500 cm^{-1} was used, and 16 scans were used for each measurement. Solid samples were crushed using the pin accessories and analyzed. The following abbreviations were used to describe the peaks: s –

strong, m – medium, w – weak. Melting points were determined by using either a melting point apparatus (Stuart Scientific Melting Point apparatus SMP10) or differential scanning calorimeter (DSC) and are uncorrected. Elemental analysis was conducted on a Thermo Scientific Flash2000 elemental analyser.

2.2. Synthesis and characterization of complexes

2.2.1. Synthesis of cyclohexyldiphenylphosphine silver(I) nitrate (1)

Solid $AgNO_3$ (0.1207 g, 0.71 mmol) was added to a solution of cyclohexyldiphenylphosphine (0.1905 g, 0.71 mmol) in acetonitrile (50 mL). The mixture was heated under reflux until all the reagents had dissolved. The solution was filtered while hot, and the solvent was reduced to 20 mL. The solution was allowed to cool to room temperature, after which colorless crystals were obtained. Melting point of 150–152 °C. IR (Solid): ν_{max} (cm^{-1}): 2924.09, 2846.93 (ν (=C–H), w), 1427.32, 1280.73 (ν (C = C aromatic), m), 1095.57–810.10 (ν (aromatic, C–H bend, meta), s), 740.67, 694.37 (ν (aromatic, C–H bend, ortho), m) 509.21, 486.06. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 1.201–1.691 (m, cyclohexyl), 7.501–7.755 (m, phenyl). ^{13}C{H}-NMR (100 MHz, $CDCl_3$) δ ppm:, 28.82 (s, cyclohexyl), 25.59 (s, cyclohexyl), 133.71 (d, aromatic), 131.08 (d, aromatic), and 129.12 (d, aromatic). ^{31}P{H} NMR (161 MHz, $CDCl_3$) δ ppm: 21.94, 25.50 (d). Anal. Calcd. for $C_{18}H_{21}AgNO_3P$: C, 49.34%; H, 4.83%; N, 3.20%. Found: C, 49.20%; H, 4.71%; N, 3.08%.

2.2.2. Synthesis of bis(cyclohexyldiphenylphosphine) silver(I) nitrate (2)

Solid $AgNO_3$ (0.1207 g, 0.71 mmol) was added to a solution of cyclohexyldiphenylphosphine (0.3810 g, 1.42 mmol) in acetonitrile (50 mL). The solution was heated under reflux overnight. The solution was filtered hot, and the solvent was reduced to ∼10 mL by means of evaporation. The solution was left to crystallize from which small white needles were isolated. Melting point of 170–172 °C. IR (Solid): ν_{max} (cm^{-1}): 2924.09, 2846.93 (ν (alkane, C–H, stretch), asymm, w), 1396.46 (ν (C = C aromatic), m), 1296.16 (s), 1180.44–817.82 (ν (aromatic, C–H bend, meta), s), 740.67, 694.37 (ν (aromatic, C–H bend, ortho), m), 509.21, 486.06 (s). ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 10.996–2.746 (cyclohexyl), 7.481–7.924 (m, phenyl). ^{13}C{H}-NMR (100 MHz, $CDCl_3$) δ ppm: 25.53 (s, cyclohexyl), 25.75 (d, cyclohexyl), 28.94 (s, cyclohexyl), 128.98 (d, aromatic), 130.81 (s, aromatic), 131.09 (s, aromatic), 131.27 (s, aromatic), 133.81 (d, aromatic). ^{31}P{H} NMR (161 MHz, $CDCl_3$) δ ppm: 17.24 (s). Anal. Calcd. for $C_{36}H_{42}AgNO_3P_2$: C, 61.20%; H, 5.99%; N, 1.98%. Found: C, 60.82%; H, 5.97%; N, 2.02%.

2.2.3. Synthesis of tris(cyclohexyldiphenylphosphine) silver(I) nitrate (3)

Solid $AgNO_3$ (0.1207 g, 0.71 mmol) was added to a solution of cyclohexyldiphenylphosphine (0.5712 g, 2.13 mmol) in acetonitrile (50 mL). The solution was heated under reflux overnight. The solution was filtered hot and the solvent was reduced to ∼10 mL by means of evaporation. The solution was left to crystallize from which small white needles were isolated. Melting point of 212–214 °C. IR (Solid): ν_{max} (cm^{-1}): 2924.09, 2846.93 (ν (alkane, C–H, stretch), asymm, w), 1435.04, 1311.59 (ν (C = C aromatic), m), 1180.44–848.68 (ν (aromatic, C–H bend, meta), s), 732.95, 694.37 (ν (aromatic, C–H bend, ortho), m), 501.49,

462.92 (s). ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 0.964-1.025 (m, cyclohexyl), 1.176–1.252 (m, cyclohexyl), 1.570 (d, cyclohexyl), 7.390 (t, phenyl), 7.455 (t, phenyl), 7.549 (t, phenyl), and 7.817 (t, phenyl). ^{13}C{H}-NMR (100 MHz, $CDCl_3$) δ ppm: 25.55 (s, cyclohexyl), 25.91 (d, cyclohexyl), 28.91 (d, cyclohexyl), 34.07 (d, cyclohexyl), 128.86 (d, phenyl), 130.34 (s, phenyl), 132.02 (d, phenyl), 133.58 (d, phenyl). ^{31}P{H} NMR (161 MHz, $CDCl_3$) δ ppm: 12.10 (s). Anal. Calcd. for $C_{54}H_{63}AgNO_3P_3$: C, 66.53%; H, 6.51%; N, 1.44%; Found: C, 65.54%; H, 6.65%; N, 1.41%.

2.2.4. Synthesis of bis(cyclohexyldiphenylphosphine) silver(I) chloride (4)

Solid AgCl (0.1018 g, 0.71 mmol) was added to a solution of cyclohexyldiphenylphosphine (0.3810 g, 1.42 mmol) in acetonitrile (50 mL). The solution was heated under reflux overnight. The solution was filtered hot and the solvent was reduced to ∼10 mL by means of evaporation. The solution was left to crystallize from which small white needles were isolated. Melting point of 165–167 °C. IR (Solid): ν_{max} (cm^{-1}): 2924.09, 2846.93 (ν (alkane, C–H, stretch), asymm, w), 1481.33, 1435.04 (ν (C = C aromatic), m), 1180.44-848.68 (ν (aromatic, C–H bend, meta), s), 740.67, 694.37 (ν (aromatic, C–H bend, ortho), m), 510.49 (s). ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 0.956 (s, cyclohexyl), 1.224 (d, cyclohexyl), 1.546 (t, cyclohexyl), 2.062 (s, cyclohexyl), 2.640 (d, cyclohexyl), 7.384 (t, phenyl), 7.437 (t, phenyl), 7.512 (d, phenyl), and 7.786 (t, phenyl). ^{13}C{H}-NMR (100 MHz, $CDCl_3$) δ ppm: 25.42 (s, cyclohexyl), 25.84 (d, cyclohexyl), 28.71 (d, cyclohexyl), 34.02 (d, cyclohexyl), 128.79 (d, phenyl), 130.38 (s, phenyl), 130.67 (d, phenyl), 132.04 (d, phenyl), 133.75 (d, phenyl) ^{31}P{H} NMR (161 MHz, $CDCl_3$) δ ppm: 13.35 (s). Anal. Calcd for $C_{36}H_{42}AgClP_2$: C, 63.59%; H, 6.23%; Found: C, 63.91%; H, 6.29%.

2.2.5. Synthesis of tris(cyclohexyldiphenylphosphine) silver(I) chloride (5)

Solid AgCl (0.1018 g, 0.71 mmol) was added to a solution of cyclohexyldiphenylphosphine (0.5715 g, 2.13 mmol) in acetonitrile (50 mL). The solution was heated under reflux overnight. The solution was filtered hot and the solvent was reduced to ∼10 mL by means of evaporation. The solution was left to crystallize from which small white needles were isolated. Melting point of 190-192 °C. IR (Solid): ν_{max} (cm^{-1}): 2924.09, 2846.93 (ν (alkane, C–H, stretch), asymm, w), 1481.33, 1427.32 (ν (C = C aromatic), m), 995.27, 848.68 (ν (aromatic, C–H bend, meta), s), 740.67, 694.37 (ν (aromatic, C–H bend, ortho), m) 501.49, 462.92. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 1.145–1.243 (m, cyclohexyl), 1.557 (t, cyclohexyl), 2.065 (s, cyclohexyl), 2.521 (s, cyclohexyl), 7.354–7.429 (m, phenyl), 7.501–7.534 (m, phenyl), 7.682 (t, phenyl), and 7.804–7.838 (m, phenyl). ^{13}C{H}-NMR (100 MHz, $CDCl_3$) δ ppm: 24.34 (s, cyclohexyl), 25.48 (s, cyclohexyl), 25.86 (d, cyclohexyl), 28.75 (d, cyclohexyl), 34.08 (d, cyclohexyl), 128.64 (d, phenyl), 129.89 (s, phenyl), 130.62 (d, phenyl), 131.40 (d, phenyl), 133.15 (d, phenyl), 133.59 (d, phenyl). ^{31}P{H} NMR (161 MHz, $CDCl_3$) δ ppm: 8.68 (s). Anal. Calcd. for $C_{54}H_{63}AgClP_3$: C, 68.39%; H, 6.70%; Found: C, 69.03%; H, 6.73%.

2.2.6. Synthesis of tris(cyclohexyldiphenylphosphine) silver(I) bromide (6)

Solid AgBr (0.1334 g, 0.71 mmol) was added to a solution of cyclohexyldiphenylphosphine (0.5715 g, 2.13 mmol) in acetonitrile (50 mL). The solution was heated under reflux overnight. The solution was filtered hot and the solvent was reduced to ∼10 mL by means of

evaporation. The solution was left to crystallize from which small white needles were isolated. Melting point of 170–172 °C. IR (Solid): ν_{max} (cm^{-1}): 2924.09, 2846.93, 2322.29 (ν (alkane, C–H, stretch), asymm, w), 1481.33, 1427.32 (ν (C = C aromatic), m), 1087.85, 995.27 (ν (aromatic, C–H bend, meta), s), 740.67, 694.37 (ν (aromatic, C–H bend, ortho), m), 501.49, 462.92 (s). ^{1}H-NMR (400 MHz, $CDCl_3$) δppm: 1.150–1.904 (m, cyclohexyl), 2.064 (s, cyclohexyl), 2.588 (s, cyclohexyl), 7.359–7.436 (m, phenyl), 7.506–7.536 (m, phenyl), 7.715 (t, phenyl), and 7.820 (t, phenyl). ^{13}C{H}-NMR (100 MHz, $CDCl_3$) δ ppm: 24.34 (s, cyclohexyl), 25.49 (d, cyclohexyl), 25.86 (d, cyclohexyl), 28.66 (d, cyclohexyl), 128.64 (s, phenyl), 128.71 (d, phenyl), 130.20 (s, phenyl), 130.64 (d, phenyl), 131.45 (s, phenyl), 132.27 (s, phenyl), 133.68 (d, phenyl). ^{31}P{H} NMR (161 MHz, $CDCl_3$) δ ppm: 9.37 (s). Anal. Calcd. for $C_{54}H_{63}AgBrP_3$: C, 65.33%; H, 6.40%; Found: C, 65.29%; H, 6.39%.

2.3. Single-crystal XRD analysis

Suitable crystals of **5**, **6**, and **5'** obtained were harvested for single-crystal X-ray diffraction (SXRD) analysis. All measurements were made on a Rigaku XtaLAB Synergy R diffractometer with a rotating-anode X-ray source and a HyPix CCD detector. Data reduction and absorption were carried out using the CrysAlisPro (version 1.171.40.23a) software package [69]. All X-ray diffraction measurements were performed at 150.0(1) K using an Oxford Cryogenics Cryostat. All structures were solved by direct methods with SHELXT-2013 [70] and refined using the SHELXL-2013 [71] algorithm. All H atoms were placed in geometrically idealized positions and constrained to ride on their parent atoms. For data collection and refinement parameters, see Tables S1 and S2 in the Supporting Information. The X-ray crystallographic coordinates for all structures have been deposited at the Cambridge Crystallographic Data Centre (CCDC) with deposition numbers CCDC 1480482, 2183297, and 2205381 for **5**, **6**, and **5'**, respectively. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

2.4. Biological studies

2.4.1. Complex preparation

All six complexes were prepared in cell culture grade dimethyl sulfoxide (DMSO) (Darmstadt, Germany), and the DMSO concentration did not exceed 1% during treatment. Stock solutions of 1 mM for both the silver(I) complexes and the uncoordinated ligand were prepared and heated for 1 h and 30 min at 70 °C to ensure complete solubility. Of all the silver salts ($AgNO_3$, AgCl, and AgBr) used for this study, $AgNO_3$ was the only salt that was soluble in DMSO, of which a 1 mM stock was also prepared. The heated stock solutions were then diluted into media to obtain a working concentration of 10 µM of each silver(I) complex, including that of the uncoordinated ligand and $AgNO_3$. Single crystal XRD and NMR studies confirmed that no conformational changes were seen for the complexes after heating. As done previously [28, 34], the 10 µM served as a screening concentration to eliminate non-selective complexes.

2.4.2. Culturing of cell lines

For this study, four malignant and one non-malignant cell lines were used. The malignant cells included human esophageal carcinoma (SNO), human breast adenocarcinoma (MCF-7), human melanoma (A375) and human lung carcinoma (A549). A human embryonic kidney cell line (HEK293) was used as the non-malignant control. The SNO, MCF-7, A375, A549, and HEK293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Highveld Biological, Kelvin, RSA), supplemented with 10% fetal calf serum (FCS) (Sciencecell™ research labs, Carlsbad, USA), 1.6% penicillin/streptomycin/fungizone (Highveld Biological) and 0.4% gentamicin sulphate (Lonzo, Wadeville, USA). They were subcultured every 48 h and incubated at 37 °C under a 5% CO_2 humidified atmosphere.

2.4.3. Cell treatments

Cells were trypsinized and seeded (2×10^5 cells/mL) in 96 well plates with a final concentration of 2×10^4 cells per well. The SNO, MCF-7, A375, and A549 cells were then incubated for 24 h, while the HEK293 cells were incubated for 48 h pre-treatment. The spent media was removed, and the cells were treated in new media with either 10 µM of **1**–**6**, the uncoordinated ligand or $AgNO_3$ for 24 h. A vehicle (mock) control (1% DMSO) and a positive cell death control [100 µM cisplatin (CDDP) (Molekula, Dorset, UK)] were included. Cisplatin was prepared in 0.9% NaCl and heated overnight at 20 °C to ensure proper solubilization.

2.4.4. Cell viability after treatment

The viability of the cells was determined using an alamarBlue® proliferation assay (Serotec, UK). The alamarBlue® dye (10%) was incubated in the 96 well plates with the seeded cells (including 'blank' controls containing media only) at 37 °C for 2 h. The fluorescence was measured with a Synergy HT Multi-Detection Microplate reader (BioTek, Winooski, Vermont) at an ex wavelength of λ 530 nm and emission at λ 590 nm. This assay is principled on the metabolic oxidation-reduction reaction of resazurin to a reduced resorufin product that is directly proportional to the number of viable cells.

Table 1. Cell viability of malignant and non-malignant cells after 24 h of treatment with either 10 µM uncoordinated ligand or $AgNO_3$. The standard error of the mean (±SEM) is indicated (n = 9)

	Cell viability (±SEM)	
Cell line	10 µM ligand	10 µM $AgNO_3$
SNO	103.70% (±3.97%)	30.54% (±1.18%) [34]
MCF-7	93.96% (±2.25%)	95.00% (±1.36%)
A375	129.74% (±4.24%)	106.03% (±2.56%)
A549	93.27% (±2.13%)	99.91% (±2.72%)
HEK293	127.15% (±3.85%)	42.61% (±1.89%)

2.4.5. Statistical analysis

The data obtained for the viability assay was analyzed using Microsoft Excel and the Student's *t*-test. The average viability (%) was calculated with respect to the vehicle (mock)

control. All the data were further analyzed for the standard error of the mean (±SEM) and is represented as the error bars in Figure 5 or as a value in Table 1. The *p*-values were deemed highly significant at <0.001*** with respect to the mock-control where *n* = 9 (number of repeats).

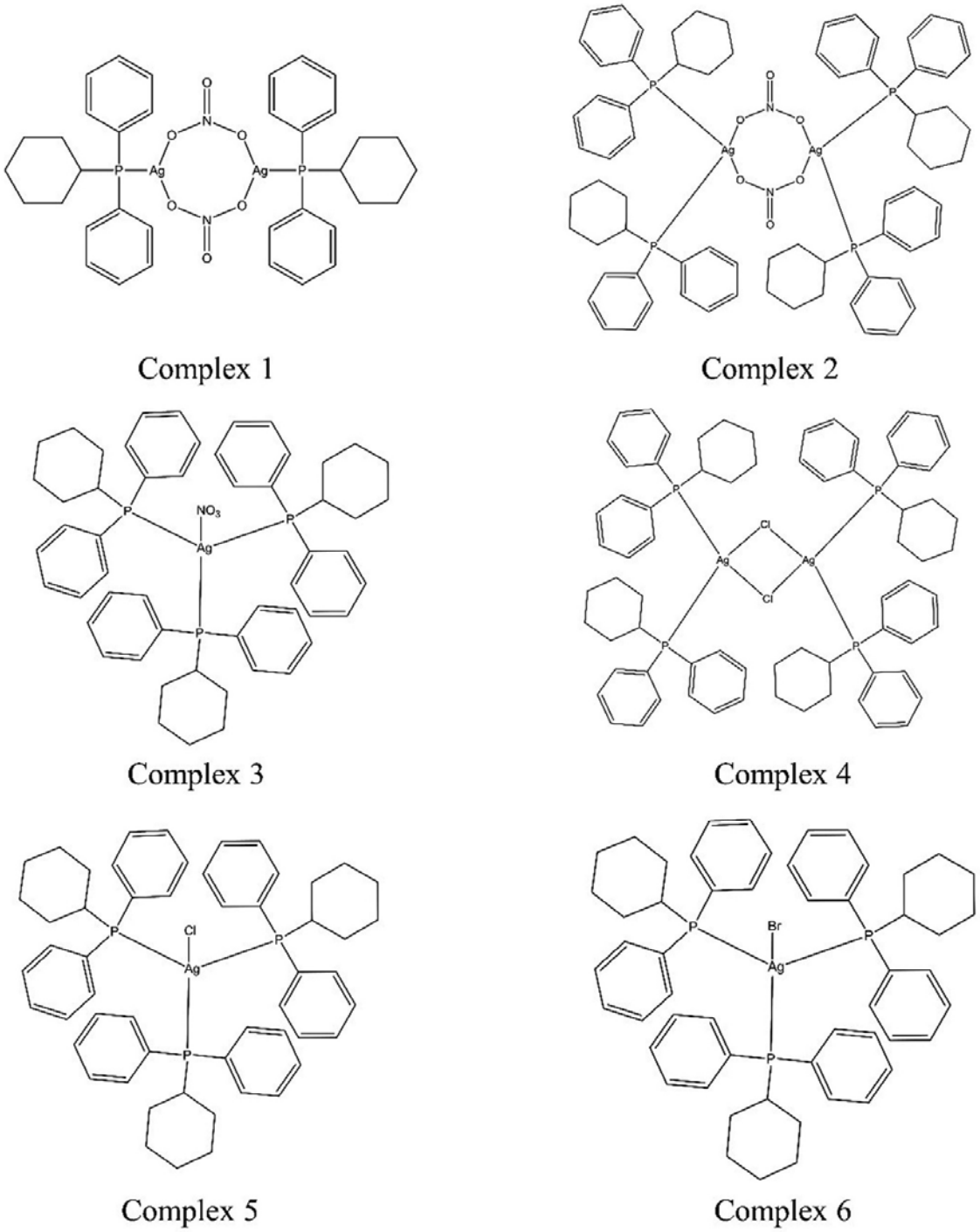


Figure 1. Schematic structures of different silver(I) cyclohexyldiphenylphosphine complexes **1**–**6**. The complexes were synthesized using different metal-to-ligand ratios (1:1–1:3) and different metal precursors ($AgNO_3$, AgCl, and AgBr).

3. Results and discussion

3.1. Synthesis and characterization of Ag(I) complexes

The adducts of various silver(I) salts and cyclohexyldiphenylphosphine in a 1:1–1:3 ratio were prepared using acetonitrile as the solvent. Figure 1 represents the schematic structures of the silver(I) complexes. The reaction requires the dissolution of the phosphine ligand in acetonitrile, and upon addition of the silver salt, the mixture is heated under reflux until all solids are dissolved. Yields of silver(I) complexes **2**–**6** were above 70%, however, for **1** the yield was 59%. The following spectroscopic techniques were used to characterize all six complexes: FTIR, ^{1}H, ^{13}C{H}, and ^{31}P{H}-NMR, single-crystal X-ray diffraction, as well as by elemental analysis (%C, %H, and %N).

From FTIR analysis, all six complexes showed the alkane C–H stretching frequency between υ 2800–2950 cm^{-1}, which is attributed to the cyclohexyl ring of the phosphine ligand. Aromatic C = C signal around υ 1370 cm^{-1} and sharp aromatic C–H bending frequencies around υ 1300 cm^{-1} due to the phenyl rings of the phosphine ligand were also observed. The cyclohexyl aliphatic protons appeared between δ 1.0 and 2.4 ppm in ^{1}H-NMR characterization with integrations of 10H for **1**, 20H for **2** and **4**, and 30H for **3**, **5,** and **6**. The aromatic protons with the corresponding integrations appeared between δ 7.2 and 7.6 ppm.

The structures of the six complexes were also confirmed by ^{13}C{H}-NMR with the cyclohexyl carbons appearing between δ 25 and 36 ppm and the aromatic carbons between δ 128 and 134 ppm. Elemental analyses of the complexes were all accurate and within the calculated elemental composition, confirming the proposed structures of the complexes.

3.2. X-Ray crystallography

The molecular structures of **5** and **6** were obtained from single-crystal XRD analysis and are shown in Figure 2 (fully labelled figures included in the Supplementary Information). The structures of **5** and **6** are isostructural to one another, with each having a molecule of acetonitrile co-crystallized in the structure. Each of the complexes crystallized with a cubic morphology in the triclinic space group *P*-1, with a Z value of 2 obtained in each case, indicating two molecules per unit cell. Other silver(I) cyclohexylphenylphosphine complexes reported in literature crystallize in the monoclinic space group $P2_1/n$, with Z = 4 [33]. The unit cell of **6** was only slightly larger than that of **5** (volume of 2462.77(5) Å^3 (**5**) versus 2491.47(8) Å^3 (**6**)), due to the larger bromido ligand in **6** as opposed to the chlorido ligand in **5**. Each of the structures revealed a distorted tetrahedral arrangement around the silver center with a halide and three $PCyPh_2$ ligands making up the coordination sphere. The distortion is indicated by the deviation from the ideal tetrahedral angle of 109.5° by the bond angles of X-Ag1-P1 (X = halogen) and P-Ag1-P (average) ranging between 105.050(14)–105.267(15)° and 110.104(13)–117.165(13)°, respectively. Expectedly, it does appear as if the average X-Ag1-P bond angle is decreased from ideality to accompany the simultaneous coordination of three sterically demanding $PCyPh_2$ ligands. The effect of the halogen on the structure is notable from the Ag1-X bond lengths of 2.6124(4) Å (**5**, Cl) and 2.7239(2) Å (**5**, Br). Despite this difference, no mentionable differences in the average Ag–P bond distances

are observed (2.530 Å (**5**) and 2.534 Å (**6**)). An acetonitrile molecule is observed to crystallize in an identical position in each of the structures.

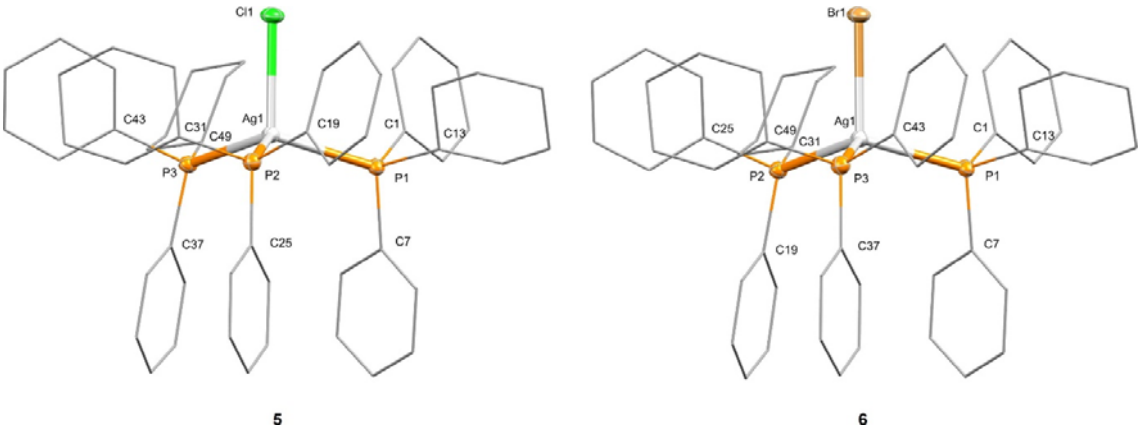


Figure 2. Molecular structures of **5** and **6**, $[Ag(PPh_2Cy)_3X]$ (X = Cl, Br), with thermal ellipsoids drawn at 50% probability level. For clarity purposes, the cyclohexyl- and phenyl rings of the phosphine groups are shown as the wireframe presentations, and hydrogen atoms as well as one CH_3CN solvent molecule are omitted from each structure.

Complex **5**, in DMSO, was heated to 70 °C (as with the biological testing) to ensure that no structural changes take place. During the process of recrystallization, as well as the biological testing process (*vide infra*) of **5**, single crystals were obtained and analyzed (XRD) to reveal a structure (**5'**) polymorphic to the structure of **5**. It is also interesting to note that acetonitrile co-crystallized molecules remain within the structure, despite exposure to DMSO. The latter may indicate a steric constraint in terms of the close packing of the adjacent molecules where the smaller and linear acetonitrile molecule is a better fit as opposed to the bulkier DMSO molecule. In the structure of **5'**, two complex molecules, along with one disordered acetonitrile molecule, are contained in the asymmetric unit (only one complex molecule and one acetonitrile in the structure of **5** and **6**). The structure of **5'** again crystallizes in the triclinic *P*-1 space group, with a unit cell volume less than double that of **5** (2462.77(5) $Å^3$ (**5**) and 4807.55(8) $Å^3$ (**5'**)) (Figure 3). Comparing bond lengths and angles in the structures of **5'** and **5**, slight contractions are seen in Ag1-Cl1 = 2.5746(4) Å (2.6124(4) Å in **5**), and the average Ag–P = 2.521 Å (2.530 Å in **5**) bond lengths. Overall, it does seem that the complex and solvent molecules assume a more condensed packing in **5'** as opposed to **5**. The packing diagram comparison of **5** and **5'** is shown in Figure 4. From viewing the crystallographic *a*, *b*, and *c* axes it is clear how the packing differs between the structures of **5** and **5'**. However, both structures contain corrugated planes with protruding chlorido groups that may provide a hydrophilic surface between the series of planes (*b* and *c* axes in **5**, *a* and *c* axes in **5'**). It is also interesting to note that although the acetonitrile molecules fill the small solvent-accessible voids within each structure, they do not appear in these channels or between the planes observed in the packing of either **5** or **5'**. Neither hydrogen bonding interactions nor pi-pi stacking interactions were observed in any of the structures discussed above.

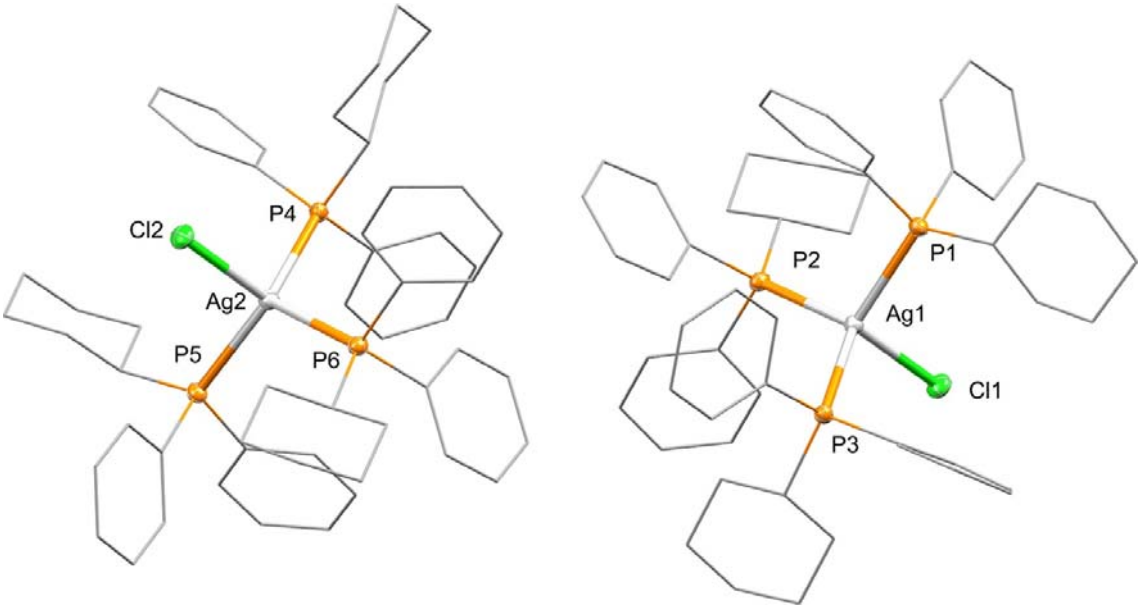


Figure 3. Molecular structure of **5'**, polymorphic to the structure of **5**, with thermal ellipsoids drawn at 50% probability level. For clarity purposes, the cyclohexyl- and phenyl rings of the phosphine groups are shown as the wireframe presentations, and hydrogen atoms as well as one CH_3CN solvent molecule (per complex molecule) are omitted.

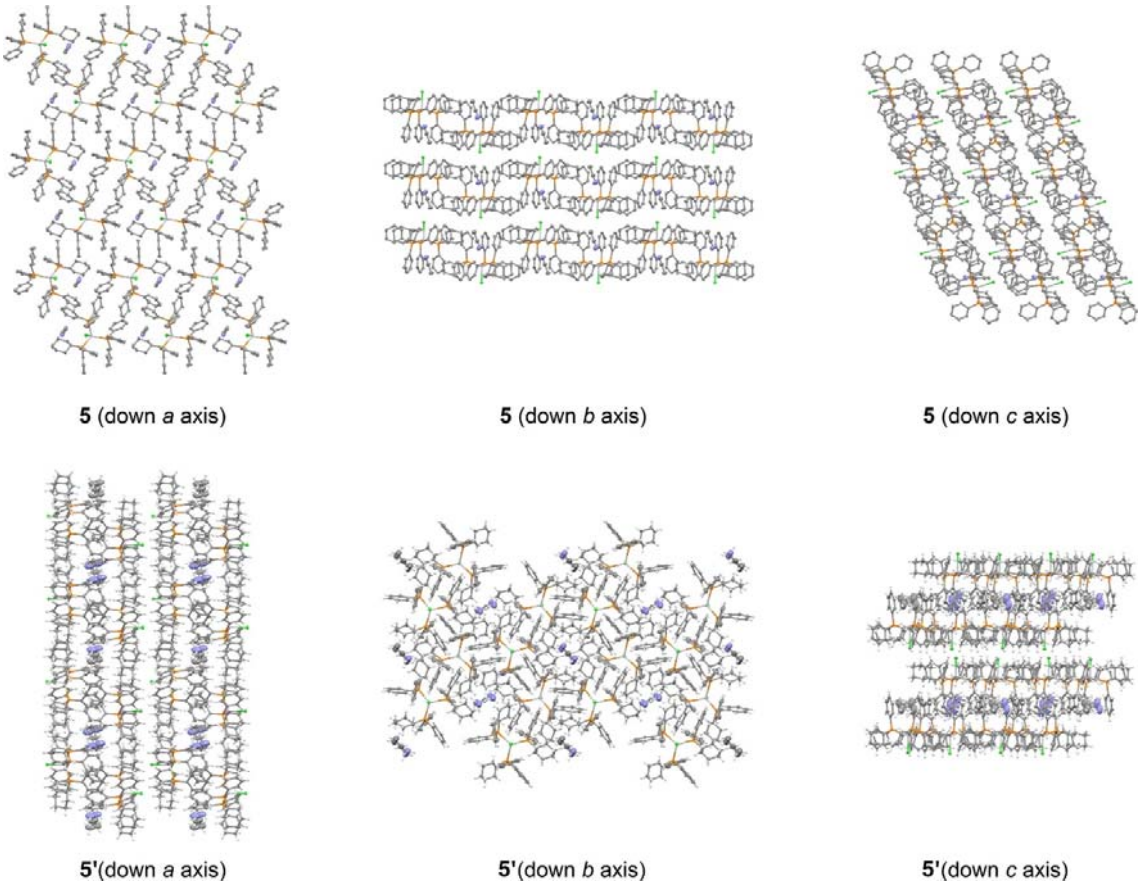


Figure 4. Packing diagrams of **5** and **5'** with thermal ellipsoids drawn at 50% probability level.

3.3. Anti-cancer studies

The fully characterized silver(I) cyclohexyldiphenylphosphine complexes were tested for their cytotoxicity and selectivity in various cell models. The viability of the cells was determined utilizing alamarBlue® cell proliferation assay 24 h post-treatment with 10 µM of **1**–**6**. Cisplatin, at a concentration of 100 µM, was used as a reference treatment or positive control (Figure 5).

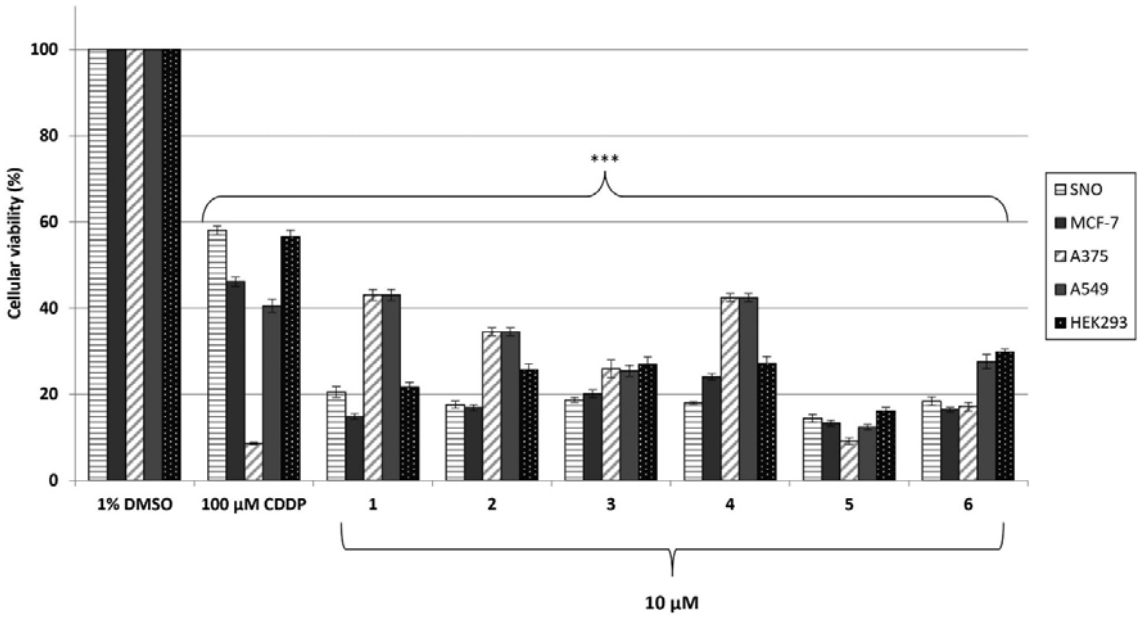


Figure 5. Cell viability of SNO, MCF-7, A375, A549, and HEK293 cells after 24 h of treatment, determined by an alamarBlue® proliferation assay. Cells were treated with 10 µM of **1**–**6**. A vehicle (mock) control, 1% DMSO, and a positive cell death control [100 µM cisplatin (CDDP)] were included. The percentage viabilities were calculated relative to the vehicle control (100%). The error bars were constructed based on the standard error of the mean (±SEM), where $n = 9$. The p-values (***<0.001) were calculated using the Student's t-test to determine the significant difference between treated and mock-treated cells.

All complexes showed a significant ($p < 0.001$) decrease in the viability of the malignant cells. Complexes **1**–**3** were synthesized using $AgNO_3$, however, different metal-to-ligand ratios of silver to phosphine ligand were used: 1:1 (complex **1**), 1:2 (complex **2**), and 1:3 (complex **3**). These complexes showed comparable cytotoxicities in the SNO cells with viabilities of 20.51%, 17.60% and 18.86%, respectively. A similar trend was observed in the SNO and MCF-7 cells treated with **4** (18.00% or 24.05%) and **5** (14.39% or 13.31%). These complexes were synthesized using AgCl salt with a metal-to-ligand ratio of 1:2 and 1:3. The cytotoxicity decreased as the ratio of the complexes increased for the MCF-7, A375, and A549 cells treated with **1**–**3**. This was also observed for the A375 and A549 cells after being treated with **4** and **5**. When comparing all the complexes containing the same metal-to-ligand ratio (1:3), **3**, **5**, and **6** are the most cytotoxic to all the cell lines, followed by **2** and **4**, having a ratio of 1:2.

Overall, the complexes under study were more cytotoxic to the SNO, MCF-7, and A549 cells than cisplatin, except for the A375 cells, where cisplatin was the most toxic. Zartilas *et al*. (2009) reported that tetrameric and monomeric tri(*p*-tolyl)-phosphine complexes containing either AgCl, AgBr, and AgI salts induced cell death in rat sarcoma cancer cells, leukemia cancer cells, and human T-lymphocyte cells [65]. The cytotoxic activity of the 1:1 complex was either comparable or higher when compared to the reported 1:3 complex. Silver(I) thiocyanate complexes (1:1, 1:2, and 1:3) containing either a benzyldiphenylphosphine ligand [29], cyclohexylphosphine ligand [33], *p*-substituted phenyl diphenyl phosphine ligand [31] or different substituted triphenylphosphines [34] has shown to be cytotoxic to both malignant esophageal and breast cell lines. In a different study, silver(I) nitrate was coordinated to different ratios of PPh_3 (1:1, 1:2, 1:3 and 1:4) [34]. Their cytotoxicity was screened in an esophageal cancer cell line [34]. After being treated for 24 h (with 10 µM of the complexes), the 1:3 ratio complex was the most cytotoxic (10.86%) followed by the 1:2 ratio (17.44%). The complex at the ratio of 1:4 was the least cytotoxic (72%) followed by the 1:1 ratio (30.18%). It, therefore, appears that the cell death-inducing ability of the silver(I) complexes is dependent on the metal-to-ligand ratio with those coordinated in a 1:2 and 1:3 manner being the most active in cancer cells.

Furthermore, the cytotoxicity and selectivity of **1**–**6** were determined by exposing non-malignant HEK293 cells to the same conditions as described for the malignant cells (Figure 5). All six complexes induced significant ($p < 0.001$) cell death in more than 70% of the HEK293 cells. This signifies that the complexes studied herein, selectivity is lower than related complexes where more than 70% and 35% of the non-malignant skin and kidney cells were alive, respectively, after being treated with 10 µM for 24 h [30].

The effect of the uncoordinated ligand, cyclohexyldiphenylphosphine, and the $AgNO_3$ salt was subsequently evaluated in all the cell lines (Table 1). Viability studies using AgCl and AgBr were excluded due to their less soluble nature in DMSO. The ligand was minimally toxic to all the cell lines with viabilities exceeding 93% and even 120% for the A375 and HEK293 cells. The silver salt, $AgNO_3$, displayed a low toxicity profile for MCF-7, A375, and A549 cells with viabilities higher than 95%. In contrast, studies reported previously show that $AgNO_3$ is more toxic to the SNO cells with a viability of 30.54% [34]. The same was observed for the non-malignant cell line where the HEK293 cells viability was 42.61% after 24 h.

Overall, it seems that the degree of toxicity observed was due to the functional coordinated complexes and not the uncoordinated ligand or silver salt. However, based on this study, complexes coordinated with $AgNO_3$ should not be considered for SNO cells due to the higher degree of toxicity observed with the uncoordinated silver salt.

4. Conclusion

Silver(I) cyclohexyldiphenylphosphine complexes were synthesized and characterized by FT-IR, NMR, and SXRD. Suitable crystals were only obtained for **5** and **6**, and the crystal structures were determined by single-crystal X-ray diffraction. All six complexes were evaluated for their anti-cancer properties in a range of cell models. The alamarBlue® assay confirmed a significant decrease in the cell viability of both malignant and non-malignant

cell lines. The cytotoxicity towards the malignant cells depends on the ratio of the metal to the ligand in the complex. Since low dose application and the selective nature of compounds (i.e. discriminate between non-malignant and malignant cells) are deemed ideal properties for use as anti-cancer agents, we conclude that the complexes described herein are not suitable candidates as anti-cancer agents.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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