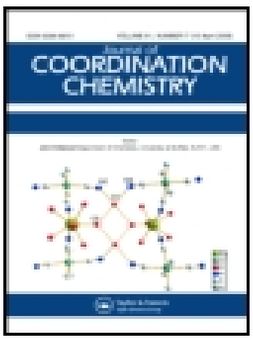


Journal of Coordination Chemistry

ISSN: 0095-8972 (Print) 1029-0389 (Online) Journal homepage: https://www.tandfonline.com/loi/gcoo20

Synthesis, characterization, and evaluation of silver(I) complexes with mixed-ligands of thiosemicarbazones and diphenyl(*p*-tolyl)phosphine as biological agents

Syahrina Nur 'Ain Abdul Halim, Fariza Juliana Nordin, Mohd Ridzuan Mohd Abd Razak, Nur Rahimah Fitrah Mohd Sofyan, Siti Nadiah Abdul Halim, Nor Fadilah Rajab & Rozie Sarip

To cite this article: Syahrina Nur 'Ain Abdul Halim, Fariza Juliana Nordin, Mohd Ridzuan Mohd Abd Razak, Nur Rahimah Fitrah Mohd Sofyan, Siti Nadiah Abdul Halim, Nor Fadilah Rajab & Rozie Sarip (2019): Synthesis, characterization, and evaluation of silver(I) complexes with mixed-ligands of thiosemicarbazones and diphenyl(*p*-tolyl)phosphine as biological agents, Journal of Coordination Chemistry, DOI: 10.1080/00958972.2019.1577400

To link to this article: https://doi.org/10.1080/00958972.2019.1577400

JOURNAL OF COORDINATION CHEMISTRY
https://doi.org/10.1080/00958972.2019.1577400

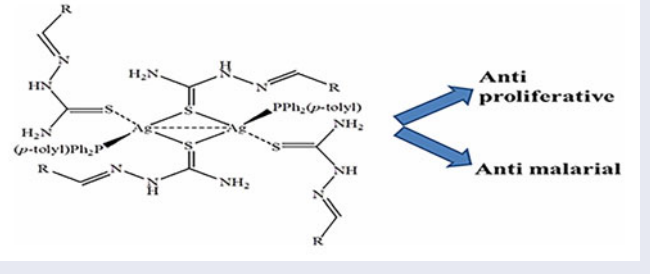
Synthesis, characterization, and evaluation of silver(I) complexes with mixed-ligands of thiosemicarbazones and diphenyl(*p*-tolyl)phosphine as biological agents

Syahrina Nur 'Ain Abdul Halim[a], Fariza Juliana Nordin[b], Mohd Ridzuan Mohd Abd Razak[c], Nur Rahimah Fitrah Mohd Sofyan[a], Siti Nadiah Abdul Halim[a], Nor Fadilah Rajab[b] and Rozie Sarip[a]

[a]Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia; [b]Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia; [c]Bioassay Unit, Herbal Medicine Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia

ABSTRACT

Five new silver(I) complexes were synthesized with mixed ligands of thiosemicarbazone derivatives and diphenyl(*p*-tolyl)phosphine in search of new biologically active compounds. A CHN elemental analysis, powder X-ray diffraction (PXRD) data and several spectroscopic techniques such as Fourier-transform infrared spectroscopy, energy-dispersive X-ray, ^{1}H, ^{13}C, and $^{31}P\{^{1}H\}$ NMR were performed to elucidate the structure of these complexes. Elemental analysis suggested that the stoichiometry of the complexes formed by the reaction of silver nitrate with thiosemicarbazone in the presence of (*p*-tolyl)PPh_2 was indeed 1:2:1 molar ratio. The silver ions were discovered to be coordinated to the sulfur of thiosemicarbazone and phosphorus of (*p*-tolyl)PPh_2, having a tetrahedral geometry based on the spectroscopic data obtained. The PXRD patterns were studied to see the degree of crystallinity of the complexes. The *in vitro* antiproliferative activity of these complexes was investigated toward the MDA-MB-231 and MCF-7 breast cancer cell lines, as well as the HT-29 colon cancer cell line, which yielded IC_{50} values in low micromolar range. The antiplasmodial activity of these complexes was also examined against chloroquine-resistant *Plasmodium falciparum* parasite which demonstrated good activity and further tested for their selectivity index.



ARTICLE HISTORY
Received 9 November 2018
Accepted 14 January 2019

KEYWORDS
Thiosemicarbazone; silver complex; anticancer; antimalarial; phosphine

CONTACT Rozie Sarip rozie@um.edu.my Department of Chemistry, Faculty of Science, University of Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia

1. Introduction

Thiosemicarbazone is classified as Schiff-base compound which functions as chelating agent for numerous metal ions. Owing to the presence of sulfur and imine nitrogen atoms, the various binding modes reaffirm its versatility [1–3]. Therefore, these ligands exhibit diverse biological properties such as antimalarial [4–6], anticancer [7, 8] and antiprotozoan through the inhibition of cysteine proteases and other targets [9–12], which have been of great interest.

Although as free ligands, the thiosemicarbazones revealed good antimalarial activities, certain studies found that the introduction of transition metals would enhance the properties of these ligands. For instance, Khanye *et al.* [13] found that the coordination of gold to thiosemicarbazone, compared to its free ligands, significantly improved the antiplasmodial activity. Meanwhile, instead of employing gold, Adams *et al.* [14] also achieved similar trend of antiplasmodial activity despite the utilization of various thiosemicarbazone derivatives with the adoption of palladium. Nevertheless, it was proved that the combination of metal and thiosemicarbazone derivatives in complexes enhanced the antiproliferative ability [15, 16].

Hence, a thorough biological study on thiosemicarbazone metal complexes is of increasing importance given that such metal coordination may positively affect the antimalarial and antiproliferative activities. Other than thiosemicarbazone, transition metal complexes with phosphine also revealed fascinating biological activities; for example, coinage metals encompassing tertiary phosphine and diphosphines were scrutinized for their anticancer properties [17–21] and displayed good activities. Following that, the antimalarial activity of phosphine complexes against murine malaria parasite, specifically *Plasmodium berghei*, was also examined, which demonstrated the potential development of transmission-blocking antimalarial compound.

Furthermore, Molter *et al.* [22] discovered that the synthesized gold complex with mixed ligands of thiosemicarbazone and phosphine revealed rather similar IC_{50} value to that of chloroquine. Hence, this study synthesized mixed-ligands system of silver(I) complexes and evaluated their anticancer and antimalarial properties. To date, several structural studies of silver(I) thiosemicarbazone complexes were reported [23–26] but studies on biological activities mainly focus on antibacterial properties [27, 28]. A thorough search of relevant literatures revealed that there were no empirical findings on anticancer and antimalarial activities of silver(I) complexes with both thiosemicarbazone and phosphine ligands. Herein, this study evaluated the synthesis of silver(I) complexes with mixed-ligands of thiosemicarbazone derivatives and diphenyl(*p*-tolyl)phosphine and their *in vitro* antiproliferative and antiplasmodial activities.

2. Experimental

2.1. Materials and instrumentation

All solvents and reagents in this study were of analytical grade and commercially purchased from Sigma Aldrich Ltd., unless stated otherwise. The thiosemicarbazide, aromatic aldehydes, silver nitrate, diphenyl(*p*-tolyl) phosphine, acetic acid, diethyl ether,

ethanol, methanol and acetonitrile were used without purification. The CHN analyses were performed by Perkin Elmer CHNS/O 2400 Series II. The infra-red (IR) spectra were determined using a Perkin Elmer Spectrum One FT-IR spectrophotometer (ATR) at a frequency range of 450–4000 cm^{-1}. JEOL FT-NMR ECX 400 (ECX 400) was employed to measure the NMR spectra of ^{1}H, ^{13}C and $^{31}P\{^{1}H\}$ at 400 MHz in deuterated solvents without internal reference. The presence of metals and other elements were detected by energy-dispersive X-ray spectroscopy (EDX). Powder X-ray diffraction (PXRD) was recorded on an X-ray diffractometer (PANalytical, Netherlands) with Cu Kα characteristic radiation ($\lambda = 0.154$ nm) at the voltage of 40 kV and current of 40 mA and the scanning rate was 4.25°/min and the scanning of 2θ was from 0° to 90° at room temperature (25 °C).

2.2. Synthesis of thiosemicarbazone ligands

As described in the literature [29], the ligands were synthesized with minor modifications. In general, thiosemicarbazide (6.0 mmol, 0.55 g) was dissolved in ethanol (30 mL) with the addition of an appropriate amount of aromatic benzaldehyde (6.3 mmol) and a few drops of acetic acid. Subsequently, the mixture was refluxed for *ca.* 6 h. The formed precipitates were filtered and dried at room temperature.

2.2.1. 4-Hydroxy-benzaldehyde thiosemicarbazone [L1]

Yield 47%; m.p. 230 °C. Anal. Calcd for $C_8H_9N_3OS$: C, 49.21; H, 4.65; N, 21.52. Found: C, 49.11; H, 4.63; N, 20.83. IR data (cm^{-1}): ν(N–H) 3471; ν(N–H) 3359; ν(S–H) 2751; ν(C = N) 1578. ^{1}H NMR (400 MHz, DMSO), δ (ppm): 11.20 (1H, s, N–H); 9.82 (1H, s, N–H); 8.01 (1H, s, S–H); 7.91 (1H, s, N = CH); 6.73–7.78 (4H, m, Ar–H); 7.78 (1H, s, Ar–OH). ^{13}C NMR (400 MHz, DMSO), δ (ppm): 177.93 (C–S); 159.76 (C–OH); 143.20 (N = C–Ar); 116.06–129.58 (C_{Ar}).

2.2.2. 2,4-Dihydroxy-benzaldehyde thiosemicarbazone [L2]

Yield 56%; m.p. 238 °C. Anal. Calcd for $C_8H_9N_3O_2S$: C, 45.49; H, 4.29; N, 19.89. Found: C, 45.38; H, 4.22; N, 19.81. IR data (cm^{-1}): ν(N–H_2) 3348, 3254; ν(N–H) 3479; ν(C = S) 876; ν(C = N) 1584. ^{1}H NMR (400 MHz, DMSO), δ (ppm): 11.13 (1H, s, N–H); 9.70 (2H, s, N–H_2); 8.20 (1H, s, N = CH); 6.21–7.64 (3H, m, Ar–H); 7.91 (1H, s, Ar–OH); 7.70 (1H, s, Ar–OH). ^{13}C NMR (400 MHz, DMSO), δ (ppm): 177.50 (C = S); 160.09, 158.50 (C–OH); 141.17 (C = N); 102.77–128.83 (C_{Ar}).

2.2.3. 5-Bromo-2-hydroxy-benzaldehyde thiosemicarbazone [L3]

Yield 85%; m.p. 250 °C. Anal. Calcd for $C_8H_8BrN_3OS$: C, 35.05; H, 2.94; N, 15.33. Found: C, 35.01; H, 2.49; N, 14.91. IR data (cm^{-1}): ν(N–H) 3356; ν(N–H) 3469; ν(S–H) 2833; ν(C = N) 1595. ^{1}H NMR (400 MHz, DMSO), δ (ppm): 11.374 (1H, s, N–H); 10.19 (1H, s, N–H); 8.24 (1H, s, N = CH); 6.76–8.16 (2H, m, Ar–H); 8.16–8.11 (3H, overlap peaks of Ar–OH, Ar–H and S–H). ^{13}C NMR (400 MHz, DMSO), δ (ppm): 178.35 (C–S); 156.06 (C–OH); 137.79 (C = N); 133.73–111.64 (C_{Ar}).

2.2.4. 2-Hydroxy-4-methoxy-benzaldehyde thiosemicarbazone [L4]

Yield 69%; m.p. 212 °C. Anal. Calcd for $C_9H_{11}N_3O_2S$: C, 47.99; H, 4.92; N, 18.65. Found: C, 47.51; H, 5.24; N, 18.76. IR data (cm^{-1}): ν(N–H) 3245; ν(N–H) 3455; ν(S–H) 2815; ν(C = N) 1544. ^{1}H NMR (400 MHz, DMSO), δ (ppm):11.21 (1H, s, N–H); 9.90 (1H, s, N–H); 8.23 (1H, s, N = CH); 6.36–6.40 (2H, m, Ar–H); 7.75–7.78 (2H, overlap peaks of Ar–OH and Ar–H); 7.97 (1H, s, S–H); 3.69 (3H, s, O–CH_3). ^{13}C NMR (400 MHz, DMSO), δ (ppm): 177.69 (C–S); 162.38 (C–OCH_3); 158.39 (C–OH); 140.68 (C = N); 128.75–101.29 (C_{Ar}); 55.66 (O–CH_3).

2.2.5. 5-Bromo-2-hydroxy-3-methoxy benzaldehyde thiosemicarbazone [L5]

Yield 95%; m.p. > 250 °C. Anal. Calcd for $C_9H_{10}BrN_3O_2S$: C, 35.54; H, 3.31; N, 13.81. Found: C, 35.54; H, 3.11; N, 12.81. IR data (cm^{-1}): ν(N–H) 3352; ν(N–H) 3450; ν(S–H) 2813; ν(C = N) 1529. ^{1}H NMR (400 MHz, DMSO), δ (ppm): 11.39 (1H, s, N–H); 9.429 (1H, s, N–H); 8.29 (1H, s, N = CH); 7.03–7.79 (2H, Ar–H); 8.09 (2H, overlap peaks of Ar–OH and S–H); 3.78 (3H, s, O–CH_3). ^{13}C NMR (400 MHz, DMSO), δ (ppm): 178.30 (C–S); 149.46 (C–OCH_3); 145.78 (C–OH); 137.82 (C = N); 145.78–111.30 (C_{Ar}); 56.80 (O–CH_3).

2.3. Synthesis of silver complexes

$AgNO_3$ (0.1 mmol, 0.02 g) was dissolved in a solvent mixture (5 mL) of acetonitrile and methanol (ratio of 2:3). Then separately, the synthesized thiosemicarbazone (0.2 mmol) was dissolved in the same solvent mixture and was added dropwise to the silver solution. Following that, the reaction mixture was refluxed for 3 h at 50 °C. The reaction mixture was further treated with diphenyl(*p*-tolyl)phosphine (0.1 mmol, 0.03 g) in the mixture of acetonitrile and methanol (5 mL) and the refluxing continued for another 2 h. The solution was then filtered to remove any impurities and then dried *in vacuo*. The outline of the reaction as shown in Figure 1 and the structure of the product was proposed referring to the previous study [30] and spectroscopic data attained as no crystal data could be obtained to date.

2.3.1. $[Ag_2(PPh_2(p\text{-tolyl}))_2(4\text{-hydroxy-benzaldehyde thiosemicarbazone})_4](NO_3)_2$ [1]

Yield 26%; m.p. 130–134 °C. Anal. Calcd for $C_{70}H_{70}Ag_2N_{12}O_4P_2S_4$: C, 54.27; H, 4.55; N, 10.85. Found: C, 53.64; H, 4.14; N, 11.37. IR data (cm^{-1}): ν(N–H) 3407; ν(N–H) 3278; ν(S–H) overlap with broad N–H and O–H peak; ν(C = N) 1591; ν(P–C_{Ar}) 1095; ν(NO_3^-) 1305. ^{1}H NMR (400 MHz, DMSO), δ (ppm): 11.64 (4H, s, N–H); 9.97 (4H, s, N–H); 7.93 (4H, s, N = CH); 6.75–7.62 (44H, m, Ar–H); 8.55 (4H, s, S–H); 8.32 (4H, s, Ar–OH); 2.24 (6H, s, Ar–CH_3). ^{13}C NMR (400 MHz, DMSO), δ (ppm): 175.20 (C–S); 160.27 (C–OH); 145.76 (C = N); 116.11–141.12 (C_{Ar}); 21.41 (Ar–CH_3). ^{31}P{^{1}H} NMR (400 MHz, DMSO), δ (ppm): 8.84.

2.3.2. $[Ag_2(PPh_2(p\text{-tolyl}))_2(2,4\text{-dihydroxy-benzaldehyde thiosemicarbazone})_4](NO_3)_2$ [2]

Yield 40%; m.p. 136–142 °C. Anal. Calcd for $C_{70}H_{70}Ag_2N_{12}O_8P_2S_4$: C, 52.11; H, 4.37; N, 10.42. Found: C, 51.85; H, 4.32; N, 10.29. IR data (cm^{-1}): ν(N–H_2) 3278, 3171; ν(N–H) 3414; ν(C = S) 849; ν(C = N) 1600; ν(P–C_{Ar}) 1096; ν(NO_3^-) 1372. ^{1}H NMR (400 MHz, DMSO), δ (ppm): 11.51 (4H, s, N–H); 9.86 (8H, d, J = 13.3 Hz, N–H_2); 8.27 (4H, s, N = CH);

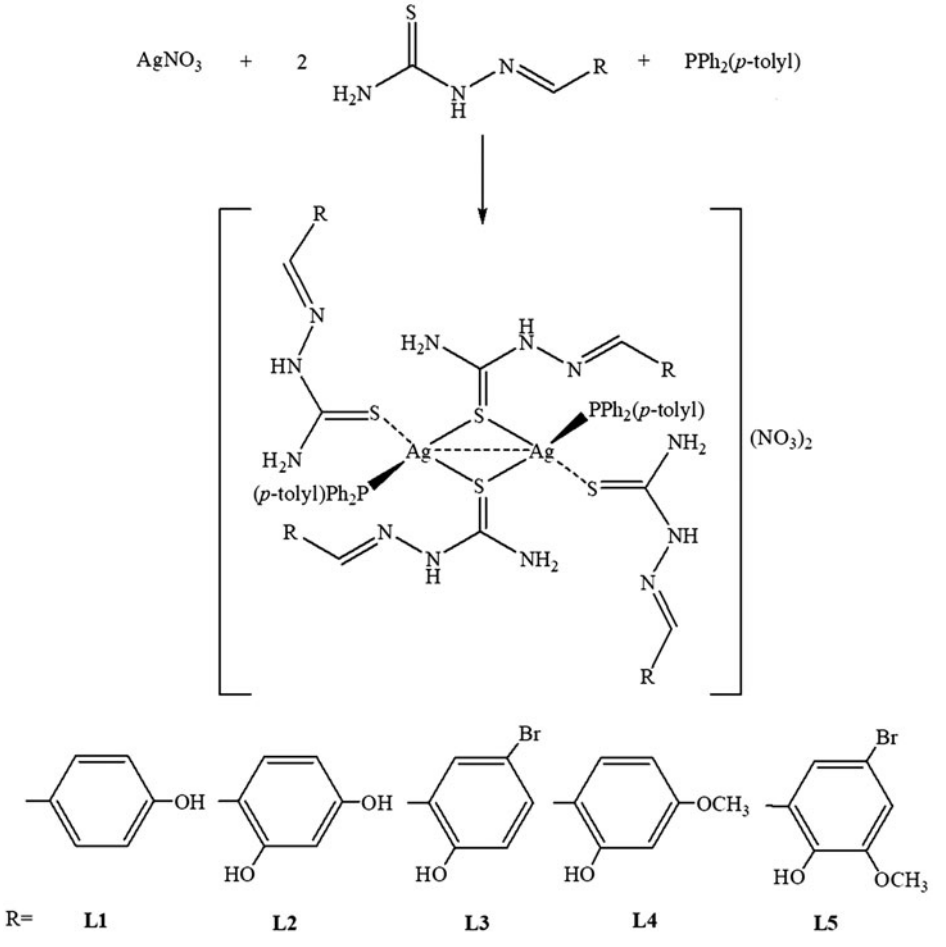


Figure 1. Synthetic route for mixed-ligands silver complexes.

6.23–7.71 (40H, m, Ar–H); 8.32, 8.20 (4H, s, Ar–OH); 2.24 (6H, s, Ar–CH_3). ^{13}C NMR (400 MHz, DMSO), δ (ppm): 175.20 (C = S); 161.67, 158.98 (C–OH); 143.87 (C = N); 102.75–141.29 (C_{Ar}); 21.40 (Ar–CH_3). ^{31}P{^{1}H} NMR (400 MHz, DMSO), δ (ppm): 8.30.

2.3.3. *[Ag_2(PPh$_2$(p-tolyl))$_2$(5-bromo-2-hydroxy-benzaldehyde thiosemicarbazone)$_4$] (NO_3)$_2$ [3]*

Yield 29%; m.p. 120–124 °C. Anal. Calcd for $C_{70}H_{66}Ag_2Br_4N_{12}O_4P_2S_4$: C, 45.08; H, 3.57; N, 9.01. Found: C, 44.88; H, 3.56; N, 8.61. IR data (cm^{-1}): ν(N–H) 3322; ν(N–H) 3409; ν(S–H) overlap with broad N–H and O–H peak; ν(C = N) 1602; ν(P–C_{Ar}) 1094.06; ν(NO_3^-) 1316.04. ^{1}H NMR (400 MHz, DMSO), δ (ppm): 11.71 (4H, s, N–H); 10.34 (4H, s, N–H); 8.29 (4H, s, N = CH); 6.79–8.16 (41H, m, Ar–H); 8.57 (4H, s, S–H); 8.51 (4H, s, Ar–OH); 2.26 (5.95H, s, Ar–CH_3). ^{13}C NMR (400 MHz, DMSO), δ (ppm): 175.69 (C–S); 156.44 (C–OH); 141.29 (C = N); 111.63–140.42 (C_{Ar}); 21.42 (Ar–CH_3). ^{31}P{^{1}H} NMR (400 MHz, DMSO), δ (ppm): 8.30.

2.3.4. $[Ag_2(PPh_2(p\text{-tolyl}))_2(2\text{-hydroxy-4-methoxy-benzaldehyde thiosemicarbazone})_4](NO_3)_2$ [4]

Yield 39%; m.p. 122–126 °C. Anal. Calcd for $C_{74}H_{78}Ag_2N_{12}O_8P_2S_4$: C, 53.24; H, 4.71; N, 10.07. Found: C, 52.97; H, 4.26; N, 9.73. IR data (cm^{-1}): ν(N–H) 3414; ν(N–H) 3281; ν(S–H) overlap with broad N–H and O–H peak; ν(C = N) 1627; ν(P–C_{Ar}) 1095; ν(NO_3^-) 1324. ^{1}H NMR (400 MHz, DMSO), δ (ppm): 11.51 (4H, s, N–H); 10.02 (4H, s, N–H); 8.27 (4H, s, N = CH); 6.38–7.81 (41H, m, Ar–H); 8.39 (4H, s, S–H); 8.18 (4H, s, Ar–OH); 3.70 (12H, s, O–CH_3); 2.26 (6H, s, Ar–CH_3). ^{13}C NMR (400 MHz, DMSO), δ (ppm): 175.10 (C–S); 158.73 (C–OH); 162.84 (C–OCH_3); 142.36 (C = N); 101.24–141.22 (C_{Ar}); 55.71 (O–CH_3); 21.42 (Ar–CH_3). ^{31}P {^{1}H} NMR (400 MHz, DMSO), δ (ppm): 8.07.

2.3.5. $[Ag_2(PPh_2(p\text{-tolyl}))_2(5\text{-bromo-2-hydroxy-3-methoxy-benzaldehyde thiosemicarbazone})_4](NO_3)_2$ [5]

Yield 46%; m.p. 126–130 °C. Anal. Calcd for $C_{74}H_{74}Ag_2Br_4N_{12}O_8P_2S_4$: C, 44.78; H, 3.76; N, 8.47. Found: C, 45.06; H, 3.42; N, 7.84. IR data (cm^{-1}): ν(N–H) 3424; ν(N–H) 3288; ν(S–H) overlap with broad N–H and O–H peak; ν(C = N) 1597; ν(P–C_{Ar}) 1093; ν(NO_3^-) 1311. ^{1}H NMR (400 MHz, DMSO), δ (ppm): 11.71 (4H, s, N–H); 10.34 (4H,s, N–H); 8.35 (4H, s, N = CH); 7.09–7.79 (36H, m, Ar–H); 8.58 (4H, s, S–H); 8.51 (4H, s, Ar–OH); 3.80 (12H, s, O–CH_3); 2.26 (6H, s, Ar–CH_3). ^{13}C NMR (400 MHz, DMSO), δ (ppm): 175.67 (C–S); 146.22 (C–OH); 149.54 (C–OCH_3); 141.34 (C = N); 111.35–140.56 (C_{Ar}); 21.49 (Ar–CH_3). ^{31}P {^{1}H} NMR (400 MHz, DMSO), δ (ppm): 8.84.

2.4. Biological assays

2.4.1. Antiproliferative assay

A sulforhodamine B (SRB) assay was performed to determine the inhibition concentration (IC_{50}) of all compounds, as described in previous studies [31]. Each cell was treated at varied concentrations: 5.053–161.686 μM (for **1**), 4.843–154.960 μM (for **2**), 4.189–134.055 μM (for **3**), 4.680–149.752 μM (for **4**) and 3.936–125.944 μM (for **5**). After 48 h, each cell was fixed in the plate with 50 μL of 50% (w/v) trichloroacetic acid (TCA) solution and incubated for 1 h at 4 °C. Following that, these plates were washed with tap water for five times then air dried. Then, the cells were stained with 100 μL of 0.4% (w/v) SRB staining solution and incubated for 10 min at room temperature. Subsequently, the plates were washed with 1% (v/v) acetic acid for three times (to remove unbound stains) and air dried. Then, 200 μL of 10 mM Trizma base was added into each well then were shaken well for 10 min. The absorbance was read at 490 nm using a microplate reader. Meanwhile, the IC_{50} values were calculated based on the following formula: IC_{50} = (OD sample/OD control) × 100. It should be noted that these experiments were triplicated.

2.4.2. *In vitro P. falciparum* HRPII assay

All compounds were evaluated *in vitro* for antiplasmodial activity using HRPII assay [32, 33] as described elsewhere, with certain modifications [34]. Briefly, ring-infected red blood cells (RBCs) with 0.05% parasitemia and 1.5% haematocrit were exposed with serially diluted compounds in a candle jar for 72 h at 37 °C. The final tested

concentrations ranged between 0.25 and 15.7 μg/mL. The negative control was the infected RBC without compounds or replaced with sterile H_2O. After 72 h of incubation, the test plates were kept overnight at −80 °C. The test plates were thawed to lyse the infected RBCs at room temperature. The parasite-compound exposure activity (end point) was measured using HRPII assay, as described by Noedl *et al.* [33, 35]. The collected data were then transferred to HN-NonLin software (malaria.farch.net) to get 50% effective concentration (EC_{50}) values directly from the graph.

2.4.3. *In vitro* cytotoxicity assay

The MDBK cells were maintained in complete DMEM culture medium containing 25 mM HEPES, 0.4% sodium bicarbonate ($NaHCO_3$) and 100 U of Penstrep (100 U penicillin and 100 U streptomycin) supplemented with 10% fetal bovine serum (FBS). The cytotoxicity of the compounds were measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [36]. Briefly, the MDBK cells (1×10^3 cells per well) were exposed with serially diluted (two-fold serial dilutions) compounds with the range of final concentration between 0.78 and 25 μg/mL in triplicates. The positive control for cell growth was the cell suspension without test substance, whereas the negative control was the cell suspension with 0.05% of Triton X 100. The culture was incubated for 72 h in a CO_2 incubator (CO_2 concentration of 5% at 37 °C). Subsequently, 50 μL of MTT solution (5 mg MTT in 1 mL PBS and 2.5 mL DMEM media) was added into each well. Likewise, the plates were further incubated for 4 h in a CO_2 incubator (CO_2 concentration of 5% at 37 °C). The medium was then removed and replaced with 200 μL of DMSO to dissolve the MTT formazan product. The solution was subsequently mixed for 15 min and once for 30 s before the measurement of absorbance at 540 nm was taken with microplate reader (FLUOstar Omega, Germany). Both growth inhibition and 50% cytotoxic concentration (CC_{50}) values were estimated in percentage based on dose–response curve. A selectivity index (SI), which corresponded to the ratio between the antiplasmodial and cytotoxic activities, was calculated according to the following formula:

$$SI_{Plasmodium} = \frac{CC_{50\ \text{normal cell lines}}}{EC_{50\ \text{Plasmodium}}}$$

3. Results and discussion

During the synthesis, **1** and **2** produced clear solution throughout the experiment. However, **3**, **4** and **5** produced precipitates after reacting with thio-ligand for few minutes. As the reaction progressed, the precipitates were reduced upon the addition of diphenyl(*p*-tolyl)phosphine ligand. However, those three complexes did not produce clear solution. Hence, this is presumably contributed by the presence of another substituent in the aromatic ring, besides the hydroxyl group as in complexes **1** and **2**.

Thiosemicarbazone ligands exhibit thione–thiol tautomerism, as shown in Figure 2. Depending on the reaction condition, this reversible reaction occurs in numerous thioamide-type derivatives as majority give rise to S-alkyl and N-alkyl derivatives [37]. The spectroscopic data suggested that all synthesized thiosemicarbazone ligands and their corresponding complexes exist in the thiol formation except for **L2** and its

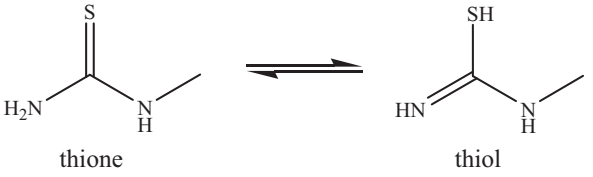


Figure 2. Thione–thiol tautomerism.

corresponding complex **2**. It is postulated that the occurrence of tautomerism is contributed by the presence of various functional groups in the aromatic ring, which subsequently promotes conjugation and electron delocalization in these compounds.

Up to this point, it was revealed that the synthesized silver(I) complexes were non-hygroscopic which it would not decompose after being stored for an extended duration. It was reaffirmed by periodic monitoring of its NMR spectrum.

3.1. Spectroscopic data analysis

Several spectroscopic techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR and ^{31}P{^{1}H} NMR were employed to characterize the synthesized thiosemicarbazone ligands and their complexes. The information of complex coordination through the shape and intensity of *d–d* bands was acquired using ultraviolet–visible (UV–vis) spectroscopy. However, this is not applicable for silver(I) complexes as the *d–d* transition of Ag(I) is principally forbidden [27]. Therefore, EDX spectroscopy was utilized to detect the presence of Ag(I) instead.

3.1.1. FT-IR spectroscopy

The IR spectra of the complexes validated the coordination of silver to thiosemicarbazone ligand, which revealed the presence of ν(C = N) vibrational modes in the complexes **1**–**5**. Nevertheless, a very weak band around 2800–2500 cm^{-1} of ν(S–H) was observed in **L1**, **L3**, **L4** and **L5** shows that the ligands exist as thiols. However, this band was not present in the spectrum of their corresponding complexes. A study by Lobana *et al.* (2009) [26] shows the presence of intramolecular and intermolecular hydrogen-bonding of hydroxyl and amino groups in silver thiosemicarbazone complex contributes to band broadening in the 3600–3000 cm^{-1} region, which causes overlapping of the bands around this region; thus, this may be the plausible rationalization for the disappearance of ν(S–H) in the complex. Meanwhile, ν(S–H) was not present in **L2** or its corresponding complex (**2**), which suggested that it appears in thione form. Thioamide band, ν(C = S), was located at 848.87 cm^{-1} for **2**, which demonstrated the shift to lower energy region (875.60 cm^{-1}) compared to that of **L2**. This could be due to the weakening of C = S bond with the coordination of silver *via* sulfur atom. The coordination of phosphorus to silver center was confirmed by the presence of characteristic peak ν(P–C_{ph}) in the region of 1091–1095 cm^{-1}. The sharp band around 1300 cm^{-1}, attributed to NO_3^- bend peak, demonstrated the presence of non-coordinated NO_3^- (outside the coordination sphere) in all five complexes in this study.

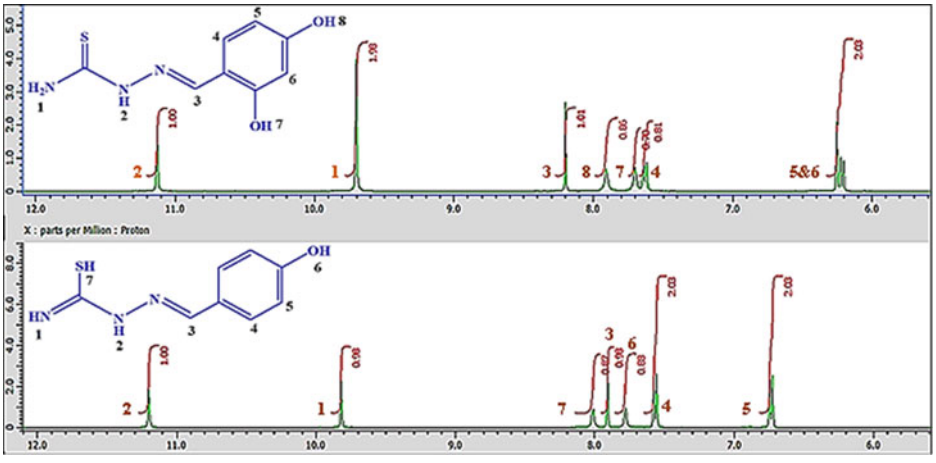


Figure 3. Comparison of ^{1}H NMR thione–thiol tautomerism between **L1** and **L2**.

3.1.2. NMR spectroscopy

The absence of NH_2 signal for **L1**, **L3**, **L4** and **L5** and their corresponding complexes of **1**, **3**, **4** and **5** indicated that they exist as thiols. Furthermore, the presence of S–H signal around 7.97–8.11 ppm for these ligands and 8.39–8.59 ppm for their corresponding complexes reaffirmed the formation of thiol tautomer. However, the NH_2 signal for **L2** was present at 9.70 ppm, whereas the NH_2 signal for its complex (**2**) was present at 9.86 ppm. Accordingly, the absence of S–H peak suggested that they appear as thiones instead. Figure 3 reveals the comparison of ^{1}H NMR thione–thiol tautomerism between **L1** and **L2**.

The spectra of silver(I) complexes closely resembled their respective free ligands but the major shift of S–H signal downfield in these complexes reaffirmed the occurrence of coordination of ligands to silver ions through sulfur atom of thiol without being deprotonated. Minor downfield shift of protons attached to N_1, N_2 of thiosemicarbazone and hydroxyl group in these complexes resulted upon their participation in hydrogen bonding, namely, intermolecular, intramolecular or both. The bonding of phosphine was proved by the presence of extra aromatic protons in the region of 7.15–7.50 ppm in these complexes, which were absent in the ligands of thiosemicarbazone. The latter conformation of the complex formation is in agreement with Altaf *et al.* [30].

Compared to the free ligands, there was an upfield shift in ^{13}C spectra of these complexes for thiocarbonyl carbon signal with the coordination of metal to the ligand through sulfur atom. The presence of *p*-tolyl methyl resonance in the region of 21.26–21.42 ppm distinguished the coordination of phosphine ligand to these complexes. The ^{13}C NMR signal for aromatic group of diphenyl(*p*-tolyl)phosphine was discovered in the form of doublet, specifically for C(-P), C-2 and C-3. Meanwhile, C-4 appeared as singlet. These results are indeed proven to be observed in other previous related study [38].

The chemical shifts in ^{31}P{^{1}H} NMR for all silver(I) complexes displayed one sharp singlet peak, which were attributed to diphenyl(*p*-tolyl)phosphine. This one peak confirmed the chemical environment of phosphorus atoms is chemically equivalent, hence

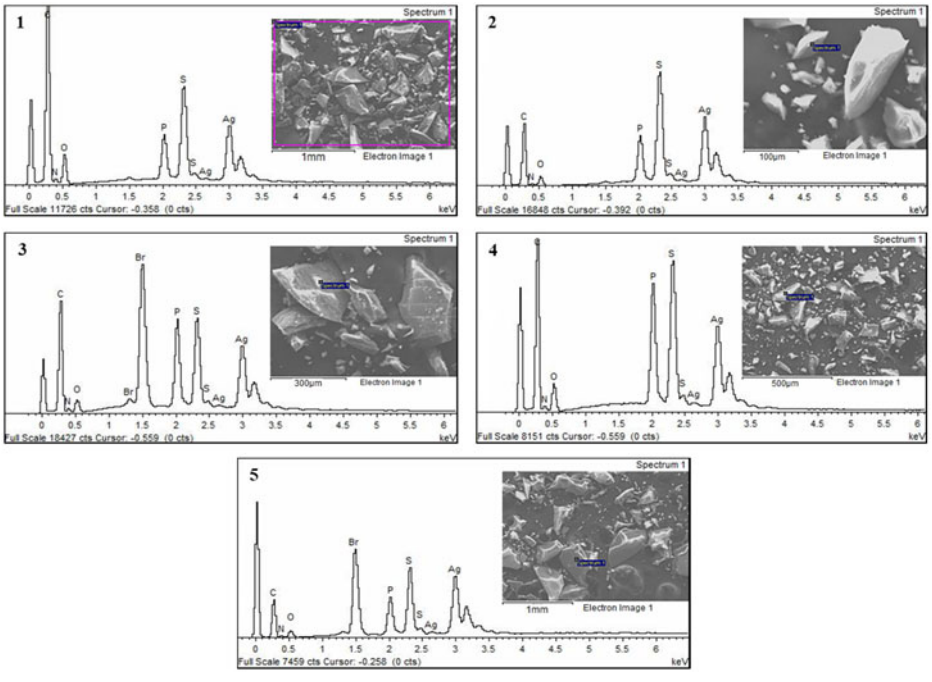


Figure 4. EDX spectra of complexes **1**–**5**.

the geometry around silver(I) ion should be tetrahedral and not square planar [39]. Compared to its free phosphine ligand, the resonance of phosphorus in these complexes shifted downfield, which confirmed the coordination of metal to ligand. Considering the limited studies on silver(I) diphenyl(*p*-tolyl)phosphine complex, the obtained chemical shifts of $^{31}P\{^{1}H\}$ for these complexes were compared to the observed PPh_3 values [30]. It should be noted that the observed values were rather similar and conclusively, the obtained results are in agreement with one another.

3.1.3. EDX analysis

The obtained complexes were subjected to EDX analysis to validate the presence of metal in the compound, as shown in Figure 4. The EDX analysis showed the presence of silver metal and other components that are present in all these synthesized complexes. Hence, this indicated successful metal–ligand complexation.

3.1.4. X-ray diffraction analysis

Several techniques were applied to crystallize these complexes to obtain suitable single-crystal for analysis including the previous successful mentioned methods [30, 38, 40]. Regrettably, to date, none of these methods were able to generate crystals that are suitable for single-crystal X-ray diffraction.

Therefore, PXRD has been carried out to study the degree of crystallinity of the metal complexes by observing the diffraction pattern in the range of 5–40° (2θ). Figure 5 shows the broad peaks in low intensities for the synthesized silver complexes **2**, **3**, **4** and **5** indicating their amorphous nature. Meanwhile, silver complex **1** displays crystalline nature based on the diffraction pattern as shown in Figure 6. Although it

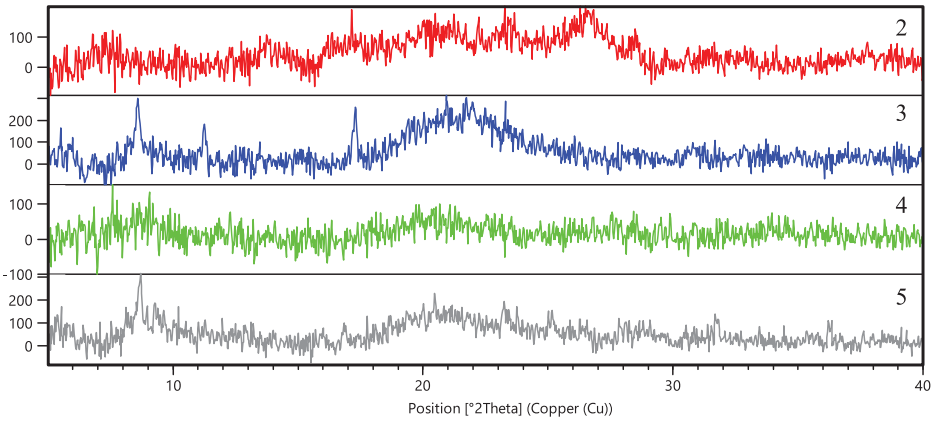


Figure 5. PXRD pattern of silver complexes **2**–**5**.

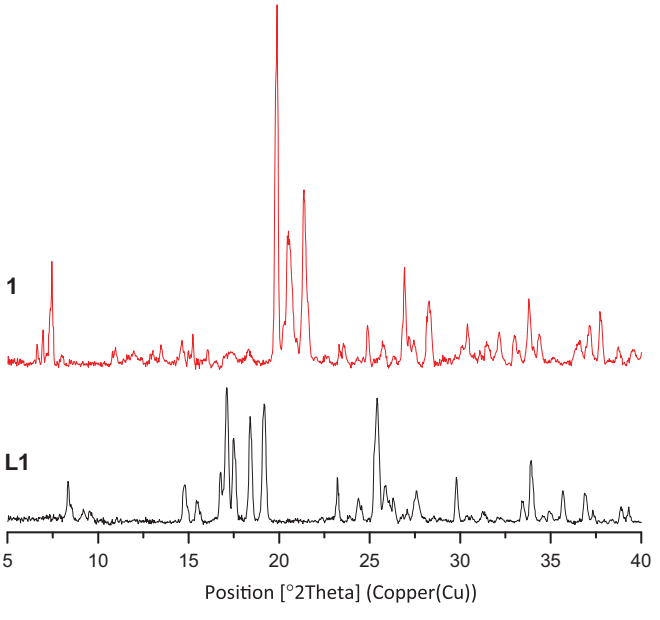


Figure 6. PXRD pattern of silver complex **1**.

was crystalline, unfortunately, the data were not sufficient to be analyzed by the single-crystal X-ray diffraction.

3.2. Antiproliferative activity

Transition metals such as silver have been long regarded as antimicrobial agents but their potential in cancer therapeutics has been underexplored [41, 42]. The *in vitro* anticancer activity of silver was previously reported [43]. Essentially, the advantage of silver is that it has lower toxicity compared to other metals such as platinum (e.g. cisplatin) [44]. Meanwhile, extensive antiproliferative activity of thiosemicarbazones was found on different tumor cell lines and display common features of other compounds

TABLE 1. The antiproliferative activities of silver complexes **1**–**5** (IC_{50} in μM).

Silver complex	MDA-MB-231	HT-29	MCF-7
1	4.45 ± 1.88	2.84 ± 0.27	3.89 ± 2.11
2	3.26 ± 1.37	2.83 ± 0.32	4.53 ± 1.86
3	4.66 ± 1.53	3.11 ± 0.56	3.74 ± 1.29
4	2.55 ± 0.67	2.20 ± 0.60	3.80 ± 1.88
5	3.68 ± 1.63	2.52 ± 0.68	3.20 ± 1.55

TABLE 2. The antimalarial, cytotoxicity (EC_{50} in μM) and selectivity index (SI) of silver complexes **1**–**5**.

Silver complex	HRPII	Normal MDBK	Selectivity index (SI)
1	1.45 ± 0.31	0.64 ± 0.06	0.44
2	1.16 ± 0.32	0.58 ± 0.08	0.50
3	2.02 ± 1.07	0.51 ± 0.02	0.25
4	1.69 ± 1.54	0.54 ± 0.02	0.32
5	0.75 ± 0.42	0.77 ± 0.16	1.03

with carcinogenic potency [45, 46]. On the other hand, it was demonstrated that silver phosphine compounds exerted *in vitro* antiproliferative effect [47, 48]. Based on IC_{50} values, all the synthesized compounds in this study displayed significantly high potential as antiproliferative agents for breast, MDA-MB-231, MCF-7 and colorectal HT-29 cell lines (Table 1). Complex **4** which consists of methoxy and hydroxyl group was found to have the best activity for colon cancer, HT-29 with IC_{50}=2.20 ± 0.60 μM and one of the breast cancer cell lines which is MDA-MB-231 with IC_{50}=2.55 ± 0.67 μM as compared to the other complexes. Meanwhile, breast cancer MCF-7 shows the best result with complex **5** with IC_{50}=3.20 ± 1.55 μM in the presence of bromine, hydroxyl and methoxy moieties. In comparison with clinically used metal compounds such as cisplatin, these compounds showed greater antiproliferative effect toward MDA-MB-231 (IC_{50}=25.28 μM) [49], MCF-7 (IC_{50}=35 μM) [50] and HT-29 (IC_{50}=5.28 μM) [51]. Thus, in-depth studies on these compounds as metallotherapeutic agents for cancer diseases are required.

3.3. Antiplasmodial activity

The obtained EC_{50} values of the complexes are shown in Table 2. All the complexes **1**–**5** showed significantly promising antiplasmodial activity with the obtained EC_{50} values ranged between 0.75 and 2.02 μM. Among these silver(I) complexes, **5** was found to be the most potent with the presence of hydroxyl, methoxy and bromine moieties in the aromatic ring of thiosemicarbazone. On the contrary, the absence of bromine (in **3**) and methoxy (in **4**) in the aromatic ring demonstrated almost two-fold reduction in the antiplasmodial activity. However, the antiplasmodial activity was maintained for complexes **1** and **2** with the presence of either one or two hydroxyl groups in the aromatic ring of thiosemicarbazone. In comparison with the commercialized antimalarials, the EC_{50} values of the silver complexes **1**–**5** were higher as most of the antimalarials such as pyrimethamine (antifolate), chloroquine (4-aminoquinoline) and artemisinin derivatives (endoperoxides) scored EC_{50} values lower than 0.1 μM against the drug-sensitive *P. falciparum* and more than 0.1 μM against the drug-resistant *P. falciparum* [52]. Nevertheless, the importance of each functional group in enhancing the

selectivity of silver complexes was unclear, which should be promptly addressed in future studies.

To know the safety of these complexes, the cytotoxicity against MDBK cells was further examined. The selectivity indices were calculated (Table 2) for all silver(I) complexes which revealed low SI values (<1). In other words, lack of selectivity was demonstrated as the inhibitory activity against the *P. falciparum* parasite was lower than the normal mammalian cell line, MDBK cells. Studies on silver compounds against malaria have been limited. A related study conducted in 1975 discovered that the splenectomized mice with the infection of *P. berghei* were cured when silver sulfadiazine was administered orally and subcutaneously in doses not exceeding 1050 mg/kg/day for 5 days to these CF-1 mice [53]. Following in 2013, another study reported that silver(I) complexes with *N*-heterocyclic carbine ligands showed promising *in vitro* antiplasmodial activity but with further testing, these compounds exhibited strong hemolytic properties on parasite culture even with the weakest doses, which indicated toxic activity [54]. In short, this study revealed that the toxicity of silver was debatable despite the recognition of silver as one of the least toxic metals [55].

4. Conclusion

A series of silver(I) complexes with mixed-ligands of thiosemicarbazone derivatives and diphenyl(*p*-tolyl)phosphine were synthesized. The silver atom was coordinated to the sulfur atom of thiosemicarbazone and phosphorus atom of diphenyl(*p*-tolyl) phosphine. Moreover, the obtained spectroscopic data agreed with suggested structures. Evaluation of these complexes against three cancer cell lines revealed potential in the antiproliferative activity as these complexes yielded low IC_{50} values (2.2–4.6 μM) in all cell lines. The antiplasmodial activity of these complexes against chloroquine-resistant *P. falciparum* parasite were also examined, which demonstrated good activity, but unfortunately they were not selective. Further modification on the structural frame of thiosemicarbazone or phosphine may improve the SI value.

Acknowledgement

This work was supported by the Research Grant University of Malaya (UMRG) - AFR (Frontier Science), Grant No. RG357-15AFR and Fundamental Research Grant Scheme (FRGS), Grant No. FP047-2014A.

Disclosure statement

No potential conflict of interest was reported by the author.

References

[1] T.S. Lobana, R. Sharma, G. Bawa, S. Khanna. *Coord. Chem. Rev.*, 253, 977 (2009).
[2] J.S. Casas, M.S. García-Tasende, J. Sordo. *Coord. Chem. Rev.*, 209, 197 (2000).
[3] T.S. Lobana, S. Khanna, R. Sharma, G. Hundal, R. Sultana, M. Chaudhary, R. Butcher, A. Castineiras. *Cryst. Growth Des.*, 8, 1203 (2008).

[4] R.B. de Oliveira, E.M. de Souza-Fagundes, R.P.P. Soares, A.A. Andrade, A.U. Krettli, C.L. Zani. *Eur. J. Med. Chem.*, 43, 1983 (2008).
[5] J.P. Mallari, W.A. Guiguemde, R.K. Guy. *Bioorg. Med. Chem. Lett.*, 19, 3546 (2009).
[6] R. Pingaew, S. Prachayasittikul, S. Ruchirawat. *Molecules*, 15, 988 (2010).
[7] D.B. Lovejoy, D.R. Richardson. *Blood*, 100, 666 (2002).
[8] A. Walcourt, M. Loyevsky, D.B. Lovejoy, V.R. Gordeuk, D.R. Richardson. *Int. J. Biochem. Cell Biol.*, 36, 401 (2004).
[9] X. Du, C. Guo, E. Hansell, P.S. Doyle, C.R. Caffrey, T.P. Holler, J.H. McKerrow, F.E. Cohen. *J. Med. Chem.*, 45, 2695 (2002).
[10] I. Chiyanzu, E. Hansell, J. Gut, P.J. Rosenthal, J.H. McKerrow, K. Chibale. *Bioorg. Med. Chem. Lett.*, 13, 3527 (2003).
[11] D.C. Greenbaum, Z. Mackey, E. Hansell, P. Doyle, J. Gut, C.R. Caffrey, J. Lehrman, P.J. Rosenthal, J.H. McKerrow, K. Chibale. *J. Med. Chem.*, 47, 3212 (2004).
[12] N. Fujii, J.P. Mallari, E.J. Hansell, Z. Mackey, P. Doyle, Y.M. Zhou, J. Gut, P.J. Rosenthal, J.H. McKerrow, R.K. Guy. *Bioorg. Med. Chem. Lett.*, 15, 121 (2005).
[13] S.D. Khanye, G.S. Smith, C. Lategan, P.J. Smith, J. Gut, P.J. Rosenthal, K. Chibale. *J. Inorg. Biochem.*, 104, 1079 (2010).
[14] M. Adams, L. Barnard, C. de Kock, P.J. Smith, L. Wiesner, K. Chibale, G.S. Smith. *Dalton Trans.*, 45, 5514 (2016).
[15] S. Halder, S.M. Peng, G.H. Lee, T. Chatterjee, A. Mukherjee, S. Dutta, U. Sanyal, S. Bhattacharya. *New J. Chem.*, 32, 105 (2008).
[16] D. Kovala-Demertzi, M.A. Demertzis, J.R. Miller, C. Papadopoulou, C. Dodorou, G. Filousis. *J. Inorg. Biochem.*, 86, 555 (2001).
[17] C. Santini, M. Pellei, G. Papini, B. Morresi, R. Galassi, S. Ricci, F. Tisato, M. Porchia, M.P. Rigobello, V. Gandin, C. Marzano. *J. Inorg. Biochem.*, 105, 232 (2011).
[18] J.J. Liu, P. Galettis, A. Farr, L. Maharaj, H. Samarasinha, A.C. McGechan, B.C. Baguley, R.J. Bowen, S.J. Berners-Price, M.J. McKeage. *J. Inorg. Biochem.*, 102, 303 (2008).
[19] M.C. Keage, J. Mark, P. Papathanasiou, G. Salem, A. Sjaarda, G.F. Swiegers, P. Waring, S.B. Wild. *Met. Based Drugs*, 5, 217 (1998).
[20] S.J. Berners-Price, R.J. Bowen, P. Galettis, P.C. Healy, M.J. McKeage. *Coord. Chem. Rev.*, 185–186, 823 (1999).
[21] S.J. Berners-Price, P.J. Sadler. Bioinorganic Chemistry. Springer Berlin Heidelberg, Berlin, Heidelberg (1988), pp. 27–102.
[22] A. Molter, J. Rust, C.W. Lehmann, G. Deepa, P. Chiba, F. Mohr. *Dalton Trans.*, 40, 9810 (2011).
[23] A. Castineiras, R. Pedrido. *Inorg. Chem.*, 47, 5534 (2008).
[24] T.S. Lobana, G. Bhargava, V. Sharma, M. Kumar. *Indian J. Chem. A*, 42, 309 (2003).
[25] T.S. Lobana, S. Khanna, A. Castineiras. *Inorg. Chem. Commun.*, 10, 1307 (2007).
[26] T.S. Lobana, S. Khanna, G. Hundal, P. Kaur, B. Thakur, S. Attri, R.J. Butcher. *Polyhedron*, 28, 1583 (2009).
[27] N.V. Loginova, T.V. Koval'chuk, A.T. Gres, N.P. Osipovich, G.I. Polozov, Y.S. Halauko, Y.V. Faletrov, H.I. Harbatsevich, A.V. Hlushko, I.I. Azarko, Y.V. Bokshits. *Polyhedron*, 88, 125 (2015).
[28] E. Shahsavani, A.D. Khalaji, N. Feizi, M. Kucerakova, M. Dusek. *Inorg. Chim. Acta*, 429, 61 (2015).
[29] A. Benmohammed, O. Khoumeri, A. Djafri, T. Terme, P. Vanelle. *Molecules*, 19, 3068 (2014).
[30] M. Altaf, H. Stoeckli-Evans, A. Cuin, D.N. Sato, F.R. Pavan, C.Q.F. Leite, S. Ahmad, M. Bouakka, M. Mimouni, F.Z. Khardli, T.B. Hadda. *Polyhedron*, 62, 138 (2013).
[31] V. Vichai, K. Kirtikara. *Nat. Protoc.*, 1, 1112 (2006).
[32] H. Noedl, J. Bronnert, K. Yingyuen, B. Attlmayr, H. Kollaritsch, M. Fukuda. *Antimicrob. Agents Chemother.*, 49, 3575 (2005).
[33] H. Noedl, W.H. Wernsdorfer, R.S. Miller, C. Wongsrichanalai. *Antimicrob. Agents Chemother.*, 46, 1658 (2002).

[34] M.R. Mohd Abd Razak, A. Afzan, R. Ali, N.F. Amir Jalaluddin, M.I. Wasiman, S.H. Shiekh Zahari, N.R. Abdullah, Z. Ismail. *BMC Complement Altern. Med.*, 14, 492 (2014).
[35] H. Noedl, W.H. Wernsdorfer, H. Kollaritsch, S. Looareesuwan, R.S. Miller, C. Wongsrichanalai. *Wiener Klinische Wochenschrift*, 115, 23 (2003).
[36] T. Mosmann. *J. Immunol. Methods*, 65, 55 (1983).
[37] C.N.R. Rao, R. Venkataraghavan, T.R. Kasturi. *Can. J. Chem.*, 42, 36 (1964).
[38] S. Nawaz, A.A. Isab, K. Merz, V. Vasylyeva, N. Metzler-Nolte, M. Saleem, S. Ahmad. *Polyhedron*, 30, 1502 (2011).
[39] A. Dehno Khalaji, E. Shahsavani, N. Feizi, M. Kucerakova, M. Dusek, R. Mazandarani. *Comptes Rendus Chimie*, 20, 534 (2017).
[40] T.S. Lobana, S. Khanna, G. Hundal, B.J. Liaw, C.W. Liu. *Polyhedron*, 27, 2251 (2008).
[41] B. Desoize. *Anticancer Res.*, 24, 1529 (2004).
[42] C.N. Banti, S.K. Hadjikakou. *Metallomics*, 5, 569 (2013).
[43] W.J. Youngs, N. Robishaw, M.J. Panzner, K. Hindi, D.A. Medvetz, J. Youngs, C. Tessier, A. Ditto, Y.H. Yun, J. Bauer. *Nanotech Conf. Expo*, 2, 5 (2009).
[44] S. Rafique, M. Idrees, A. Nasim, H. Akbar, A. Athar. *Biotechnol. Mol. Biol. Rev.*, 5, 38 (2010).
[45] S. Arora, S. Agarwal, S. Singhal. *Int. J. Pharm. Pharm. Sci.*, 6, 34 (2014).
[46] M. Serda, D.S. Kalinowski, N. Rasko, E. Potůčková, A. Mrozek-Wilczkiewicz, R. Musiol, J.G. Małecki, M. Sajewicz, A. Ratuszna, A. Muchowicz. *PLoS One*, 9, e110291 (2014).
[47] S. Zartilas, S.K. Hadjikakou, N. Hadjiliadis, N. Kourkoumelis, L. Kyros, M. Kubicki, M. Baril, I.S. Butler, S. Karkabounas, J. Balzarini. *Inorg. Chim. Acta*, 362, 1003 (2009).
[48] L. Kyros, N. Kourkoumelis, M. Kubicki, L. Male, M.B. Hursthouse, I.I. Verginadis, E. Gouma, S. Karkabounas, K. Charalabopoulos, S.K. Hadjikakou. *Bioinorg. Chem. Appl.*, 2010, 12 (2010).
[49] S. Wang, J. Xie, J. Li, F. Liu, X. Wu, Z. Wang. *Am. J. Cancer Res.*, 6, 1108 (2016).
[50] H. Mansouri-Torshizi, E. Rezaei, F. Kamranfar, M. Heidari Majd. *Adv. Pharm. Bull.*, 6, 449 (2016).
[51] B. Xu, F. Chu, Y. Zhang, X. Wang, Q. Li, W. Liu, X. Xu, Y. Xing, J. Chen, P. Wang, H. Lei. *Int. J. Mol. Sci.*, 16, 21035 (2015).
[52] M. Delves, D. Plouffe, C. Scheurer, S. Meister, S. Wittlin, E.A. Winzeler, R.E. Sinden, D. Leroy. *PLoS Med.*, 9, e1001169 (2012).
[53] M.S. Wysor. *Chemotherapy*, 21, 302 (1975).
[54] C. Hemmert, A. Fabie, A. Fabre, F. Benoit-Vical, H. Gornitzka. *Eur. J. Med. Chem.*, 60, 64 (2013).
[55] K.M. Hindi, M.J. Panzner, C.A. Tessier, C.L. Cannon, W.J. Youngs. *Chem. Rev.*, 109, 3859 (2009).