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Exploring the Versatility of Cycloplatinated Thiosemicarbazones as Antitumor and Antiparasitic Agents

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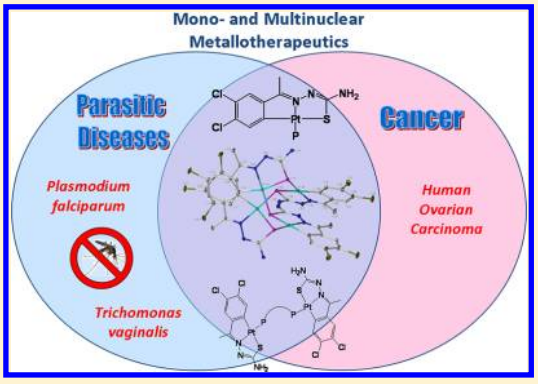
Supporting Information

ABSTRACT: Tridentate cycloplatinated thiosemicarbazone complexes have been prepared from a biologically significant ligand, 3,4-dichloroacetophenone thiosemicarbazone (**1**). The tetranuclear complex **2** was prepared by reaction of the ligand with $K_2[PtCl_4]$. Two mononuclear (**3** and **4**) and two dinuclear (**5** and **6**) complexes were isolated upon cleavage of the Pt−$S_{bridging}$ bonds of the tetranuclear complex **2** with the appropriate phosphane ligand. Each complex was characterized using various analytical and spectroscopic techniques, and the molecular structures of **2**−**4** were also elucidated. The *in vitro* antiparasitic activities of these complexes against *Plasmodium falciparum* strains (D10 (chloroquine sensitive) and Dd2 (chloroquine resistant)) and *Trichomonas vaginalis* have been determined. Preliminary studies into their potential plasmodial target in the form of β-hematin formation inhibition assays were also completed. Preliminary results suggest that ligand **1** and complex **3** do not hinder formation of β-hematin. The antiproliferative activity of the complexes against the cisplatin-senstive A2780 and cisplatin-resistant A2780cisR human ovarian cancer cell lines has been evaluated. The complexes were found to exhibit moderate to weak inhibitory activities.



INTRODUCTION

Thiosemicarbazones are a class of Schiff base compounds that contain a thiourea moiety. They are well-known for their biological activity as antiparasitics,[1−6] antibacterial[7−9] and antitumoral agents.[10−14] As chemotherapeutics their mode of action is believed to be through the inhibition of ribonucleotide reductase, thus arresting DNA synthesis.[15] As antiparasitics, thiosemicarbazones may affect processes, some associated with hemoglobin (Hb) digestion in the food vacuole of the parasite through several possible inhibitory mechanisms of action. Their ability to chelate endogenous metals such as Fe(III) can inhibit parasite growth by withholding it from metal-dependent enzymes such as ribonucleotide reductase and enzymes in the heme biosynthetic pathway.[4,16] Alternatively, the resulting chelate complexes could inhibit cysteine proteases, effectively enhancing the natural inhibitory effect of endogenous metals on the protease catalytic site.[17] Another possible mechanism of cysteine protease inhibition could be through covalent modification of the cysteine thiol groups within the parasite via electrophilic centers (the thione carbon and/or imine carbon) of the thiosemicarbazone moiety. All of the above inhibitory mechanisms would compromise the parasite's ability to degrade host hemoglobin required for (parasite) protein synthesis.[18−21]

The ability of thiosemicarbazones to form stable complexes with a variety of metals is also believed to be of great importance to their biological activities. The lipophilic nature of the complex might be enhanced in comparison to the free ligand, and certain long-term side effects may decrease or be altogether avoided, since the coordinated ligand of the metal complexes can dissociate and the metal ion can then interact with the organism.[22−24] In addition, the metal complex could exhibit bioactivities not shown by the free thiosemicarbazone ligand and coordination could yield a significant reduction in drug resistance.[25] Research into the synthesis and application of thiosemicarbazone metal complexes as biological agents is thus

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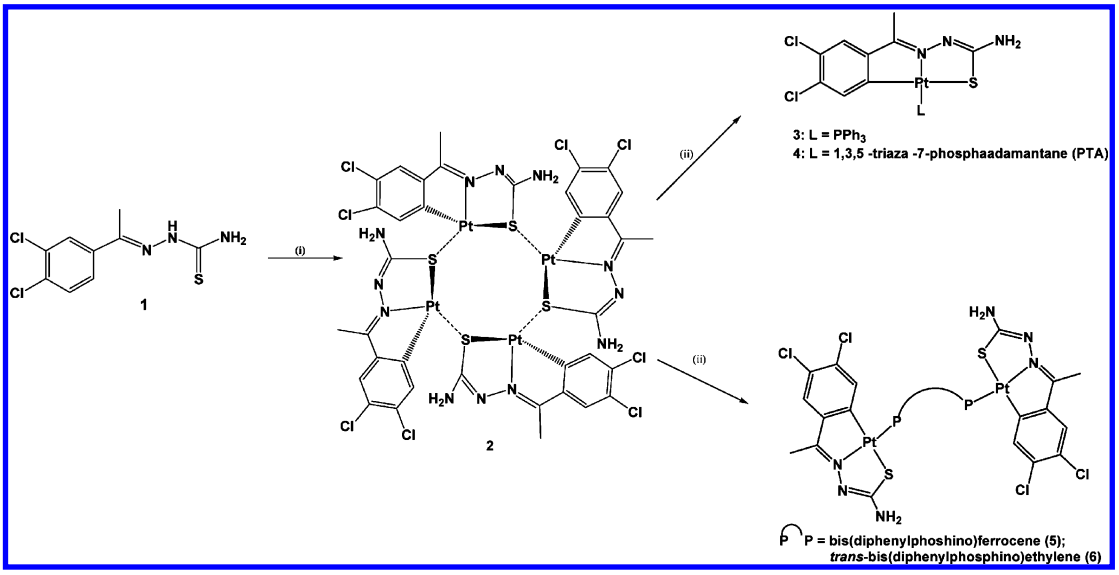
Scheme 1



Reaction conditions: (i) $K_2[PtCl_4]$, EtOH–H_2O, 50 °C, 5 days; (ii) L or P^P, acetone, room temperature, 3 h.

an area of great promise, and there have been several reports on this subject published by us[26−29] as well as others.[30−37]

The discovery of cisplatin and its analogues as antitumoral agents had a profound influence on establishing the field of metal complexes in medicine,[38] and the amount of research already accomplished within this area is substantial.[39−44] Despite the considerable quantity of work completed, there are still many little-researched avenues such as the preparation of mono- and polynuclear complexes from biologically active hybrid ligands. Hybrid ligands are defined as bis- or polydentate molecules which contain at least two different functionalities containing donor atoms capable of binding to a metal.[45] Thiosemicarbazones are prime hybrid candidates, as they fulfill this basic criterion. Recently, we have explored the chemistry and application of biologically significant thiosemicarbazones and their platinum group metal (PGM) complexes. Our studies have included the synthesis of ruthenium-, palladium-, and platinum-containing thiosemicarbazones.[26−29] To demonstrate the versatility of these complexes, we have extended our studies into their biological and catalytic applications and the results so far have been promising.[27−29,46,47] Our studies of their inhibitory effects against malarial and *T. vaginalis* parasitic strains are among the first to be reported in the literature for palladium(II) thiosemicarbazone complexes.[28,29,48] In light of these results, together with recent research carried out in other laboratories,[33,49−54] we have been encouraged to expand our studies into the biological relevance of thiosemicarbazone PGM complexes.

As part of our continuing search for improved metallotherapeutics, we have now turned our attention to cycloplatinated thiosemicarbazone complexes. In this paper, we report the synthesis and structural characterization of five new cycloplatinated thiosemicarbazone complexes. The preliminary biological screening of these complexes for inhibitory effects against chloroquine-resistant and chloroquine-sensitive *Plasmodium falciparum* strains, *Trichomonas vaginalis*, and cisplatin-resistant (A2780cisR) and cisplatin-sensitive ovarian (A2780) cancer cell lines is also reported.

RESULTS AND DISCUSSION

Synthesis and Characterization. The tetranuclear complex **2** was prepared by stirring ligand **1** and $K_2[PtCl_4]$ in an ethanol–water mixture at 50 °C for 5 days (Scheme 1). The mono- and diplatinum complexes were synthesized by cleavage of the bridging Pt–S bonds of **2** with either a mono- or diphosphane in acetone at room temperature.

On examination of the ^{1}H NMR spectral data for complex **2**, the absence of proton resonances for the thioamide and ortho protons (observed at 10.20 and 8.20 ppm, respectively, for **1**) presents evidence of the coordination of the ortho carbon to platinum and the formation of a second imine bond, thus suggesting that sulfur bonds to the metal in the thiolato form. In comparison to the tetranuclear complex **2**, a slight upfield shift is observed for the thiosemicarbazone aromatic protons of complexes **3**, **5**, and **6**. In complexes **3**, **5**, and **6**, platinum satellites are observed for the proton of the carbon adjacent to the ortho-metalated carbon. Figure 1 shows the proton NMR spectrum for complex **3**, where these satellite peaks are clearly

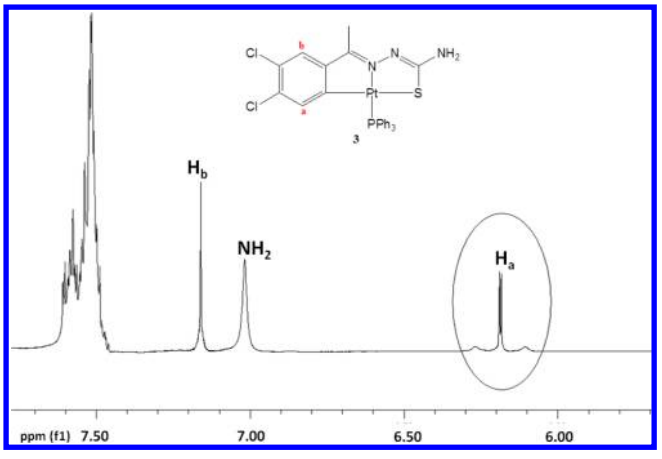


Figure 1. Expansion of the ^{1}H NMR aromatic region of complex **3**. The proton resonance with Pt satellites is circled.

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visible. A coupling constant of 20−25 Hz is observed, and this corresponds to a ^{3}J coupling of ^{195}Pt.[55,56]

A downfield shift of the azomethyl protons by approximately 0.25 ppm is observed for **3**−**6** in comparison to **2**. This is consistent with deshielding of these protons upon coordination of the imine nitrogen to the metal. For complex **4**, two sets of resonances are observed for the PTA ancillary ligand. A singlet at 4.20 ppm is assigned to the CH_2 protons adjacent to phosphorus. The CH_2 protons of the N−CH_2−N moiety usually exhibit two doublets due to geminal coupling between $H_{equatorial}$ and H_{axial}.[57,58] In complex **4**, these protons are observed as a quartet, possibly due to the influence of platinum.

In the $^{13}C\{^{1}H\}$ NMR spectra, the thiolato carbon resonance shifts from 179.1 ppm in the free ligand **1** to 166.6 ppm in complex **2**, further confirming deprotonation and coordination of sulfur in the thiolato form. The *ortho* carbon resonance shifts downfield from 128.0 to 153.8 ppm, displaying evidence of cyclometalation, and the imine carbon also shifts downfield as a result of coordination of the imine nitrogen to platinum. For complexes **3**−**6**, the thioamide (C−S) carbon resonates at approximately 177.0 ppm, similar to that of the tetranuclear complex **2**. The ortho carbon is observed between 154.0 and 155.0 ppm. The chloro-substituted carbons of the thiosemicarbazone ligand appear to be equivalent and are observed at ca. 131.0 ppm for all complexes except complex **4**. In complex **4**, two distinct resonances are seen at 135.3 and 133.15 ppm. The $^{31}P\{^{1}H\}$ NMR analysis for complexes **3**−**5** revealed one singlet for the phosphorus nuclei with platinum satellites due to platinum−phosphorus coupling. A ^{1}J coupling constant of between 1680 and 1888 Hz was observed, and this is consistent with the literature values for analogous cycloplatinated compounds.[59] In complex **6**, where the bridging ligand is *trans*-bis(diphenylphosphino)ethylene, the phosphorus nucleus resonates as a triplet with platinum satellites and is a consequence of phosphorus−phosphorus and phosphorus−platinum coupling. For complex **2**, infrared spectral analysis revealed two strong absorption bands (three observed for the ligand) in the N−H region at 3321 and 3149 cm^{-1}, agreeing with the presence of two N−H bonds for the primary amine in the complex after cyclometalation. Absorption bands at 1598 and 1561 cm^{-1} correspond to two C═N bonds, the high-frequency band being assigned to the newly formed imine and the lower energy band to the metal-coordinated imine (via nitrogen). For the mononuclear (**3** and **4**) and dinuclear (**5** and **6**) complexes, similar absorption bands are observed.

X-ray Structure Analysis. Single crystals of complexes **2**−**4** suitable for single-crystal X-ray diffraction were obtained by exposing dimethyl sulfoxide solutions of each complex to air. Crystal data for complexes **2**·3DMSO,[60] **3**·2DMSO,[61] and **4**[62] are summarized in the reference section, and Table 1 gives selected bond angles and lengths.

Complex **2** (Figure 2) crystallizes with three solvent molecules of DMSO with a monoclinic system. Each platinum center is bridged by a sulfur of the adjacent Pt−thiosemicarbazone unit to form an eight-membered Pt_4S_4 core, around which the four thiosemicarbazone ligands reside. Each metal center is coordinated to the imine nitrogen, ortho carbon, and sulfur of one thiosemicarbazone ligand, and the fourth coordination site is occupied by the sulfur of the adjacent thiosemicarbazone ligand. A slightly distorted square-planar geometry is observed around each metal center.

The bond lengths observed between platinum and each coordinated atom agree with those reported for similar tetranuclear platinum thiosemicarbazone complexes.[63] A lengthening of the C−S bond for S(1A)−C(9A) and S(1B)−C(9B) is observed with values for the bond distances in close agreement with the bond length expected for a C−S single bond (1.82 Å) rather than a normal C═S double bond (1.56 Å),[64] corroborating that sulfur coordinates to palladium in the thiolato form.[65] The bond angles N(1A)−Pt(1A)−S(1B)#2, N(1B)−Pt(1B)−S(1A), C(1A)−Pt(1A)−S(1A), and C(1B)−Pt(1B)−S(1B) are all less than 180°, confirming the slight deviation from the expected square-planar geometry around platinum.

Table 1. Selected Bond Lengths (Å) and Angles (deg) for 2−4

	2·3DMSO			
	A	B	3·2DMSO	4
Pt1−C1	2.004(3)	1.999(4)	2.039(3)	2.027(3)
Pt1−N1	1.996(3)	1.995(3)	2.038(2)	2.035(2)
Pt1−S1	2.3169(8)	2.3513(8	2.2359(7)	2.3322(11)
Pt1−P1			2.2359(7)	2.2101(7)
N1−C7	1.307(4)	1.302(5)	1.298(3)	1.301(3)
N2−C9	1.293(5)	1.300(5)	1.316(4)	1.313(3)
C1−Pt1−S1	164.12(11)	100.70(3)	163.57(8)	163.74(7)
C1−Pt1−P1			97.00(8)	98.56(8)
N1−Pt1−C1	80.61(13)	81.42(15)	80.78(10)	80.73(9)
N1−Pt1−S1	83.97(9)	84.08(9)	82.80(7)	83.07(6)
N1−Pt1−P1			177.17(7)	168.56(6)
P1−Pt1−S1			99.43(3)	97.59(3)

The mononuclear complexes **3** and **4** (parts a and b, respectively, of Figure 3) crystallize in the orthorhombic and monoclinic systems, respectively. The molecular structure of each complex confirms that the platinum−sulfur bridging bonds in complex **2** are cleaved and the tridentate coordination of the thiosemicarbazone remains intact. As with **2**, there is a distorted square-planar coordination sphere around the metal and this is confirmed by the slight deviation of the bond angles observed around platinum for complexes **3** and **4**.

The bond distances observed between the metal and the coordinated carbon, nitrogen, and sulfur atoms in **3** and **4** are similar to those in the tetranuclear complex **2**, indicating that the electron density distribution between platinum and the thiosemicarbazone ligand does not change after cleavage. These bond distances are also in close agreement with similar mononuclear platinum thiosemicarbazone complexes.[59,66]

***In Vitro* Antitumor Activity.** The *in vitro* antiproliferative effects of compounds **1**−**6** were evaluated against the cisplatin-sensitive (A2780) and cisplatin-resistant (A2780cisR) ovarian cancer cell lines. Table 2 summarizes the cytotoxic values obtained.

Ligand **1** exhibited the highest activity out of all the compounds tested against both cell lines. Complexes **2**−**5** are moderately cytotoxic against both cell lines, with complex **4** showing the lowest IC_{50} values. Complexes **2**, **4**, and **5** are less active against the cisplatin-resistant (A2780cisR) cell line, while complex **3** is more active in this cell line in comparison to A2780. While none of the compounds tested showed activities comparable to cisplatin against the A2780 cell line, ligand **1** shows a markedly better activity than cisplatin against the A2780cisR line. Metalation of the thiosemicarbazone ligand seems to have an adverse effect on cytotoxicity, and the number of platinum thiosemicarbazone moieties does not appear to influence the biological activity. The tetraplatinum complex **2** is

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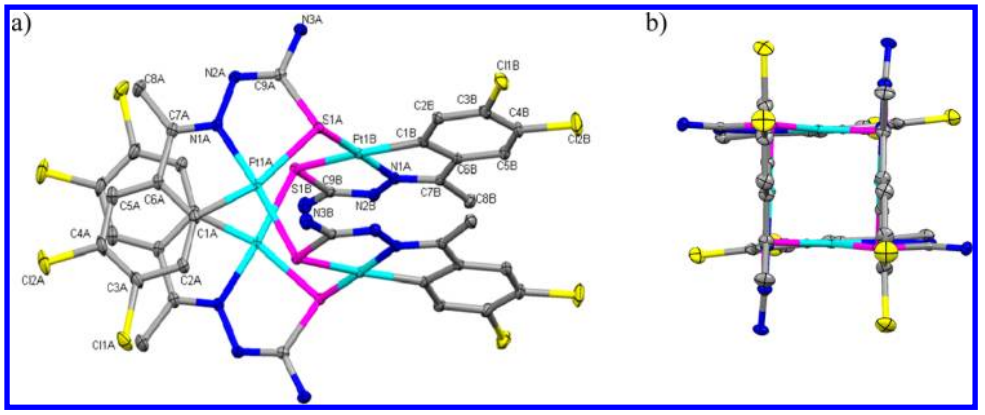

Figure 2. (a) Molecular structure of **2**·3DMSO with ellipsoids at the 25% probability level and another half-molecule generated via the symmetry code $1 - x, y, 0.5 - z$. Solvent molecules and hydrogen atoms have been omitted for clarity. (b) View of complex **2** down the 010 plane, showing the ring formed by the Pt_4S_4 core. Atom labeling, solvent molecules, and hydrogen atoms are omitted for clarity.

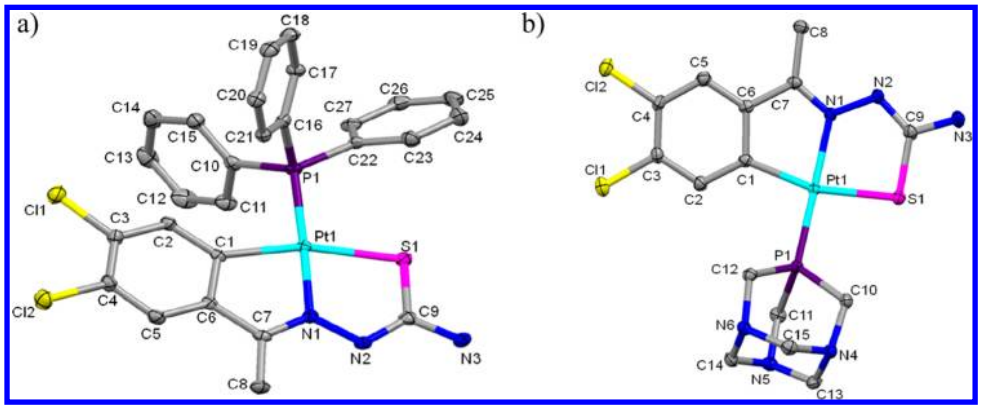

Figure 3. (a) Structure of complex **3**·2DMSO with ellipsoids at the 30% probability level. Solvent molecules and hydrogen atoms have been omitted for clarity. (b) Structure of complex **4** with ellipsoids at the 40% probability level. Hydrogen atoms are omitted for clarity.

Table 2. IC_{50} Determinations for 1−6 against A2780 and A2780cisR Cancer Cell Lines

	IC_{50}[a] (μM)	
compd	A2780	A2780*cis*R
1	11.3 ± 2.7	13.8 ± 2.6
2	51.5 ± 1.0	67 ± 10
3	129 ± 31	99.0 ± 4.7
4	21.2 ± 1.2	32 ± 7
5	36.5 ± 3.6	>200
6	>200	175 ± 1.7
cisplatin[b]	1.5	25

[a]IC_{50}: minimum concentration for 50% cell death. [b]IC_{50} values for cisplatin were determined using the same experimental assay and protocol as for **1**−**6** but were not determined at the same time as compounds **1**−**6**.

more active than complex **6** (containing the bis-(diphenylphosphino)ethylene spacer) but is less active than the dppf-containing complex **5**. The type of ancillary ligand used in the design of platinum thiosemicarbazone chemotherapeutics may play a key role in enhancing activity. The mononuclear complex **4**, containing the most hydrophilic phosphane ligand, showed the best activity, followed by **2**, which has no phosphane ligands. Complex **6** shows moderate activity against the A2780 line yet was not active in the cisplatin-resistant cell line.

***In Vitro* Antiparasitic Activity.** The antiparasitic activity of complexes **2**−**6** *in vitro* against two *Plasmodium falciparum* strains, D10 (chloroquine-sensitive) and Dd2 (chloroquine-resistant), and the parasite *Trichomonas vaginalis* was also determined. For the *in vitro* assays, the complexes were tested as suspensions if not completely dissolved in the assay medium. Table 3 gives the antiplasmodial data ascertained for complexes **2**−**6**.

Complexes **2**−**4** shows only moderate activity compared to chloroquine, while **5** and **6** show no activity at the highest compound concentration tested. Complex **3**, which contains the triphenylphosphane ancillary ligand, shows better activity compared to **4** (containing a PTA ancillary ligand) against both

Table 3. Antiplasmodial Data Obtained for Complexes 2−6

	IC_{50}[a] (μM)	
complex	D10	Dd2
2	32.29 ± 2.28	not tested
3	19.93 ± 3.74	14.47 ± 1.98
4	21.42 ± 1.22	24.90 ± 3.24
5	not active[b]	not tested
6	not active[b]	not tested
chloroquine	0.012 ± 0.001	0.146 ± 2.08

[a]The minimum compound concentration required for 50% inhibition in vitro. [b]Up to the highest compound concentration tested (100 μg/cm^3).

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P. falciparum strains. The PTA ligand is widely used in the design and synthesis of metallotherapeutics, as it is a water-soluble compound that is believed to enhance the hydrophilicity of the metal complex.[67,68] Against the Dd2 strain, complex **3** shows enhanced activity (IC_{50} = 14.47 μM) in comparison to the D10 strain. None of the compounds tested show activities comparable to chloroquine against both strains.

In contrast to the inhibitory studies for *P. falciparum*, complex **4** is the only compound that showed a significant percentage inhibition (92.5%) of the *Trichomonas vaginalis* parasite (Table 4). Metronidazole, the current FDA-approved

Table 4. Preliminary Inhibition Data of Compounds 1−6 against *T. vaginalis*

compd	inhibition at 100 μM (%)	IC_{50} T1 strain (μM)
1	51.3	
2	68.7	
3	42.3	
4	92.5	21.1
5	24.5	
6	42.3	
metronidazole[a]	100.0	0.72

[a]Current FDA-approved treatment for *T. vaginalis* infections.

treatment, exhibited a 100% inhibition at this concentration (100 μM), comparable to that for **4**. Since **4** was deemed to be the most potent inhibitor out of all compounds tested, its IC_{50} value was determined and it was found to be 21.1 μM. Although this compound is not as potent as metronidazole, its inhibitory effect suggests that their targets are necessary for parasite viability.

Cysteine proteases are integral to the parasitic life cycle. They are involved in several parasite functions, including host invasion, nutrition, and protein processing.[69] Thiosemicarbazones are believed to inhibit parasite growth through reversible interactions with cysteine proteases in the parasite, and a recent study of the interactions of Pt(II) complexes and cysteine proteases revealed that Pt(II) complexes can bind to the active site cysteine of different cysteine proteases.[70] Furthermore, it was found that the presence of an aromatic ring enhanced the inhibitory activity of these complexes in comparison to the clinical drugs cisplatin and carboplatin. However, the type and rate of active site cysteine binding were unique for each cysteine protease. Different cysteine proteases have structurally different folds, and these can sterically influence how the complex binds to the cysteine protease targets within each parasite.[70] This could account for the contrasting activities observed for complexes **3** and **4** against the *P. falciparum* and *T. vaginalis* parasite strains. Moreover, it has been shown that the PTA ligand forms specific interactions within the active site of a cysteine protease when bound to a different metal center.[71]

β-Hematin Inhibition Assay. In the life cycle of the *Plasmodium falciparum* parasite, degradation of the infected host's hemoglobin to provide essential amino acids for its growth and nutrition is a vital step.[72] A side product of this hemoglobin digestion is free heme, which is toxic to the parasite. The parasite removes this threat by conversion of the free heme into a crystalline solid known as hemozoin, which is nontoxic to the parasite. Thus, this process is one potential target in the design of possible antiplasmodial therapeutics. The clinically used drugs amodiaquine and chloroquine are believed to inhibit the formation of hemozoin.[73] The ability of a potential drug to inhibit formation of hemozoin can be measured using a β-hematin (synthetic hemozoin) inhibition assay. Complex **3** exhibits the best antiplasmodial activity against the two malarial strains tested and was thus chosen, along with ligand **1**, for β-hematin inhibition studies. Compounds **1** and **3** were tested using a modified NP-40 detergent mediated hematin formation screen.[74] Figure 4 shows

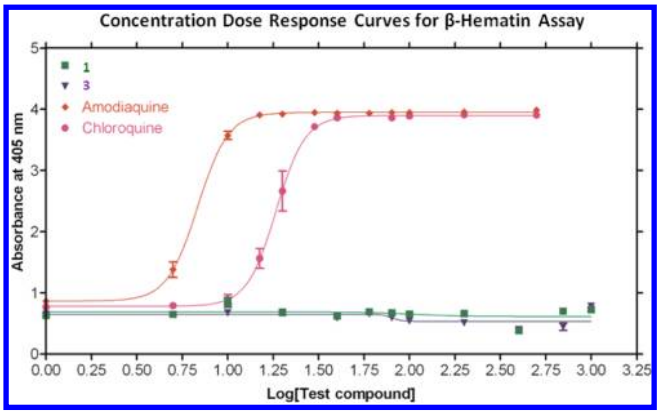

Figure 4. Concentration dose−response curves for NP-40 detergent mediated β-hematin assays of **1**, **3**, amodiaquine, and chloroquine.

the dose−response curves obtained for compounds **1** and **3** along with amodiaquine and chloroquine. Neither ligand **1** nor complex **3** is found to hinder β-hematin formation at the concentrations tested, in comparison to amodiaquine and chloroquine. This result suggests that arylthiosemicarbazones and their cycloplatinated complexes target other processes within the *Plasmodium falciparum* parasite, possibly cysteine protease function.

CONCLUSIONS

Five new cycloplatinated thiosemicarbazone complexes (**2**−**6**) have been prepared using the ligand 3,4-dichloroacetophenonethiosemicarbazone (**1**). The molecular structures of **2**−**4** show that the ligand coordinates to platinum in a tridentate manner via the ortho carbon, imine nitrogen, and thiolato sulfur. All of the compounds prepared were screened for antiparasitic activity against *Plasmodium falciparum* and *Trichomonas vaginalis* strains and antitumor activity against cisplatin-sensitive and -resistant human ovarian cancer cell lines. They were found to exhibit moderate to low parasite inhibition and antiproliferative activities. When ligand **1** and complex **3** were evaluated for their ability to inhibit β-hematin formation, it was found that the compounds do not target this process. Thiosemicarbazones and their metal complexes may act upon cysteine protease functions within the parasite, and a study of their interaction with different cysteine protease enzymes must be carried out to validate this hypothesis.

EXPERIMENTAL SECTION

General Methods. Thiosemicarbazide, 3,4-dichloroacetophenone, triphenylphosphane, 1,3,5-triaza-7-phosphaadamantane, bis-(diphenylphosphino)ferrocene, and *trans*-bis(diphenylphosphino)-ethylene were purchased from Sigma-Aldrich and used without further purification. Potassium tetrachloroplatinate was kindly donated by AngloAmerican Platinum Ltd. Ligand **1**, 3,4-dichloroacetophenone-thiosemicarbazone, was prepared using a previously reported literature method.[6] All solvents used were analytical grade and were dried over molecular sieves. Nuclear magnetic resonance (NMR) spectra were

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recorded on a Varian Unity XR400 (^{1}H at 399.95 MHz, ^{13}C at 100.58 MHz, ^{31}P at 161.90 MHz), Varian Mercury XR300 (^{1}H at 300.08 MHz, ^{13}C at 75.46 MHz, ^{31}P at 121.47 MHz), or Bruker Biospin GmbH (^{1}H at 400.22 MHz, ^{13}C at 100.65 MHz, ^{31}P at 162.00 MHz) spectrometer at ambient temperature. Chemical shifts for ^{1}H and ^{13}C{^{1}H} NMR are reported using tetramethylsilane (TMS) as the internal standard, and ^{31}P{^{1}H} NMR spectra were measured relative to H_3PO_4 as the external standard. NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d_6) unless otherwise stated. Infrared absorptions (IR) were measured on a Perkin-Elmer Spectrum 100 FT-IR spectrometer as KBr pellets. Microanalyses for C, H, and N were carried out using a Thermo Flash 1112 Series CHNS-O analyzer, and melting points were determined using a Büchi B-540 melting point apparatus. Mass spectrometry determinations were carried out on all new compounds using either electron impact (EI) on a JEOL GC Matell instrument or electrospray ionization (ESI) on a Waters API Quattro Micro instrument in either the positive or negative mode.

Synthesis of [Pt(3,4-dichloroacetophenone thiosemicarbazone)]$_4$ (2). $K_2[PtCl_4]$ (0.862 g, 2.08 mmol) was dissolved in deionized water (10 cm^3), and ethanol (150 cm^3) was added. The ligand **1** (0.520 g, 2.08 mmol) was then added to the resulting milky orange suspension. The reaction mixture was heated to 50 °C for 5 days before being cooled to room temperature. The orange-yellow product (**2**) was isolated by vacuum filtration, washed with ethanol (3 × 10 cm^3), and dried. Yield: 0.880 g, 23%. Mp: 226−228 °C dec without melting. ^{1}H NMR: δ/ppm 7.47 (s, 4H, Ar H), 7.25 (br s, 8H, NH_2), 6.74 (s, 4H, Ar H), 2.08 (s, 12H, CH_3). ^{13}C NMR: δ/ppm 166.6 (C−S), 165.0 (C═N), 153.8 (Ar C), 149.2 (Ar C), 130.88 (2C, Ar C), 126.4 (Ar C), 124.5 (Ar C), 13.2 (CH_3). IR: ν/cm^{-1} 3321 (N−H stretch), 3149 (N−H stretch), 1598 (C═N stretch), 1561 (C═N stretch). Anal. Found: C, 25.42; H, 2.36; N, 8.34. Calcd for $C_{36}H_{28}Cl_8N_{12}Pt_4S_4{\cdot}C_4H_{18}O$: C, 25.35; H, 2.44; N, 8.87. ESI-MS: m/z 1821.8 ([$C_{36}H_{28}Cl_8N_{12}Pt_4S_4$ + H]$^+$, 20%).

General Method for Synthesis of Mono- and Bis-Phosphane Pt(II) Thiosemicarbazone Complexes. The tetrameric complex **2** (1 mol equiv) was suspended in acetone (15 cm^3). For complexes **3** and **4**, the monophosphane (4 mol equiv) was added, and for complexes **5**−**7**, the bis(diphenylphoshane) (2 mol equiv) was added and the reaction mixture was stirred at room temperature for 3 h. The product was collected as an orange solid by filtration, washed with acetone (2 × 5 cm^3), and dried under vacuum.

Synthesis of Complex ***3***. Triphenylphosphane (0.145 g, 0.552 mmol) was reacted with complex **2** (0.255 g, 0.140 mmol). The product (**3**) was isolated as an orange amorphous solid (0.198 g, 50%). Mp: 299−301 °C dec without melting. ^{1}H NMR: δ/ppm 7.46−7.59 (m, 15H, PPh_3 H), 7.16 (s, 1H, Ar H), 7.02 (s, 2H, NH_2), 6.19 (s, 1H, ^3J(Pt−H) = 25.5 Hz, Ar H), 2.35 (s, 3H, CH_3). ^{13}C NMR: δ/ppm 177.5 (C−S), 167.1 (C═N), 155.2 (Ar C), 152.7 (Ar C), 134.4−134.3 (PPh_3 C), 131.8 (2C, Ar C), 129.05 (PPh_3 C), 130.3 (Ar C), 125.5 (Ar C), 13.6 (CH_3). ^{31}P NMR: δ/ppm 25.70 (PPh_3, ^1J(Pt−P) = 1888 Hz). IR: ν/cm^{-1} 3472 (N−H stretch), 3340 (N−H stretch), 1598 (C═N stretch), 1579 (C═N stretch). Anal. Found: C, 45.47; H, 3.15; N, 5.63. Calcd for $C_{27}H_{22}Cl_2N_3PPtS$: C, 45.20; H, 3.09; N, 5.86. EI-MS: m/z 716.85 [M − H]$^+$, 100%.

Synthesis of Complex ***4***. 1,3,5-Triaza-7-phosphaadamantane (0.0383 g, 0.244 mmol) was reacted with complex **2** (0.102 g, 0.0558 mmol). The product (**4**) was isolated as an orange amorphous solid (0.102 g, 74%.). Mp: 303−304 °C dec without melting. ^{1}H NMR: δ/ppm 7.19−7.20 (m, 4H, Ar H, NH_2), 4.51 (q, 6H, $^3J(H_{\beta(axial)}H_{\beta(equatorial)})$ = 3.10, 13.48 Hz, PTA H), 4.20 (s, 6H, PTA H), 2.28 (s, 3H, CH_3). ^{13}C NMR: δ/ppm 177.7 (C−S), 166.7 (C═N), 154.8 (Ar C), 152.6 (Ar C), 135.3 (Ar C), 133.15 (Ar C), 127.9 (Ar C), 125.7 (Ar C), 72.43 (PTA C), 51.0 (PTA C), 13.6 (CH_3). ^{31}P NMR: δ/ppm −65.70 (PTA, ^1J(Pt−P) = 1680 Hz). IR: ν/cm^{-1} 3413 (N−H stretch), 3257 (N−H stretch), 1621 (C═N stretch), 1563 (C═N stretch). Anal. Found: C, 29.63; H, 3.23; N, 13.91. Calcd for $C_{15}H_{19}Cl_2N_6PPtS$: C, 29.42; H, 3.13; N, 13.72. ESI-MS: m/z 613.0 [M + H]$^+$, 100%.

Synthesis of Complex ***5***. Bis(diphenylphosphino)ferrocene (0.0657 g, 0.118 mmol) was reacted with complex **2** (0.106 g, 0.0580 mmol). The product (**5**) was isolated as an orange amorphous solid (0.120 g, 70%). Mp: 225−227 °C. ^{1}H NMR: δ/ppm 7.42−7.54 (m, 20H, PPh_2 H), 7.17 (s, 2H, Ar H), 7.15 (s, 4H, NH_2), 6.13(s, 2H, Ar H, ^3J(Pt−H) = 20.0 Hz), 5.04 (br s, 4H, Cp H), 4.23 (br s, 4H, Cp H), 2.36 (s, 6H, CH_3). ^{13}C NMR: δ/ppm 177.1 (C−S), 166.5 (C═N), 154.1 (Ar C), 152.0 (Ar C), 132.9 (PPh_2 C), 131.0 (2C, Ar C), 128.0 (PPh_2 C), 126.7 (Ar C), 124.8 (Ar C), 73.5−74.9 (Cp C), 13.1 (CH_3). ^{31}P NMR: δ/ppm 11.62 (PPh_2, ^1J(Pt−P) = 1881.8 Hz). IR: ν/cm^{-1} 3475 (N−H stretch), 3383 (N−H stretch), 1595 (C═N stretch), 1573 (C═N stretch). Anal. Found: C, 42.36; H, 2.71; N, 5.53. Calcd for $C_{52}H_{42}Cl_4FeN_6P_2Pt_2S_2$: C, 42.64; H, 2.89; N, 5.74. ESI-MS: m/z 1465.0 [M + H]$^+$, 100%.

Synthesis of Complex ***6***. *trans*-Bis(diphenylphosphino)ethylene (0.0468 g, 0.1180 mmol) was reacted with complex **2** (0.102 g, 0.0557 mmol). The product (**6**) was isolated as an orange amorphous solid (0.0976 g, 67%). Mp: 312−314 °C. ^{1}H NMR: δ/ppm 7.31−7.52 (m, 22H, H_{α}, PPh_2 H), 7.13(s, 2H, Ar H), 7.12 (s, 4H, NH_2), 6.30 (s, 2H, Ar H, ^3J(Pt−H) = 24.1 Hz), 2.33 (s, 6H, CH_3). ^{13}C NMR: δ/ppm 177.3 (C−S), 167.4 (C═N), 154.6 (Ar C), 152.4 (Ar C), 135.0 (Ph_2P−C═), 134.0 (PPh_2 C), 132.11 (2C, Ar C), 129.3 (PPh_2 C), 127.5 (Ar C), 125.7 (Ar C), 13.7 (CH_3). ^{31}P NMR: δ/ppm 16.25 (t, PPh_2, ^3J(P−P) = 22.80 Hz, ^1J(Pt−P) = 1870.0 Hz). IR: ν/cm^{-1} 3456 (N−H stretch), 3324 (N−H stretch), 1627 (C═N stretch), 1606 (C═N stretch). Anal. Found: C, 40.67; H, 2.95; N, 6.65. Calcd for $C_{44}H_{36}Cl_4N_6P_2Pt_2S_2$: C, 40.44; H, 2.78; N, 6.43. ESI-MS: m/z 1307.0 [M + H]$^+$, 100%.

X-ray Structure Analysis. Crystals suitable for single-crystal X-ray diffraction of complexes **2**·3DMSO, **3**·2DMSO, and **4** were grown from the DMSO-d_6 NMR samples by exposure of the solutions to air. X-ray single-crystal intensity data were collected on a Nonius Kappa-CCD diffractometer using graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å). The temperature was controlled by an Oxford Cryostream cooling system (Oxford Cryostat). The strategy for the data collections was evaluated using the Bruker Nonius "Collect" program. Data were scaled and reduced using DENZO-SMN software.[75] Absorption corrections were performed using SADABS.[76] The structure was solved by direct methods and refined employing full-matrix least squares with the program SHELXL-97[77] refining on F^2. Packing diagrams were produced using the program PovRay and graphic interface X-seed.[78] For **2**·3DMSO, all non-hydrogen atoms, except the carbon and oxygen atoms on one of the DMSO molecules, were refined anisotropically. All hydrogen atoms were positioned geometrically with an N−H distance of 0.88 Å and C−H distances ranging from 0.95 to 0.98 Å and refined as riding on their parent atoms, with U_{iso}(H) = 1.2−1.5[U_{eq}(C or N)]. In the asymmetric unit, one DMSO molecule is on general positions. Another molecule is on a 2-fold rotation axis, and it is disordered and shows high thermal motions; therefore the carbon and oxygen atoms were refined isotropically and the hydrogen atoms were not placed. The complex **2** main molecule is located at special positions on a 2-fold rotation axis. The structure was refined successfully to an R factor of 0.0204. For **3**·2DMSO, all non-hydrogen atoms were refined anisotropically. All the hydrogen atoms, except those on the N3 atom, were placed in idealized positions in a riding model with U_{iso} set at 1.2 or 1.5 times those of their parent atoms and fixed C−H bond lengths ranging from 0.95 to 0.98 Å. The hydrogen atoms H3A and H3B on N3 were located in the difference electron density maps and refined with simple bond length constraints. One DMSO molecule was disordered over two positions with a site occupancy of 0.5 each. For complex **4**, all non-hydrogen atoms were refined anisotropically. All the hydrogen atoms were placed in idealized positions in a riding model with U_{iso} set at 1.2 or 1.5 times those of their parent atoms and restrained with fixed bond lengths ranging from 0.95 to 0.99 Å for C−H and 0.88 Å for N−H.

***P. falciparum* in Vitro Assay.** The test samples were tested in triplicate on one occasion against chloroquine-sensitive (CQS) D10 strain and chloroquine-resistant (CQR) Dd2 strain of *Plasmodium falciparum*. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method.[79] Quantitative assessment of antiplasmodial activity in vitro was determined via the

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parasite lactate dehydrogenase assay using a modified method.[80] The test samples were prepared to a 20 mg/cm^3 stock solution in 100% DMSO and sonicated to enhance solubility. Samples were tested as a suspension if not completely dissolved. Stock solutions were stored at −20 °C. Further dilutions were prepared on the day of the experiment. Chloroquine diphosphate (Sigma) was used as the reference drug in all experiments. A full dose−response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50} value). Test samples were tested at a starting concentration of 100 μg/cm^3, which was then serially diluted 2-fold in complete medium to give 10 concentrations, with the lowest concentration being 0.2 μg/cm^3. The same dilution technique was used for all samples. CQ was tested at a starting concentration of 100 ng/cm^3 against the CQR strain and 1000 ng/cm^3 against the CQS strain. The highest concentration of solvent (0.5%) to which the parasite was exposed had no measurable effect on the parasite viability (data not shown). The IC_{50} values were obtained using a nonlinear dose−response curve fitting analysis via Graph Pad Prism v.4.0 software.

***T. vaginalis in Vitro* Assay.** Cultures of *T. vaginalis* T1 strain were grown in 5 cm^3 completed TYM Diamond's media in a 37 °C incubator for 24 h. One hundred millimolar stocks of the compounds were made by dissolving in DMSO, were screened against T1 stain of *T. vaginalis*. Samples were tested as a suspension if not completely dissolved. Cells untreated and inoculated with 5 μL of DMSO were used as controls. A 5 μL portion of 100 mM stock of the compound library was inoculated for a final concentration of 100 μM. Results were calculated on the basis of counts utilizing a hemocytometer after 24 h. For IC_{50} values, increasing concentrations of the compound (0−100 μM) were tested for inhibitory activity, and concentrations that inhibited at approximately 50% were then obtained by linear regression analysis. These predicted IC_{50} values were then confirmed by direct testing on *T. vaginalis* strain T1 as described above.

A2780 and A2780cisR Cancer *in Vitro* Assay. The human A2780 and A2780cisR ovarian carcinoma cells were obtained from the European Collection of Cell Cultures (Salisbury, U.K.). Cells were grown routinely in RPMI-1640 medium with 10% fetal calf serum (FCS) and antibiotics at 37 °C and 5% CO_2. Cytotoxicity was determined using the MTT assay (MTT = 3(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide). Cells were seeded in 96-well plates as monolayers with 100 cm^3 of cell solution (approximately 20 000 cells) per well and preincubated for 24 h in medium supplemented with 10% FCS. Compounds were prepared as a DMSO solution and then dissolved in the culture medium and serially diluted to the appropriate concentration, to give a final DMSO concentration of 1%. A 100 cm^3 portion of the drug solution was added to each well and the plates were incubated for another 72 h. Subsequently, MTT (5 mg/cm^3 solution) was added to the cells, and the plates were incubated for a further 2 h. The culture medium was aspirated, and the purple formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in DMSO. The optical density, directly proportional to the number of surviving cells, was quantified at 540 nm using a multiwell plate reader, and the fraction of surviving cells was calculated from the absorbance of untreated control cells. The evaluation is based on means from three independent experiments, each comprising three microcultures per concentration level.

Detergent-Mediated Assay for β-Hematin Inhibitors. The β-hematin formation assay method described by Carter et al.[74] was modified for manual liquid delivery. Stock solutions of the test compounds were prepared at 10, 2, and 0.4 mM by dissolving each sample in DMSO with sonication. Test compounds were delivered to a 96-well plate in triplicate from 0 to 500 μM (final concentration) with a total DMSO volume of 10 μL in each well. Deionized H_2O (70 μL) and NP-40 (20 μL; 30.55 μM) were then added. A 25 mM hematin stock solution was prepared by sonicating hemin in DMSO, for complete dissolution, and then suspending 177.76 μL of this in a 2 M acetate buffer (pH 4.8). The homogeneous suspension (100 μL) was then added to the wells to give final buffer and hematin concentrations of 1 M and 100 μM, respectively. The plate was covered and incubated at 37 °C for 5−6 h in a water bath. Analysis of the assay was carried out using the pyridine−ferrichrome method developed by Ncokazi and Egan.[81] A solution of 50% (v/v) pyridine, 30% (v/v) H_2O, 20% (v/v) acetone, and 0.2 M HEPES buffer (pH 7.4) was prepared and 32 μL added to each well to give a final pyridine concentration of ±5% (v/v). Acetone (60 μL) was then added to assist with hematin dispersion. The UV−vis absorbance of the plate wells was read on a SpectraMax plate reader. Sigmoidal dose−response curves were fitted to the absorbance data using GraphPad Prism v3.02.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

CIF files giving crystallographic data for complexes **2**·3DMSO, **3**·2DMSO, and **4**. This material is available free of charge via the Internet at http://pubs.acs.org. CCDC 866790, 866791, and 866792 also contain supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, U.K.; fax (internat.) +44-1223/336-033; e-mail deposit@ccdc.cam.ac.uk).

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) De Oliveira, R. B.; De Souza-Fagundes, E. M.; Soares, R. P. P.; Andrade, A. A.; Krettli, A. U.; Zani, C. L. *Eur. J. Med. Chem.* **2008**, *43*, 1983.

(2) Duffy, K. J.; Shaw, A. N.; Delorme, E.; Dillon, S. B.; Erickson-Miller, C.; Giampa, L.; Huang, Y.; Keenan, R. M.; Lamb, P.; Liu, N.; Miller, S. G.; Price, A. T.; Rosen, J.; Smith, H.; Wiggall, K. J.; Zhang, L.; Luengo, J. I. *J. Med. Chem.* **2002**, *45*, 3573.

(3) Abid, M.; Agarwal, S. M.; Azam, A. *Eur. J. Med. Chem.* **2008**, *43*, 2035.

(4) Walcourt, A.; Loyevsky, M.; Lovejoy, D. B.; Gordeuk, V. R.; Richardson, D. R. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 401.

(5) Fujii, N.; Mallari, J. P.; Hansell, E. J.; Mackey, Z.; Doyle, P.; Zhou, Y. M.; Gut, J.; Rosenthal, P. J.; Mckerrow, J. H.; Guy, R. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 121.

(6) Du, X.; Guo, C.; Hansel, E.; Doyle, P. S.; Caffery, C. R.; Holler, T. P.; Mckerrow, J. H.; Cohen, F. E. *J. Med. Chem.* **2002**, *45*, 2695.

(7) Khan, S. A.; Yusuf, M. *Eur. J. Med. Chem.* **2009**, *44*, 2597.

(8) Khan, S. A.; Kumar, P.; Joshi, R.; Iqbal, P. F.; Saleem, K. *Eur. J. Med. Chem.* **2008**, *43*, 2029.

(9) Costello, C.; Karpanen, T.; Lambert, P. A.; Mistry, P.; Parker, K. J.; Rathbone, D. L.; Ren, J.; Wheeldon, L.; Worthington, T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1708.

(10) Kovala-Demertzi, D; Demertzis, M. A.; Filou, E.; Pantazaki, A. A.; Yadav, P. N.; Miller, J. R.; Zheng, Y.; Kyriakidis, D. A. *Biometals* **2003**, *16*, 411.
(11) Kolesar, J. M.; Schelman, W. R.; Geiger, P. G.; Holen, K. D.; Traynor, A. M.; Alberti, D. B.; Thomas, J. P.; Chitambar, C. R.; Wilding, G.; Antholine, W. E. *J. Inorg. Biochem.* **2008**, *102*, 693.
(12) Petering, H. G.; Buskirk, H. H.; Underwood, G. E. *Cancer Res.* **1964**, *24*, 367.
(13) Liu, M.-C.; Lin, T.-S.; Sartorelli, A. C. *J. Med. Chem.* **1992**, *35*, 3672.
(14) Dilovic, I.; Rubcic, M.; Vrdoljak, V.; Pavelic, S. K.; Kralj, M.; Piantanida, I.; Cindric, M. *Bioorg. Med. Chem.* **2008**, *16*, 5189.
(15) Agrawal, K. C.; Booth, B. A.; Moore, E. C.; Sartorelli, A. C. *J. Med. Chem.* **1972**, *15* (11), 1154.
(16) Chipeleme, A.; Gut, J.; Rosenthal, P. J.; Chibale, K. *Bioorg. Med. Chem. Lett.* **2007**, *15*, 273.
(17) Sweeney, D.; Raymera, M. L.; Lockwood, T. D. *Biochem. Pharmacol.* **2003**, *66*, 663.
(18) Lew, V. L.; Tiffert, T.; Ginsburg, H. *Blood* **2003**, *101*, 4189.
(19) Francis, S. E.; Sullivan, D. J., Jr; Goldberg, D. E. *Annu. Rev. Microbiol.* **1997**, *51*, 97.
(20) Rosenthal, P. J. *Int. J. Parasitol.* **2004**, *34*, 1489.
(21) Mckerrow, J. H.; Sun, E.; Rosenthal, P. J.; Bouvier, J. *Annu. Rev. Microbiol.* **1993**, *47*, 821.
(22) Farrel, N. *Coord. Chem. Rev.* **2002**, *232*, 1.
(23) Beraldo, H.; Gambino, D. *Mini-Rev. Med. Chem.* **2004**, *4*, 31.
(24) Hughes, M. N. *The Inorganic Chemistry of Biological Processes*; Wiley: Chichester, U.K., 1981.
(25) West, D. X.; Padhye, S. B.; Sonawane, P. B. *Struct. Bonding (Berlin)* **1991**, *76*, 1.
(26) Stringer, T.; Hendricks, D. T.; Guzgay, H.; Smith, G. S. *Polyhedron* **2012**, *31* (1), 486.
(27) Stringer, T.; Chellan, P.; Therrien, B.; Shunmoogam-Gounden, N.; Hendricks, D. T.; Smith, G. S. *Polyhedron* **2009**, *28*, 2839.
(28) Chellan, P.; Nasser, S.; Vivas, L.; Chibale, K.; Smith, G. S. *J. Organomet. Chem.* **2010**, *695* (19−20), 2225.
(29) Chellan, P.; Shunmoogam-Gounden, N.; Hendricks, D. T.; Gut, J.; Rosenthal, P. J.; Lategan, C.; Smith, P. J.; Chibale, K.; Smith, G. S. *Eur. J. Inorg. Chem.* **2010**, *22*, 3520.
(30) Singh, D.; Singh, R. V. *J. Inorg. Biochem.* **1993**, *15*, 227.
(31) Maiti, A.; Guha, A. K.; Ghosh, S. *J. Inorg. Biochem.* **1988**, *33*, 57.
(32) Kasuga, N. C.; Sekino, K; Koumo, C.; Shimada, N.; Ishikawa, M.; Nomiya, K. *J. Inorg. Biochem.* **2000**, *84*, 55.
(33) Grguric-Sipka, S.; Kowol, C. R.; Valiahdi, S.-M.; Eichinger, R.; Jakupec, M. A.; Roller, A.; Shova, S.; Arion, V. B.; Keppler, B. K. *Eur. J. Inorg. Chem.* **2007**, No. 18, 2870.
(34) Kowol, C. R.; Trondl, R.; Heffeter, P.; Arion, V. B.; Jakupec, M. A.; Roller, A.; Galanski, M.; Berger, W.; Keppler, B. K. *J. Med. Chem.* **2009**, *52* (16), 5032.
(35) Popovic-Bijelic, A.; Kowol, C. R.; Lind, M. E. S.; Luo, J.; Himo, F.; Enyedy, E. A.; Arion, V. B.; Graeslund, A. *J. Inorg. Biochem.* **2011**, *105* (11), 1422.
(36) Kowol, C. R.; Trondl, R.; Arion, V. B.; Jakupec, M. A.; Lichtscheidl, I.; Keppler, B. K. *Dalton Trans.* **2010**, *39* (3), 704.
(37) Kowol, C. R.; Berger, R.; Eichinger, R.; Roller, A.; Jakupec, M. A.; Schmidt, P. P.; Arion, V. B.; Keppler, B. K. *J. Med. Chem.* **2007**, *50* (6), 1254.
(38) Lippert, B. *Cisplatin. Chemistry and Biochemistry of a Leading Anticancer Drug*; VCHA & Wiley-VCH: Zurich, 1999.
(39) Bruijnincx, P. C. A.; Sadler, P. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 197.
(40) Guo, Z.; Sadler, P. J. *Angew. Chem., Int. Ed.* **1999**, *38*, 1512.
(41) Garoufis, A.; Hadjikakou, S. K.; Hadjiliadis, N. In *Metallotherapeutic Drugs and Metal-Based Diagnostic Agents: The Use of Metals in Medicines*; Tiekink, M., Gielen, E. R. T., Eds.; Wiley: Chichester, U.K., 2005; Chapter 21, p 399.
(42) Garoufis, A.; Hadjikakou, S. K.; Hadjiliadis, N. *Coord. Chem. Rev.* **2009**, *253*, 1384.
(43) Jakupec, M. A.; Galanski, M.; Arion, V. B.; Hartinger, C. G.; Keppler, B. K. *Dalton Trans.* **2008**, No. 2, 183.
(44) Hartinger, C. G.; Phillips, A. D.; Nazarov, A. A. *Curr. Top. Med. Chem.* **2011**, *11*, 2688.
(45) Zhang, W.-H.; Chien, S. W.; Hor, T. S. A. *Coord. Chem. Rev.* **2011**, *255*, 1991.
(46) Stringer, T.; Therrien, B.; Hendricks, D. T.; Guzgay, H.; Smith, G. S. *Inorg. Chem. Commun.* **2011**, *14* (6), 956.
(47) Xie, G.; Chellan, P.; Mao, J.; Chibale, K.; Smith, G. S. *Adv. Synth. Catal.* **2010**, *352* (10), 1641.
(48) Chellan, P.; Stringer, T.; Shokar, A.; Dornbush, P. J.; Vazquez-Anaya, G.; Land, K. M.; Chibale, K.; Smith, G. S. *J. Inorg. Biochem.* **2011**, *105* (11), 1562.
(49) Raja, G.; Sathya, N.; Jayabalakrishnan, C. *J. Coord. Chem.* **2011**, *64* (5), 817.
(50) Beckford, F. A.; Leblanc, G.; Thessing, J.; Shaloski, M., Jr; Frost, B. J.; Li, L.; Seeram, N. P. *Inorg. Chem. Commun.* **2009**, *12*, 1094.
(51) Beckford, F.; Dourth, D.; Shaloski, M., Jr.; Didion, J.; Thessing, J.; Woods, J.; Crowell, V.; Gerasimchuk, N.; Gonzalez-Sarrías, A.; Seeram, N. P. *J. Inorg. Biochem.* **2011**, *105*, 1019.
(52) Kalaivani, P.; Prabhakaran, R.; Dallemer, F.; Poornima, P.; Vaishnavi, E.; Ramachandran, E.; Padma, V. V.; Renganathan, R.; Natarajan, K. *Metallomics* **2012**, *4*, 101.
(53) Vieites, M.; Smircich, P.; Pagano, M.; Otero, L.; Fischer, L. L.; Terenzi, H.; Prieto, M. J.; Moreno, V.; Garat, B.; Gambino, D. *J. Inorg. Biochem.* **2011**, *105*, 1704.
(54) Ferraz, K. S. O.; Ferandes, L.; Carrilho, D.; Pinto, M. C. X.; Leite, M. D. F.; Souza−Fagundes, E. M.; Speziali, N. L.; Mendes, I. C.; Beraldo, H. *Bioorg. Med. Chem.* **2009**, *17*, 7138.
(55) Appleton, T. G.; Hall, J. R. *Inorg. Chem.* **1971**, *10* (8), 1717.
(56) Natile, G.; Maresca, L.; Cattalini, L. *J. Chem. Soc., Chem. Commun.* **1976**, 24.
(57) Smolenski, P.; Pombeiro, A. J. L. *Dalton Trans.* **2008**, 87.
(58) Smolenski, P.; Dinoi, C.; Fatima, M.; Da Silva, C. G.; Pombeiro, A. J. L. *J. Organomet. Chem.* **2008**, *693*, 2338.
(59) Vazquez-Garcıa, D.; Fernandez, A.; Fernandez, J. J.; Lopez-Torres, M.; Suarez, A.; Ortigueira, J. M.; Vila, J. M.; Adams, H. *J. Organomet. Chem.* **2000**, *595*, 199.
(60) Crystal data for **2**·3DMSO: formula, $C_{42}H_{46}Cl_8N_{12}O_3Pt_4S_7$; formula weight, 2055.29; crystal system, monoclinic; space group, *C*2/*c*; *a* (Å), 14.7265(6); *b* (Å), 30.6788(14); *c* (Å), 14.2547(6); β (deg), 111.9020(10); *V* (Å^3), 5975.3(4); *Z*, 4; D_c (g cm^{-3}), 2.285; μ (mm^{-1}), 9.987; θ range for data collection (deg), 1.63−28.33; limiting indices, $-19 < h < 18$, $-40 < k < 40$, $-19 < l < 19$; measured reflections, 67 775; reflections used (R_{int}), 7432 (0.0358); parameters, 342; R1, 0.0204; wR2, 0.0454; goodness of fit on F^2, 1.068.
(61) Crystal data for **3**·2DMSO: formula, $C_{31}H_{34}Cl_2N_3O_2PPtS_3$; formula weight, 873.75; crystal system, orthorhombic; space group, $Pca2_1$; *a* (Å), 15.9511(4); *b* (Å), 9.2259(2); *c* (Å), 23.0990(6); β (deg), 90; *V* (Å^3), 3399.32(14); *Z*, 4; D_c (g cm^{-3}), 1.707; μ (mm^{-1}), 4.550; θ range for data collection (deg), 3.49−28.28; limiting indices, $-21 < h < 21$, $-12 < k < 12$, $-30 < l < 30$; measured reflections, 136 324; reflections used (R_{int}), 8405 (0.0498); parameters, 414; R1, 0.0214; wR2, 0.0382; goodness of fit on F^2, 1.145.
(62) Crystal data for **4**: formula, $C_{15}H_{19}Cl_2N_6PPtS$; formula weight, 612.38; crystal system, monoclinic; space group, $P2_1/c$; *a* (Å), 19.176(4); *b* (Å), 6.0493(12); *c* (Å), 17.977(4); β (deg), 114.13(3); *V* (Å^3), 1903.2(7); *Z*, 4; D_c (g cm^{-3}), 2.137; μ (mm^{-1}), 7.860; θ range for data collection (deg), 3.36−28.28; limiting indices, $-25 < h < 23$, $-8 < k < 0$, $0 < l < 23$; measured reflections, 4703; reflections used (R_{int}), 4703 (0.0000); parameters, 237; R1, 0.0182; wR2, 0.0389; goodness of fit on F^2, 1.126.
(63) Quiroga, A. G.; Perez, J. M.; Lopez-Solera, I.; Masaguer, J. R.; Luque, A.; Roman, P.; Edwards, A.; Alonso, C.; Navarro-Ranninger, C. *J. Med. Chem.* **1998**, *41*, 1399.
(64) Shutton, L. E. *Tables of Interatomic Distances and Configurations in Molecules and Ions (Supplement)*; The Chemical Society: London, 1965.

(65) Afrasiabi, Z.; Sinn, E.; Lin, W.; Ma, Y.; Campana, C.; Padhye, S. *J. Inorg. Biochem.* **2005**, *99*, 1526.
(66) Lobana, T. S.; Bawa, G.; Hundal, G; Zeller, M. *Z. Anorg. Allg. Chem.* **2008**, *634*, 931.
(67) Phillips, A. D.; Gonsalvi, L.; Romerosa, A.; Vizza, F.; Peruzzini, M. *Coord. Chem. Rev.* **2004**, *248*, 955.
(68) Bravo, J.; Bolano, S.; Gonsalvi, L.; Peruzzini, M. *Coord. Chem. Rev.* **2010**, *254*, 555.
(69) Fricker, S. P.; Mosi, R. M.; Cameron, B. R.; Baird, I.; Zhu, Y.; Anastassov, V.; Cox, J.; Doyle, P. S.; Hansell, E.; Lau, G.; Langille, J.; Olsen, M.; Qin, L.; Skerlj, R.; Wong, R. S. Y.; Santucci, Z.; Mckerrow, J. H. *J. Inorg. Biochem.* **2008**, *102*, 1839.
(70) Lo, Y.-C.; Su, W.-C.; Ko, T.-P.; Wang, N.-C.; Wang, A.H.-J. *J. Biomol. Struct. Dyn.* **2011**, *29* (2), 267.
(71) Casini, A.; Gabbiani, C.; Sorrentino, F.; Rigobello, M. P.; Bindoli, A.; Geldbach, T. J.; Marrone, A.; Re, N.; Hartinger, C. G.; Dyson, P. J.; Messori, L. *J. Med. Chem.* **2008**, *51*, 6773.
(72) Goldberg, D. E.; Slater, A. F.; Cerami, A.; Henderson, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 2931.
(73) Egan, T. J.; Marques, H. M. *Coord. Chem. Rev.* **1999**, *190*−*192*, 493.
(74) Carter, M. D.; Phelan, V. V.; Sandlin, R. D.; Bachmann, B. O.; Wright, D. W. *Comb. Chem. High Throughput Screen* **2010**, *13* (3), 285.
(75) Otwinowski, Z.; Minor, W. In *Methods in Enzymology, Macromolecular Crystallography*; Sweet, C. W., Carter, R. M., Jr., Eds.; Academic Press: New York, 1997; Vol. *276*, Part A.
(76) Sheldrick, G. M. *SADABS*; University of Göttingen, Göttingen, Germany, 1997.
(77) Sheldrick, G. M. *SHELXL-97 and SHELXS-97, Programs for Crystal Structure Refinement*; University of Göttingen, Göttingen, Germany, 1997.
(78) Barbour, L. J. *J. Supramol. Chem.* **2001**, *1*, 189.
(79) Trager, W.; Jensen, J. B. *Science* **1976**, *193* (4254), 673.
(80) Makler, M. T.; Ries, J. M.; Williams, J. A.; Bancroft, J. E.; Piper, R. C.; Gibbins, B. L.; Hinrichs, D. J. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 739.
(81) Ncokazi, K. K.; Egan, T. J. *Anal. Biochem.* **2005**, *338* (2), 306.