DOI: 10.1002/ejic.201000317

Synthesis, Structure and in Vitro Biological Screening of Palladium(II) Complexes of Functionalised Salicylaldimine Thiosemicarbazones as Antimalarial and Anticancer Agents

Prinessa Chellan,[a] Nelusha Shunmoogam-Gounden,[b] Denver T. Hendricks,[b] Jiri Gut,[c] Philip J. Rosenthal,[c] Carmen Lategan,[d] Peter J. Smith,[d] Kelly Chibale,[a,e] and Gregory S. Smith*[a]

Keywords: Palladium / Salicylaldimine / Anticancer activity / Antimalarial activity / Thiosemicarbazone

A series of mononuclear salicylaldiminato(thiosemicarbazone)palladium(II) complexes of general formula [Pd(saltsc-R)PPh_3], {H_2saltsc-R = salicylaldehyde thiosemicarbazone; R = H (**5**), 3-*tert*-butyl (**6**), 3-methoxy (**7**), 5-chloro (**8**)} have been synthesized. The palladium complexes were prepared by the reaction of the appropriate salicylaldimine thiosemicarbazone with $Pd(PPh_3)_2Cl_2$. All complexes were characterised by a range of spectroscopic and analytical techniques. The molecular structures of **6**–**8** have been determined by single-crystal X-ray diffraction analysis. The salicylaldimine thiosemicarbazones coordinate to palladium in a tridentate manner, through the phenolic oxygen, imine nitrogen and thiolate sulfur, forming five-and six-membered chelate rings within their structures. The fourth coordination site for these square-planar complexes is occupied by PPh_3. Biological activities of the thiosemicarbazone ligands and palladium complexes have been investigated toward the WHCO1 oesophageal cancer cell line and against two strains of the malaria parasite *Plasmodium falciparum*, W2 (chloroquine-resistant) and D10 (chloroquine-sensitive). The palladium(II) complexes show enhanced in vitro antiplasmodial activity in comparison with their thiosemicarbazone ligand precursors. On the other hand, in vitro anticancer activity studies on oesophageal cancer cell lines revealed a decrease in activity upon coordination of palladium to the thiosemicarbazone ligand.

Introduction

Thiosemicarbazones are known for their pharmacological properties, particularly as antiparasitic,[1–4] antibacterial[5–8] and antitumoral[9,10] agents. Research into the structure and coordination chemistry of aliphatic, aromatic, heterocyclic and other types of thiosemicarbazones and their metal complexes is well-established.[11] Generally, thiosemicarbazones act as chelating agents for various metal ions, by bonding through the sulfur atom and the imine nitrogen atom. Cadmium,[12] mercury,[12] platinum,[13] cobalt[14] and palladium[15,16] are a few of the metals that have been complexed with various types of thiosemicarbazone ligands. The study of the biological activity of transition metal complexes of thiosemicarbazones has emerged as an area of great interest, the premise being that coordination to a metal may affect biological activity.[17]

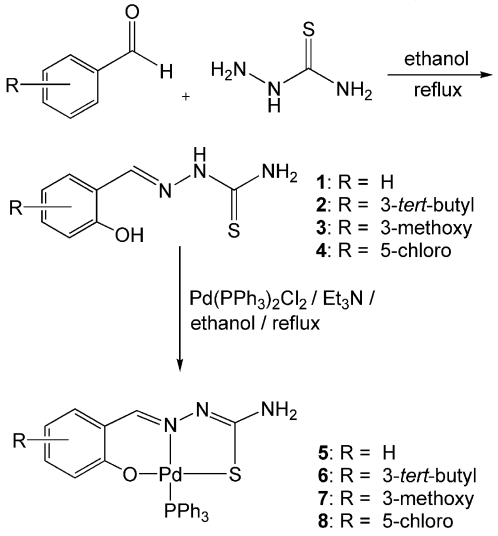
Several transition metal complexes of aromatic mono- and bidentate thiosemicarbazones have been studied for their anticancer activities.[18] Our current investigation of salicylaldehyde thiosemicarbazones stems from the incorporation of three donor atoms [O,N,S], increasing the coordination capacity of thiosemicarbazones, giving rise to chelating tridentate thiosemicarbazone metal complexes. Metal complexes of salicylaldehyde thiosemicarbazones have been studied for their antitumour activity in vitro and in some cases were found to be generally more active than the free ligand.[15,19] Das and Livingstone suggested that sulfur-containing ligands chelated to palladium(II) are better antitumor agents than those of other metals, as the palladium(II) chelates possess the proper lability to transport the metal to DNA, its primary target.[20] Palladium(II) complexes of aryl-derived thiosemicarbazones have been tested for antitumoral and anticancer activity against several cancer cell lines including, human and murine tumor cell lines that are resistant and sensitive to cisplatin,[21–23] human breast cancer[24] and bladder cancer.[24] In certain cases, the palladium(II) complexes were found to be better cytotoxic agents than cisplatin, as well as their free ligands. A tridentate

[a] Department of Chemistry, University of Cape Town,
P. B. Rondebosch 7701, South Africa
Fax: +27-21-6505195
E-mail: Gregory.Smith@uct.ac.za
[b] Division of Medical Biochemistry, University of Cape Town,
Anzio Road, Observatory 7925, South Africa
[c] Department of Medicine, San Francisco General Hospital,
University of California,
San Francisco, Box 0811, San Francisco, CA 94143, USA
[d] Division of Pharmacology, Department of Medicine, University of Cape Town,
K45, OMB, Groote Schuur Hospital, Observatory 7925, South Africa
[e] Institute of Infectious Disease and Molecular Medicine,
University of Cape Town,
Rondebosch 7701, South Africa

phenanthrenequinone thiosemicarbazone palladium(II) complex was found to exhibit superior selectivity toward breast cancer cells which have previously shown resistance to conventional chemotherapies.[25] Overall, tridentate thiosemicarbazone Pd^{II} complexes have exhibited antiproliferative activities that are comparable or better than their Pt^{II} analogues.[26–28]

Reports on the use of thiosemicarbazone metal complexes as antimalarial agents are sparse. Copper(II), nickel(II) and iron(II) complexes of 2-acetyl pyridine-derived thiosemicarbazones have been screened for antimalarial activity.[29] The Cu^{II} and Fe^{II} complexes were found to exhibit modest activity compared to their free ligands while the Ni^{II} complexes showed no activity. To the best of our knowledge, the investigation of thiosemicarbazone Pd^{II} complexes as antimalarial agents has not been reported in the literature.

With the established biological activities of thiosemicarbazones, we decided to study the synthesis and characterisation of four mononuclear thiosemicarbazonepalladium(II) complexes, containing functionalized salicylaldehyde moieties and investigate the biological activity of the thiosemicarbazone ligands and their palladium(II) complexes against the WHCO1 oesophageal cancer cell line, as well as a sensitive and resistant strain of the malaria parasite *Plasmodium falciparum*.



Scheme 1. Synthetic route to salicylaldiminato(thiosemicarbazone)palladium(II) complexes **5**–**8**.

Results and Discussion

Synthesis of Salicylaldimine Thiosemicarbazones 1–4 and Their Mononuclear Palladium(II) Complexes 5–8

The functionalised salicylaldehyde thiosemicarbazones **1**–**4** were prepared by Schiff base condensation reactions of the appropriate salicylaldehyde with thiosemicarbazide (Scheme 1). Thiosemicarbazones **1**,[30] **3**[31] and **4**[32] are known compounds and their spectroscopic data and melting points correlate with the literature. The salicylaldimine thiosemicarbazone ligands **1**–**4** synthesised were treated with the precursor palladium(II) complex, *cis*-bis(triphenylphosphane)palladium dichloride (Scheme 1) to give the mononuclear salicylaldiminato(thiosemicarbazone)palladium(II) complexes **5**–**8**. The complexes **5**–**8** are soluble in most organic solvents and show greater solubility when compared with the uncomplexed ligands **1**–**4**.

The new salicylaldimine thiosemicarbazone ligand **2** shows similar spectral properties with the known compounds **1**, **3** and **4**.[30–32] Typically, the ^1H NMR spectrum of **2** shows a broad singlet for the hydroxy proton at δ = 10.03 ppm and the hydrazinic proton occurs at δ = 11.29 ppm. The imine proton, indicative of Schiff base condensation, is seen as a singlet at δ = 8.28 ppm. In the ^{13}C NMR spectrum for ligand **2**, the thione carbon is observed at δ = 177.8 ppm and the hydroxy-substituted aromatic carbon at δ = 155.3 ppm. In the ^1H NMR spectra for palladium(II) complexes **6**–**8**, the imine proton is observed as a doublet at around 8.26 ppm. A coupling constant (^4J) of 14.14 Hz is consistent with long range coupling of the imine proton with the phosphorus nucleus of the triphenylphosphane co-ligand.[33] Peaks for the hydroxy proton and the hydrazinic proton of the free thiosemicarbazone ligand were not observed confirming coordination of the ligand via the phenolic oxygen and that sulfur coordinates to palladium in the thiolate form. All of the palladium complexes exhibit a singlet in their ^{31}P NMR spectra in the range between 24.00 and 26.00 ppm, with the exception of complex **7**, which shows a singlet further upfield (δ = 19.67 ppm) relative to the analogous complexes. The ^{13}C NMR spectra for **5**–**8** displays resonances due to the thiolate carbon at around 161 ppm and the imine carbon at around 171 ppm.

Infrared spectra for the salicylaldimine thiosemicarbazone ligands (**1**–**4**) show an absorption band assigned to the C=N stretching vibration at 1615 cm^{-1}, confirming the formation of the thiosemicarbazone, Schiff-base product. For the monuclear palladium(II) complexes **5**–**8**, two absorption bands are observed in the imine region. This is consistent with the formation of a new imine bond within the thiosemicarbazone ligand, upon coordination of palladium to sulfur in the thiolate form. The lower frequency band, observed between 1610 and 1585 cm^{-1}, is assigned to the imine bond coordinated to palladium. In all of the complexes two bands are observed for the N–H vibrations of the terminal amine between 3500 and 3250 cm^{-1}. A third band for the hydrazinic N–H is not observed and this is consistent with the loss of the hydrazinic proton and formation of a new imine bond in the coordinated thiosemicarbazone ligand.

ESI mass spectrometry further confirmed the integrity of the new mononuclear palladium(II) complexes **6**–**8**. The ESI spectrum of **6** and **7** reveals base peaks at *m*/*z* 617 and *m*/*z* 591 respectively for the $[M - H]^+$ ion. The spectrum of compound **8** displays a base peak for the mononuclear complex in its protonated form $[M + H]^+$ at *m*/*z* 597.

Molecular Structures

The molecular structures of palladium complexes **6**–**8** have been determined using single-crystal X-ray crystallography. Single crystals suitable for X-ray diffraction of the palladium complexes were obtained by slow exaporation of either 1:1 dichloromethane/hexane or $CDCl_3$/hexane solutions. Crystallographic data are listed in Table 5 (see Exp. Section) and selected interatomic distances and bond angles are summarised in Table 1. The molecular structures of complexes **6**–**8** are shown in Figures 1, 2, and 3 respectively.

Table 1. Selected bond lengths [Å] and angles [°] for palladium(II) complexes **6**–**8**.

	6	**7**	**8**
Pd1–S1	2.2432(8)	2.2426(8)	2.2478(6)
Pd1–P1	2.2833(8)	2.2782(8)	2.2779(6)
Pd1–N3	2.028(3)	2.012(2)	2.0204(19)
Pd1–O1	2.014(2)	2.033(2)	2.0164(16)
C1–S1	1.754(3)	1.754(3)	1.751(3)
C1–N2	1.303(4)	1.304(4)	1.291(3)
C2–N3	1.294(4)	1.293(4)	1.294(3)
N3–Pd1–S1	84.16(8)	84.52(7)	84.09(6)
N3–Pd1–O1	93.03(10)	92.15(9)	93.15(7)
P1–Pd1–S1	93.41(3)	93.56(3)	92.27(2)
P1–Pd1–O1	89.41(7)	90.07(6)	90.43(5)

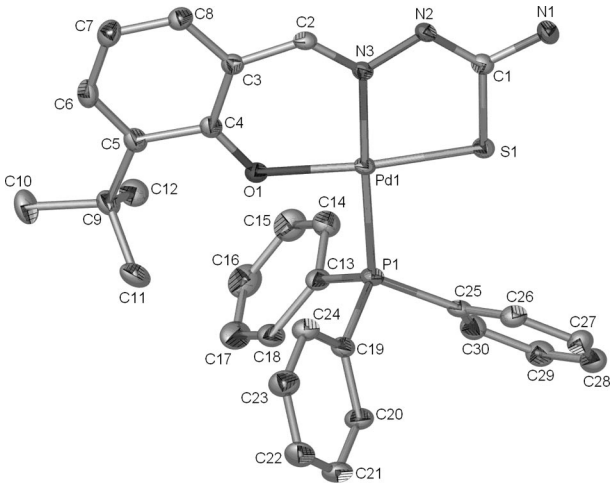


Figure 1. Molecular structure of complex **6** showing ellipsoids at the 40% probability level with hydrogen atoms and solvent molecules omitted for clarity.

For the palladium complexes **6**–**8**, the molecular structures show that the thiosemicarbazone ligand coordinates to the metal in the expected tridentate (O–N–S) fashion, via the phenolic oxygen, imine nitrogen and sulfur atom in a square-planar geometry, forming five- and six-membered chelate rings with the metal centre. The fourth coordination site is occupied by a triphenylphosphane ligand, coordinated to palladium *trans* to the nitrogen.

The bite angles formed between the metal and the coordinated ligands show that in each complex there is a slightly distorted square-planar arrangement around the metal. All of the bite angles around the metal in each complex are consistent with those observed for similar com-

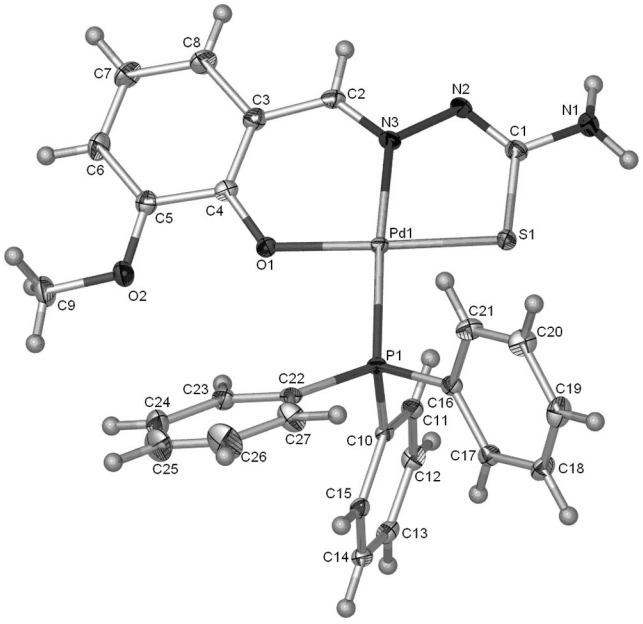


Figure 2. Molecular structure of complex **7** showing ellipsoids at the 40% probability level.

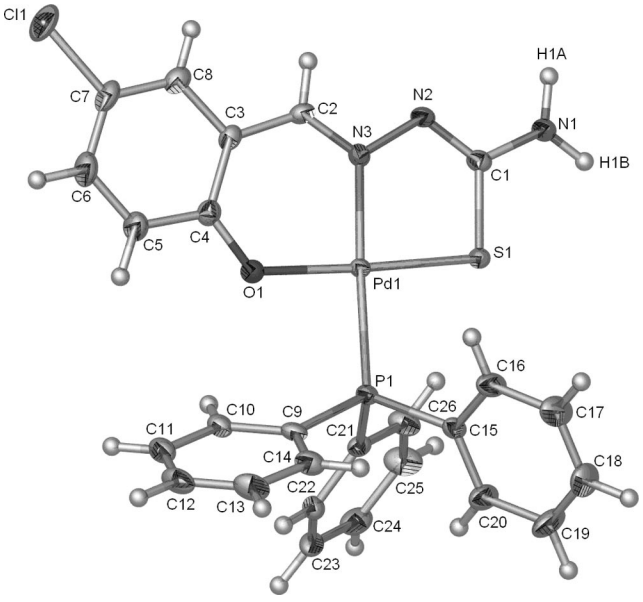


Figure 3. Molecular structure of complex **8** showing ellipsoids at the 40% probability level, with the solvent molecule (CH_2Cl_2) omitted for clarity.

plexes.[15,34,35] The bite angle that shows the least deviation from 90° is the P(1)–Pd(1)–O(1); 89.41(7) for **8**, 90.07(6) for **7** and 90.43(5) for **6**. The *trans* angles O(1)–Pd(1)–S(1) and N(3)–Pd(1)–P(1) are close to linearity in all of the molecular structures with **8** showing the least deviation from 180°.

Inspection of the bond angles formed between the metal and the coordinated atoms show that they are consistent with analogous complexes.[15,35] The Pd(1)–N(3) bond length observed in **8** and **7** are slightly longer than that of **6**. This suggests there may be greater *trans* influence exerted

by the triphenylphosphane ligand in these two complexes.[34]

The C(1)–S(1) bond has a length of approximately 1.75 Å for all three complexes, closer to the expected bond length of a typical C–S single bond (1.82 Å) than that of a C=S double bond (1.56 Å),[36] confirming that sulfur coordinates to palladium in the thiolate form.[37] Further evidence of this is obtained from the bond lengths observed for the C(1)–N(2) bond. The approximate bond length of 1.30 Å in all of the molecular structures is similar to that of the C(2)–N(3) imine bond, indicating increased double bond character and formation of a new double bond between carbon and nitrogen in the thiosemicarbazone ligand upon coordination to palladium.[15,38,39]

In Vitro Antimalarial Activity of Compounds 1–8

Compounds **1**–**8** were evaluated for in vitro antimalarial activity against both the chloroquine-resistant (W2) and -sensitive (D10) strains of *P. falciparum*, and the biological data are presented in Table 2. The control drugs used in the experiment were chloroquine (CQ) and artemisinin (ART) for the W2 strain and chloroquine for the D10 strain.

Table 2. In vitro antiplasmodial activity against *P. falciparum* strains in μM for compounds **1**–**8**.

	W2	D10	D10
	$IC_{50} \pm SD$ [μM]	Percentage survival 10 mg/mL [%]	$IC_{50} \pm SD$ [μM]
CQ[a]	0.097 ± 0.92	–	0.0598 ± 0.0148
ART[b]	0.019 ± 2.30	–	–
1	> 20.0	56.18	–
2	> 20.0	102.59	–
3	> 20.0	48.62	–
4	> 20.0	107.21	–
5	8.87 ± 2.00	26.88	9.02 ± 0.342
6	13.74 ± 2.19	51.68	–
7	10.75 ± 0.44	33.47	5.64 ± 0.442
8	> 20.0	37.67	1.38 ± 0.0453

[a] CQ = chloroquine. [b] ART = artemisinin.

The palladium(II) complexes **5**–**7**, with the exception of complex **8**, generally showed superior antiplasmodial activity in comparison to the salicyaldimine thiosemicarbazone ligands **1**–**4**, against the chloroquine-sensitive (W2) strain of the parasite. For the chloroquine-sensitive (D10) strain, the compounds were first screened for percentage parasite survival at a single concentration, before determining the IC_{50} values for selected compounds which were deemed to be active (Table 2). Generally, the palladium complexes showed enhanced activity over the analogous thiosemicarbazones against the D10 strain, and exhibited comparable antiplasmodial activity in the W2 strain. Although no clear structure–activity relationships can be gleaned from this study, it is interesting to note that for the D10 strain, the substituted aryl thiosemicarbazone palladium complexes are more active than the unsubstituted salicylaldimine(thiosemicarbazone)palladium(II) complex **5**. None of the free thiosemicarbazone ligands **1**–**4** showed appreciable activity against either strain. It is evident that chelation of the ligand to palladium enhances antimalarial activity. The differences in antiplasmodial activities observed for the complexes suggest that, i. the aromatic ring of the coordinated thiosemicarbazone ligand may be involved in the mechanism of inhibition and ii. the effect of the aryl substituent is not electronic but may be attributed to steric effects.

Antiproliferative Activity of Compounds 1–8 in Cancer Cell Lines

The salicylaldehyde-derived thiosemicarbazones ligands **1**–**4** and their corresponding Pd^{II} complexes **5**–**8** were evaluated for their antiproliferative activity in vitro against the cancer cell line WHCO1, an oesophageal cancer cell line, using Doxorubicin as the control drug. The results of cytotoxic activity in vitro are expressed as IC_{50}, the minimum compound concentration required for 50% inhibition of cell growth as compared to control untreated cells (Table 3).

Table 3. In vitro activity of compounds **1**–**8** [expressed as IC_{50} (μM)] against the WHCO1 cancer cell line.

	IC_{50} [μM]	95% confidence interval
1	n/a[a]	n/a[a]
2	1.10	0.91–1.28
3	95.13	71.61–126.40
4	10.83	9.31–12.59
5	6.68	6.35–7.03
6	54.38	15.70–188.30
7	2.56	2.33–2.82
8	24.00	19.27–29.90
Doxorubucin	0.58	0.48–0.70

[a] n/a: not active at the measured concentration.

All the compounds, except thiosemicarbazone ligand **1**, show cytotoxicity. The ligand, 3-*tert*-butyl-2-hydroxybenzaldehyde thiosemicarbazone (**2**), shows the best activity (IC_{50} = 1.10 μM) out of all compounds screened against the WHCO1 cell line. Its corresponding complex **6**, however, exhibits only moderate to weak cytotoxicity (IC_{50} = 54.38 μM). Similarly, ligand **4** shows better activity than its corresponding complex **8**. The poorer activity of these metal complexes as anticancer agents may be ascribed to their poor solubility in the cultivation medium since the metal complexes of **1** and **3** are poorly soluble under the conditions tested.

The palladium complex **7** is nearly 40 times more cytotoxic than the free ligand precursor **3**, with IC_{50} = 95.13 μM. Generally, the coordination of the thiosemicarbazone to palladium may alter the lipophilic character of the complex, thus increasing the biological activity or it may be a synergistic effect, where the ligand dissociates within the cell and interacts with the ribonucleotide reductase enzyme while the free metal ion interacts with DNA.[24,40] In the case of complex **7**, this enhanced activity may be attributed to the presence of the methoxy substituent. Interestingly, ligand **1** is not active at the maximum concentration used while its palladium complex **5** shows cytotoxicity. Coordination of

Table 4. IC_{50} values for compounds **2**, **7** and **5** against several cancer cell lines.

Cell line	Ligand **2** IC_{50} [μM]	95% confidence interval	Complex **7** IC_{50} [μM]	95% confidence interval	Complex **5** IC_{50} [μM]	95% confidence interval
WHCO1	1.10	0.91–1.28	2.56	2.33–2.82	6.68	6.35–7.03
WHCO5	4.03	3.47–4.69	2.11	0.69–6.39	n/a[a]	n/a[a]
WHCO6	6.32	5.05–7.92	12.23	5.40–27.70	43.13	15.00–124.00
KYSE30	3.02	2.21–4.13	1.48	1.11–1.96	3.76	2.63–5.39
KYSE70	7.98	6.66–9.56	n/a[a]	n/a[a]	10.28	8.14–12.97
KYSE180	7.27	6.46–8.19	5.23	n/d	6.43	5.40–7.65
KYSE410	7.46	6.48–8.59	46.02	23.51–90.11	n/a[a]	n/a[a]
KYSE450	1.64	1.33–2.02	2.19	1.42–3.36	4.11	2.86–5.91
CaSki	13.03	9.56–17.75	n/a[a]	n/a[a]	n/a[a]	n/a[a]
HeLa	0.91	0.79–1.06	n/a[a]	n/a[a]	70.61	61.81–80.67

[a] n/a: not active at the measured concentration.

thiosemicarbazone **1** to palladium clearly modifies its cytotoxic properties most likely by increasing its lipophilic nature. Of all the Pd^{II} complexes tested, complex **7** shows the greatest cytotoxic activity with an IC_{50} value of 2.56 μM.

Inspection of the results obtained for the free ligands **1**–**4** suggests that having a substituent on the aryl ring increases the activity of the compound, however, the degree of activation may be dependent on the position of the substituent, as well as the inductive effect of the substituent. Compound **3** has a strongly electron-donating substituent in position 3 on the ring yet it does not show any appreciable activity while thiosemicarbazone **2**, where the tertiary butyl group donates electron density into the ring to a lesser extent than **3**, shows the best activity. Compound **4** has an electron-withdrawing chloro substituent on the ring and it exhibits intermediate activity; better than **3**. This may be explained by the fact that the chloro group is in position 5 on the ring. It is possible that the position as well as the electronic nature of the aromatic substituent may influence the way the ligand interacts with targets inside the cell. Further studies regarding the structure–activity relationship of these compounds need to be undertaken in order to establish the actual effect of the aromatic substituent.

These observations, however, cannot be extended to their corresponding complexes which do not show particular structure–activity correlation with regard to the substituents on the ring. In a previous study, cisplatin has shown an IC_{50} value of approximately 13 μM against the WHCO1 cell line.[41] The free ligands **2** and **4** as well as palladium complexes **5** and **7** all exhibit cytotoxicities lower than this value against this cell line.

The three compounds showing the best activities, thiosemicarbazone ligand **2** and palladium(II) complexes **5** and **7**, were then further tested in two additional oesophageal cancer cell lines of South African origin (WHCO5 and WHCO6), five oesophageal cancer cell lines of Japanese origin (KYSE30, KYSE70, KYSE180, KYSE410 and KYSE450) and two cervical cancer cell lines (CaSki and HeLa). The results are presented in Table 4 along with their activity against WHCO1.

The free thiosemicarbazone ligand **2**, showed good activity against all of the cell lines screened with the best activity observed in the cervical cancer cell line HeLa. Complex **7** exhibited good IC_{50} values against cell lines WHCO1, WHCO5, KYSE30, KYSE180 and KYSE450; intermediate activity against WHCO6 and negligible activity against KYSE410 cell lines. It did not show any activity against the remaining cell lines at the highest concentrations used. Complex **5** displayed good activity in only four of the oesophageal cancer cell lines tested; was not active in the CaSki line and showed negligible activity against the HeLa cell line.

Complex **5** and similar [O,N,S] tridentate thiosemicarbazone Pd^{II} analogues have been previously screened for in vitro anticancer activity against the promyelocytic HL-60 and histiocytic lymphoma U-937 cell lines.[15] Most of these complexes exhibited IC_{50} values less than 10 μM. In addition, they were found to be more potent cytotoxic agents than the clinical drugs cisplatin, BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea], hydroxyurea and 5-FU (5-fluorouracil) which were also screened during the same experiments.[15] Similarly, in our studies, complex **5** has displayed activities lower than 10 μM in four of the oesophageal cell lines tested. Overall, thiosemicarbazone **2** was found to be a good cytotoxic agent, it consistently displayed good activity against all cell lines tested while the complexes **7** and **5** only exhibited cytotoxicity against selected cell lines.

In Vitro Apoptosis Assay

Cytotoxic agents can induce cell death through various pathways that include necrosis and apoptosis. Apoptosis is a common process of programmed cell death and is the focus of current oncology research. This process involves a series of biochemical steps resulting in morphological changes to the cell membrane including cell shrinkage, nuclear fragmentation and chromosomal DNA fragmentation.[42] PARP (Poly Adenosine-Diphosphate Ribose Polymerase) is a known caspase substrate and cleavage of PARP into two distinct molecular fragments (116 kDa and 85 kDa) serves as a marker of apoptosis. Following the results of our in vitro cytotoxicity studies, we decided to further explore PARP cleavage by Western blot analysis, in order to determine the mode of cell death induced by thio-

semicarbazone ligand **2**, the most active compound from the series of ligands and complexes synthesised. Figure 4 shows the Western blot diagrams for this experiment.

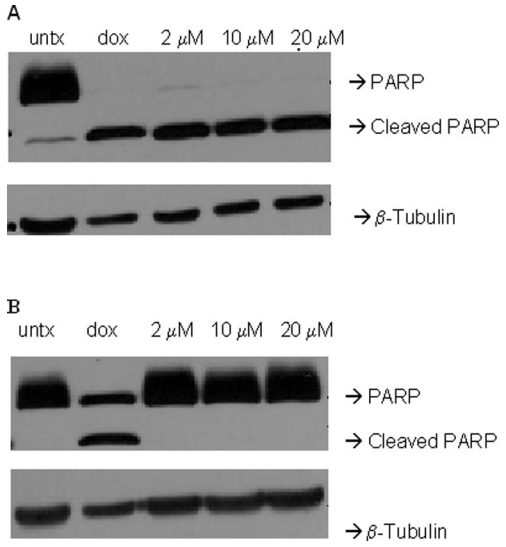


Figure 4. Western Blot diagrams for HeLa (**A**) and WHCO1 (**B**) cells treated with ligand **2** for 48 h. Dox represents the cells treated with 5 μM doxorubicin for 48 h (positive control) and Untx represents the untreated cells (negative control).

HeLa cells treated with three concentrations of thiosemicarbazone **2** showed considerable cleavage of PARP whereas WHCO1 cells treated with this compound showed no PARP cleavage. Treatment of cells with 5 μM doxorubicin served as a positive control. From these results it is evident that **2** kills HeLa cells via apoptosis. The mode of cell death in WHCO1 cells treated with **2** is clearly not apoptosis and it might be interesting to further investigate whether **2** triggers cell death in WHCO1 cells by necrosis, or autophagy (degradation of a cell by separating the contents from the rest of the cytoplasm).[43]

Conclusions

The salicylaldimine thiosemicarbazones **1**–**4** were prepared by the condensation reaction of thiosemicarbazide and the appropriate salicylaldehyde derivatives. These thiosemicarbazone ligands were complexed with *cis*-bis(triphenylphosphane)palladium(II) dichloride to produce three new mononuclear thiosemicarbazone palladium complexes **6**–**8**. The new complexes were fully characterised by ^{1}H, ^{13}C and ^{31}P NMR and IR spectroscopy, elemental analysis and mass spectrometry. The molecular structures of **6**–**8** reveal a square-planar geometry around palladium, with the thiosemicarbazone ligand bonding in a tridentate manner, forming a five- and six-membered chelate ring. The palladium(II) complexes exhibit an enhanced antiplasmodial effect over the analogous thiosemicarbazone ligand precursors when tested against both chloroquine-resistant (W2) and chloroquine-sensitive (D10) strains of *P. falciparum*. The present study also shows that the palladium(II) complexes have moderate cytotoxic properties, and in some cases, lower activities in comparison with the analogous thiosemicarbazone ligand. The thiosemicarbazone ligand **2**, containing the *tert*-butyl functionality, reveals cell death for HeLa cells to occur via apoptosis.

Experimental Section

General Procedures: All complexation reactions were performed under an atmosphere of nitrogen or argon, using a dual vacuum/nitrogen line and standard Schlenk-line techniques. All reaction solvents were dried by refluxing under an inert atmosphere over the appropriate drying agent and all samples were dried under vacuum. Reagents and solvents were purchased from commercial suppliers. $PdCl_2$ was kindly donated by Johnson–Mathey Inc. All purchased starting materials were used without further purification. The salicylaldimine thiosemicarbazone ligands **1**,[30] **3**[31] and **4**[32] and the palladium complexes, bis(triphenylphosphane)palladium(II) dichloride[44] and **5**,[15] were prepared according to the published literature procedures. Nuclear Magnetic Resonance (NMR) Spectra were recorded on a Varian Unity XR400 MHz (^{1}H at 399.95 MHz, ^{13}C at 100.58 MHz, ^{31}P at 161.90 MHz) or Varian Mercury XR300 (^{1}H at 300.08 MHz, ^{13}C at 75.46 MHz, ^{31}P at 121.47 MHz) MHz spectrometer at ambient temperature. Chemical shifts for ^{1}H and $^{13}C\{^{1}H\}$ NMR shifts are reported using tetramethylsilane (TMS) as the internal standard and $^{31}P\{^{1}H\}$ spectra were measured relative to H_3PO_4 as the external standard. Infrared absorptions (IR) were measured on a Perkin–Elmer Spectrum One FT-IR Spectrometer as KBr pellets. Microanalyses for C, H, N and S were carried out using a Fisons EA 110 elemental analyser and melting points were determined using a Kofler hot stage microscope (Reichert Thermovar). Mass Spectrometry determinations were carried out on all new compounds using electron spray ionisation on a Waters API Quattro Micro instrument in the positive mode.

Salicylaldimine Thiosemicarbazone 2: A solution of 3-*tert*-butyl-2-hydroxybenzaldehyde (0.520 g, 2.92 mmol) in ethanol (10 mL) was added dropwise to an equimolar amount of thiosemicarbazide (0.272 g, 2.98 mmol) in ethanol (20 mL). The reaction mixture was refluxed for 6 h. After cooling to room temperature, the product **2** precipitates out of solution as a white solid. The product is isolated by filtration, washed with ethanol and diethyl ether and dried in vacuo; yield 0.397 g (54%); m.p. 254–257 °C. ^{1}H NMR (300 MHz, DMSO): δ = 11.29 (s, 1 H, NN*H*CS), 10.03 (br. s, 1 H, O*H*), 8.28 (s, 1 H, *H*C=N), 7.99 (br. s, 2 H, NH_2), 7.25 (m, 2 H, Ar*H*), 6.86 (t, J = 7.69 Hz, 1 H, Ar*H*), 1.40 [s, 9 H, $C(CH_3)_3$] ppm. ^{13}C NMR (75 MHz, DMSO): δ = 177.8, 155.3, 147.0, 136.6, 129.3, 128.2, 119.1, 118.4, 34.3, 29.2 ppm. IR (KBr): $\tilde{\nu}$ = 3419 (s, O–H), 3248 (s, N–H), 3164 (s, N–H), 3038 (s, N–H), 1614 (s, C=N), 1537 (s, C=C aromatics), 1488 (m, N–N), 1293 (m, N–CS–N) 1148 (m, C=S) cm^{-1}. $C_{12}H_{17}N_3OS$ (251.36): calcd. C 57.34, H 6.81, N 16.72, S 12.76; found C 56.92, H 6.73, N 16.71, S 12.63.

General Synthesis for the Palladium(II) Complexes 6–8: The appropriate thiosemicarbazone ligand **2**–**4** (1 mol-equiv.) was added to dry ethanol (40 cm^3) under argon gas. The solution was heated to 60 °C with stirring. Triethylamine (2.1 mol-equiv.) was added followed by $Pd(PPh_3)_2Cl_2$ (1 mol-equiv.). The mixture was refluxed under argon for five hours. During this time, the product precipitated as an orange solid. The product is isolated via filtration and washed with ethanol and diethyl ether, and dried in vacuo. All of the products were recrystallised from DCM/hexane.

Spectroscopic Data for 6: *cis*-Bis(triphenylphosphane)palladium(II) dichloride (0.530 g, 0.755 mmol) was treated with 3-*tert*-butyl-2-hy-

droxybenzaldehyde thiosemicarbazone (0.216 g, 0.837 mmol); yield 0.136 g (29%); m.p. 234–236 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.26 (d, J = 14.14 Hz, 1 H, *H*C=N), 7.70–7.77 (m, 6 H, PPh_3), 7.38–7.48 (m, 9 H, PPh_3), 7.20–7.37 (m, 2 H, Ar*H*), 6.57 (t, J = 8.82 Hz, 1 H, Ar*H*), 4.59 (s, 2 H, NH_2), 0.744 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0, 162.2, 152.3, 140.3, 128.0–135.1, 118.0, 114.1 ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 24.16 (1 P, PPh_3) ppm. IR (KBr): $\tilde{\nu}$ = 3464 (m, N–H), 3391 (m, N–H), 1634 (m, C=N), 1610 (s, C=N), 1593 (s, C=C aromatics) 1435 (s, N–N) cm^{-1}. $C_{30}H_{30}N_3OPPdS$ (618.03): calcd. C 58.30, H 4.89, N 6.80, S 5.19; found C 57.73, H 4.91, N 6.39, S 4.69. ESI-MS: m/z 617 [M – H]$^+$.

Spectroscopic Data for 7: *cis*-Bis(triphenylphosphane)palladium(II) dichloride (0.516 g, 0.735 mmol) was treated with 2-hydroxy-3-methoxybenzaldehyde thiosemicarbazone (0.164 g, 0.728 mmol); yield 0.344 g (80%); m.p. 241–243 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.24 (d, J = 13.87 Hz, 1 H, *H*C=N), 7.49–7.81 (m, 6 H, PPh_3), 7.26–7.49 (m, 9 H, PPh_3), 6.96 (d, J = 8.06 Hz, 1 H, Ar*H*), 6.85 (d, J = 7.57 Hz, 1 H, Ar*H*), 6.56 (t, J = 7.79 Hz, 1 H, Ar*H*), 4.67 (s, 2 H, NH_2), 3.57 (s, 3 H, OCH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.4, 150.9, 128.3–134.8, 126.5, 117.7, 115.5, 113.9, 56.6 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 19.67 (1 P, PPh_3) ppm. IR (KBr): $\tilde{\nu}$ = 3442 (m, N–H), 3308 (w, N–H), 1642 (m, C=N), 1592 (s, C=N), 1526 (s, C=C aromatics) 1434 (s, N–N) cm^{-1}. $C_{27}H_{24}N_3O_2PPdS$ (591.96): calcd. C 54.78, H 4.09, N 7.10, S 5.42; found C 53.67, H 4.32, N 6.10, S 5.17. ESI-MS: m/z 591 [M – H]$^+$.

Spectroscopic Data for 8: *cis*-Bis(triphenylphosphane)palladium(II) dichloride (0.513 g, 0.732 mmol) was treated with 5-chlorosalicylaldehyde thiosemicarbazone (0.162 g, 0.706 mmol); yield 0.262 g (63%); m.p. 221–223 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.13 (d, J = 13.62 Hz, 1 H, *H*C=N), 7.66–7.75 (m, 6 H, PPh_3), 7.26–7.55 (m, 9 H, PPh_3), 7.23 (d, J = 2.75 Hz, 1 H, Ar*H*), 7.11 (dd, J = 2.76, 9.02 Hz, 1 H, Ar*H*), 6.59 (d, J = 9.02 Hz, 1 H, Ar*H*), 4.74 (s, 2 H, NH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.5, 161.2, 149.6, 128.3–134.7, 122.2, 118.7 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 25.23 (1 P, PPh_3) ppm. IR (KBr): $\tilde{\nu}$ = 3493 (N–H), 3385 (m, N–H), 3054 (w, C–N), 1605 (s, C=N), 1586 (m, C=N), 1529 (s, C=C aromatics), 1433 (s, N–N) cm^{-1}. $C_{26}H_{21}ClN_3OPPdS$ (596.37): calcd. C 52.36, H 3.55, N 7.05, S 5.38; found C 51.96, H 3.55, N 5.83, S 4.76. ESI-MS: m/z 597 [M + H]$^+$.

X-ray Crystallography: X-ray single-crystal intensity data were collected on a Nonius Kappa-CCD diffractometer using graphite-monochromated Mo-K_α radiation. 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 [36,45] The structure was solved by direct methods and refined employing full-matrix least-squares with the program SHELXL-97[46,47] refining on F^2. Packing diagrams were produced using the program PovRay (http://www.povray.org) and graphic interface X-seed.[48] All non-H atoms were refined anisotropically. All the hydrogen atoms, except the amino hydrogen H1A and H1B, were included in idealised positions in a riding model with U_{iso} set at 1.2 or 1.5 times those of the parent atoms (Table 5).

Crystal Data for 6–8: CCDC-719352 (for **6**), -719353 (for **7**) and -719354 (for **8**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Biological Experiments

Antimalarial Experiments: Ring stage, W2-strain *P. falciparum* parasites (1% parasitaemia, 2% haematocrit) were cultured in 0.5 mL of medium in 48-well culture dishes.[49] Inhibitors from 10 mM stocks in DMSO were added to cultured parasites to give a final concentration of 20 μM. From 48-well plates, 125 μL of culture was transferred to two 96 well plates (duplicates). Serial dilutions (1:5) of inhibitors were made to final concentrations of 10 μM, 2 μM, 0.4 μM, 80 nM, 16 nM and 3.2 nM. Cultures were maintained at 37 °C for 2 d after which the parasites were washed and fixed with 1% formaldehyde in PBS. After two days, parasitaemia was measured by flow cytometry using the DNA stain YOYO-1 as a marker for cell survival.[49] IC_{50} values for growth inhibition were determined with GraphPad Prism software from plots of percentage parasitemia of untreated control cultures against inhibitor concentration.

The test compounds were also tested in triplicate on one occasion against chloroquine-sensitive (CQS) strain of *Plasmodium falciparum* (D10). Continuous in vitro cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.[50] Quantitative assessment of antiplasmodial activity in vitro was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler.[51] The samples were prepared to a 2 mg/mL stock solution in 10% DMSO and sonicated to enhance solubility. Samples were tested as a sus-

Table 5. Crystallographic data and structure refinement parameters for palladium complexes **6**–**8**.

	6	**7**	**8**
Empirical formula	$C_{32}H_{30}Cl_6D_2N_3OPPdS$	$C_{27}H_{24}N_3O_2PPdS$	$C_{27}H_{23}Cl_3N_3OPPdS$
Formula mass	858.75	591.92	681.26
Crystal size	0.18 × 0.14 × 0.14 mm	0.12 × 0.11 × 0.09 mm	0.20 × 0.14 × 0.08 mm
Crystal system	monoclinic	monoclinic	monoclinic
Space group	$P2_1/n$	$C2/c$	$P2_1/c$
a	10.1900(2) Å	33.3399(7) Å	14.7145(4) Å
b	20.5408(4) Å	10.3708(2) Å	8.0414(1) Å
c	17.9344(3) Å	14.9613(2) Å	24.3591(6) Å
α	90°	90°	90°
β	102.543°	102.0910°	101.151°
γ	90°	90°	90°
V	3664.27(12) Å^3	5058.28(16) Å^3	2827.88(11) Å^3
Z	4	8	4
Calculated density	1.557 Mg/m^3	1.555 Mg/m^3	1.600 Mg/m^3
F(000)	1728	2400	1368
R indices, (for all data)	$R1$ = 0.0476, $wR2$ = 0.0887	$R1$ = 0.0362, $wR2$ = 0.0628	$R1$ = 0.0528, $wR2$ = 0.0883

pension if not completely dissolved. Stock solutions were stored at –20 °C. Further dilutions were prepared on the day of the experiment. Chloroquine (CQ) was used as the reference drug in all experiments. Test samples were tested at one concentration (10 μg/mL). CQ was tested at concentrations of 30, 15 and 7.5 ng/mL.

Anticancer Experiments

Cell Lines and Cell Proliferation Assays: The three oesophageal cancer cell lines, WHCO1, WHCO5 and WHCO6, were derived from biopsies of primary oesophageal squamous cell carcinomas (oesophageal cancer cells of South African origin)[52] and kindly provided by Professor Rob Veale (University of Witwatersrand, South Africa). The CaSki and HeLa cervical cancer cell lines were purchased from the American Type Culture Collection (Rockville, MD, USA) and the KYSE oesophageal squamous cell carcinoma cell lines, previously established by Shimada and co-workers,[53] were purchased from the German Resource Centre for Biological Material (http://www.dsmz.de). IC_{50} determinations were carried out using the MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Briefly, 3000 cells were seeded per well in 96-well plates. Cells were incubated at 37 °C under 5% CO_2 (24 h), after which aqueous DMSO solutions of each compound (10 μL, with a constant final concentration of DMSO: 0.2%) were plated at various concentrations. After 48 h incubation, observations were made, and MTT (10 μL) solution added to each well. After a further 4 h incubation, solubilization solution (100 μL) was added to each well, and plates were incubated overnight. Plates were read at 595 nm on a BioTek microplate reader.

Western Blot Analysis: Cells were harvested in 60 μL of radioimmuno-precipitation assay buffer [150 mmol/L NaCl, 1% Triton X-100, 0.1% SDS, 25 mmol/L Tris-HCl (pH 7.5), 1% sodium deoxycholate, 1 mmol/L Na_3VO_4, 20 μg/mL pepstatin, 1 mmol/L phenylmethylsulfonyl fluoride] with protease inhibitor (Complete tablets, Roche), sonicated for 10 s with a probe sonicator (Heat System-Ultrasonics) and centrifuged for 15 min at 13,000 *g*. The protein concentration of the lysates was determined using the BCA Protein Assay Kit (Pierce). Equal amounts of protein was electrophoresed on 10% SDS polyacrylamide gel at a constant current of 15 mA and electrophoretically transferred to a nitrocellulose membrane (Hybond-ECL; Amersham Pharmacia Biotech UK) at 100 V for 1 h. Membranes were incubated for 1 h with 5% fat-free dry milk in TBS with 0.1% Tween 20 to block nonspecific binding sites and then incubated with 1:1000 dilution of rabbit polyclonal primary antibody to poly (ADP ribose) polymerase (Santa Cruz Biotechnology) at 4 °C overnight. The immunoreactivity was detected by using peroxidase-conjugated antirabbit secondary antibody and visualized by enhanced chemiluminescence (SuperSignal West Pico Chemiluminescent Substrate; Pierce). The blots were stripped before reprobing with antibody to β-tubulin (Santa Cruz Biotechnology).

Acknowledgments

We gratefully thank the University of Cape Town, the National Research Foundation (NRF), the Medical Research Council (MRC) of South Africa and the Cancer Association of South Africa (CANSA) for financial support. AngloPlatinum Corporation and Johnson Matthey is acknowledged for the kind donation of palladium salts.

[1] E. W. Ainscough, A. M. Brodie, W. A. Denny, G. J. Finlay, J. D. Ranford, *J. Inorg. Biochem.* **1998**, *70*, 175–185.
[2] H. Beraldo, D. Gambino, *Mini-Rev. Med. Chem.* **2004**, *4*, 31–39.
[3] D. S. Kalisnowski, D. R. Richardson, *Pharmacol. Rev.* **2005**, *57*, 547–583.
[4] D. S. Kalinowski, D. R. Richardson, *Chem. Res. Toxicol.* **2007**, *20*, 715–720.
[5] J. S. Casas, M. S. García-Tasende, J. Sordo, *Coord. Chem. Rev.* **2000**, *209*, 197–261.
[6] M. A. Ali, S. E. Livingstone, *Coord. Chem. Rev.* **1974**, *13*, 101–115.
[7] M. J. M. Campbell, *Coord. Chem. Rev.* **1975**, *15*, 279–319.
[8] S. Padhye, G. B. Kauffman, *Coord. Chem. Rev.* **1985**, *63*, 127–160.
[9] A. G. Quiroga, C. N. Ranninger, *Coord. Chem. Rev.* **2004**, *248*, 119–133.
[10] S. N. Pandeya, J. R. Dimmock, *Pharmazie* **1993**, *48*, 659–666.
[11] T. S. Lobana, R. Sharma, G. Bawa, S. Khanna, *Coord. Chem. Rev.* **2009**, *253*, 977–1055.
[12] A. A. El-Asmy, O. A. El-Gammal, H. S. Saleh, *Spectrochimica Acta Part A: Mol. Biomol. Spec.* **2008**, *71*, 39–44.
[13] S. Halder, R. J. Butcher, S. Bhattacharya, *Polyhedron* **2007**, *26*, 2741–2748.
[14] R. M. El-Shazly, G. A. A. Al-Hazmi, S. E. Ghazy, M. S. El-Shahawi, A. A. El-Asmy, *J. Coord. Chem.* **2006**, *59*, 845–849.
[15] S. Halder, S.-M. Peng, G.-H. Lee, T. Chatterjee, A. Mukherjee, S. Dutta, U. Sanyal, S. Bhattacharya, *New J. Chem.* **2008**, *32*, 105–114.
[16] T. Stringer, P. Chellan, B. Therrien, N. Shunmoogam-Gounden, D. T. Hendricks, G. S. Smith, *Polyhedron* **2009**, *28*, 2839–2846.
[17] Z. Iakovidou, E. Mioglou, D. Mourelatos, A. Kotsis, M. A. Demertzis, A. Papagoegiou, J. R. Miller, D. Kovala-Demertzi, *Anticancer Drugs* **2001**, *12*, 65–70.
[18] A. Garoufis, S. K. Hadjikakou, N. Hadjiliadis, *Metallotherapeutic Drugs and Metal-based Diagnostic Agents: The Use of Metals in Medicine* (Eds.: M. Gielen, E. R. T. Tiekink), John Wiley & Sons Ltd, **2005**, chapter 21, p. 399, and references cited therein.
[19] D. Kovala-Demertzi, M. A. Demertzis, J. R. Miller, C. Papadopoulou, C. Dodorou, G. Filousis, *J. Inorg. Biochem.* **2001**, *86*, 555–563.
[20] M. Das, S. E. Livingstone, *Br. J. Cancer* **1978**, *37*, 463–466.
[21] A. G. Quiroga, J. M. Perez, I. Lopez-Solera, J. R. Masaguer, A. Luque, P. Roman, A. Edwards, C. Alonso, C. Navarro-Ranninger, *J. Med. Chem.* **1998**, *41*, 1399–1408.
[22] A. G. Quiroga, J. M. Perez, C. Alonso, C. Navarro-Ranninger, *Appl. Organomet. Chem.* **1998**, *12*, 809–813.
[23] A. G. Quiroga, J. M. Perez, E. I. Montero, C. Alonso, C. Navarro-Ranninger, *J. Inorg. Biochem.* **1999**, *75*, 293–301.
[24] D. Kovala-Demertzi, A. Alexandratos, A. Papageogiou, P. N. Yadav, P. Dalezis, M. A. Demertzis, *Polyhedron* **2008**, *27*, 2731–2738.
[25] S. Padhye, Z. Afrasiabi, E. Sinn, J. Fok, K. Mehta, N. Rath, *Inorg. Chem.* **2005**, *44*, 1154–1156.
[26] J. Patole, S. Padhye, M. S. Moodbidri, N. Shirsat, *Eur. J. Med. Chem.* **2005**, *40*, 1052–1055.
[27] A. G. Quiroga, L. Cubo, P. J. S. Miguel, V. Moneo, A. Carnero, C. Navarro-Ranninger, *Eur. J. Inorg. Chem.* **2008**, 1183–1187.
[28] D. Kovala-Demertzi, P. N. Yadav, M. A. Demertzis, M. Coluccia, *J. Inorg. Biochem.* **2000**, *78*, 347–354.
[29] J. P. Scovill, D. L. Klayman, D. G. Franchino, *J. Med. Chem.* **1982**, *25*, 1261–1265.
[30] I. D. Kostas, F. J. Andreadaki, D. Kovala-Demertzi, C. Prentjas, M. A. Demertzis, *Tetrahedron Lett.* **2005**, *46*, 1967–1970.
[31] A. P. Kumar, *Anal. Lett.* **2008**, *41*, 1022–1037.
[32] I. Yilmaz, *Heteroat. Chem.* **2003**, *14*, 617–621.
[33] J. Albert, J. Granell, J. Sales, M. Fon-Bardia, X. Solans, *Organometallics* **1995**, *14*, 1393–1404.

[34] R. Prabhakaran, S. V. Renukadevi, R. Karvembu, R. Huang, J. Mautz, G. Huttner, R. Subashkumar, K. Natarajan, *Eur. J. Inorg. Chem.* **2008**, *43*, 268–273.
[35] T. S. Lobana, G. Bawa, A. Castineiras, R. J. Butcher, *Inorg. Chem. Commun.* **2007**, *10*, 506–509.
[36] L. E. Shutton, *Tables of Interatomic Distances and Configurations in Molecules and Ions (Supplement)*, The Chemical Society, **1965**, London.
[37] Z. Afrasiabi, E. Sinn, J. Chen, Y. Ma, A. L. Rheingold, L. N. Zakharov, N. Rath, S. Padhye, *Inorg. Chim. Acta* **2004**, *357*, 271–278.
[38] P. N. Yadav, M. A. Demertzis, D. Kovala-Demertzi, S. Skoulika, D. X. West, *Inorg. Chim. Acta* **2003**, *349*, 30–36.
[39] L. Papathanasis, M. A. Demertzis, P. N. Yadav, D. Kovala-Demertzi, C. Prentjas, A. Castineiras, S. Skoulika, D. X. West, *Inorg. Chim. Acta* **2004**, *357*, 4113–4120.
[40] D. Kovala-Demerzi, A. Domopoulou, M. A. Demertzis, G. Valle, A. Papageorgiou, *J. Inorg. Biochem.* **1997**, *68*, 147–155.
[41] J. Rajput, J. R. Moss, A. T. Hutton, D. T. Hendricks, C. E. Arendse, C. Imrie, *J. Organomet. Chem.* **2004**, *689*, 1553–1568.
[42] S. W. Lowe, A. W. Lin, *Carcinogenesis* **2000**, *21*, 485–495.
[43] M. Kundu, C. B. Thompson, *Annu. Rev. Pathol. Mech. Dis.* **2008**, *3*, 427–455.
[44] W. A. Hermann, A. Slazer, *Synth. Meth. Organomet. Inorg. Chem.* **1996**, *1*, 160.
[45] Z. Otwinowski, W. Minor, *Methods in Enzymology, Macromolecular Crystallography* (Eds.: C. W. Carter Jr., R. M. Sweet), Academic Press, **1997**, vol. 276, part A, pp. 307–326.
[46] G. M. Sheldrick, *SADABS*, University of Göttingen, Germany, **1996**.
[47] G. M. Sheldrick, *SHELXL-97 and SHELXS-97*, *Program for Crystal Structure Refinement*, University of Göttingen, Germany, **1997**.
[48] L. J. Barbour, *J. Supramol. Chem.* **2001**, *1*, 189–191.
[49] P. S. Sijwali, P. J. Rosenthal, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 4384–4389.
[50] W. Trager, J. B. Jensen, *Science* **1976**, *193*, 673–675.
[51] M. T. Makler, J. M. Ries, J. A. Williams, J. E. Bancroft, R. C. Piper, B. L. Gibbins, D. J. Hinrichs, *Am. J. Trop. Med. Hyg.* **1993**, *48*, 739–741.
[52] R. B. Veale, A. L. Thornley, *S. Afr. J. Sci.* **1989**, *85*, 375–379.
[53] Y. Shimada, M. Imamura, T. Wagata, N. Yamaguchi, T. Tobe, *Cancer* **1992**, *69*, 227–284.

Received: March 19, 2010
Published Online: June 15, 2010

