Journal of Inorganic Biochemistry 105 (2011) 1562–1568

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Synthesis and *in vitro* evaluation of palladium(II) salicylaldiminato thiosemicarbazone complexes against *Trichomonas vaginalis*

Prinessa Chellan [a], Tameryn Stringer [a], Ajit Shokar [b], Padraick J. Dornbush [b], Guillermo Vazquez-Anaya [b], Kirkwood M. Land [b], Kelly Chibale [a,c], Gregory S. Smith [a,*]

[a] Department of Chemistry, University of Cape Town, Private Bag, Rondebosch 7701, South Africa
[b] Department of Biological Sciences, University of the Pacific, Stockton, CA 95211, United States
[c] Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa

ARTICLE INFO

Article history:
Received 6 May 2011
Received in revised form 29 July 2011
Accepted 29 July 2011
Available online 6 August 2011

Keywords:
Thiosemicarbazones
Pd(II) complexes
Antiparasital activity
Trichomonas vaginalis

ABSTRACT

Eight mononuclear Pd(II) complexes containing salicylaldiminato thiosemicarbazones (saltsc-R; where R = H (**1**), 3-OMe (**2**), 3-^{t}Bu (**3**) and 5-Cl (**4**)) as dinegative tridentate ligands were prepared by the reaction of the corresponding thiosemicarbazone with the precursor $Pd(L)_2Cl_2$ (L = phosphatriazaadamantane or 4-picoline) in the presence of a weak base. These complexes (**9**–**16**) were characterised by a range of spectroscopic and analytical techniques including NMR spectroscopy and X-ray diffraction. These complexes along with four other Pd(II) analogues (**5**–**8**) were screened for activity *in vitro* against the *Trichomonas vaginalis* parasite. Preliminary results show that the type of ancillary ligand as well as the substituents on the aromatic ring of the salicylaldiminato thiosemicarbazone ligand influences the antiparasitic activity of these complexes.

1. Introduction

Trichomonas vaginalis is a flagellated facultative anaerobic protozoan, which causes the sexually transmitted infection trichomoniasis in humans. Metronidazole (Fig. 1), a 5-nitroimidazole drug derived azomycin, is the current FDA-approved treatment [1]. Approximately 5% of all cases are resistant to this compound, with that percentage increasing [2]. There is therefore a need to find alternative forms of treatment due to this increase in resistance. The use of thiosemicarbazones (TSCs) as potential therapeutics is an active area of research. These Schiff-base type compounds are noted for their pharmacological properties, particularly as antiparasitic [3–8], antibacterial [9–11] and antitumoral agents [12–16]. As anticancer agents, it is believed that their mechanism of action is through the inhibition of ribonucleotide reductase [17]. These compounds have also been shown to inhibit a *Trypanosoma cruzi* derived cysteine protease, cruzain [8] and are also effective against the parasites *Plasmodium falciparum* and *Trypanosoma brucei* [18]. Thus thiosemicarbazones may be an alternative therapy against the parasite *T. vaginalis*, since it also secretes cysteine proteases [19–21].

In previous work, we synthesised a library of thiosemicarbazones and screened them against three parasitic cysteine proteases, cruzain, falcipain-2, and rhodesain and against the respective parasite sources of these three proteases, *T. cruzi, P. falciparum and T. brucei* [18]. The screens identified compounds that were effective against the enzymes and the parasites, but also some compounds that were parasiticidal despite a lack of activity against the proteases, suggesting that other mechanisms besides cysteine protease inhibition are involved. This data suggested that thiosemicarbazones represent validated drug leads that kill several species of protozoan parasites through the inhibition of cysteine proteases as well as other novel targets. The thiourea moiety of thiosemicarbazones contains several donor atoms and is thus capable of acting as a multidentate ligand toward a metal. Their metal chelating abilities are believed to partially account for their biological activity and numerous studies on the biological activity of TSC metal complexes have been published [22–28]. The study of the coordination chemistry of thiosemicarbazones has long been of interest with the earliest review being published in 1974 [29]. Since then there have been extensive reports on the synthesis of thiosemicarbazone complexes with metals including vanadium [30,31], zinc [32], cobalt [33], gold [34], nickel [26,35], silver [36], copper [37–39] and iron [40].

The synthesis and study of tridentate salicylaldiminato thiosemicarbazone Pd(II) complexes as potential biological agents have previously been reported by us [41,42] as well as others [43]. The complexes were found to be moderately cytotoxic as chemotherapeutics when tested *in vitro* [41–43]. However, our studies into their use against the malarial parasite *P. falciparum in vitro* revealed these complexes to be promising inhibitors [41]. Coupled with our earlier studies on parasiticidal thiosemicarbazone cysteine protease inhibitors against *P. falciparum*, *T. brucei* and *T. cruzi* [18], these results have thus prompted us to study the effects of these types of salicylaldiminato

* Corresponding author. Tel.: +27 21 650 5279; fax: +27 21 650 5195.
E-mail address: gregory.smith@uct.ac.za (G.S. Smith).

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doi:10.1016/j.jinorgbio.2011.07.023

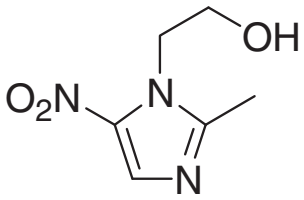


Fig. 1. Structure of Metronidazole, the current FDA-approved treatment of *T. vaginalis*.

palladium(II) complexes on the causative agents of other parasitic diseases exemplified by *T. vaginalis*.

Herein, we report the synthesis, structural characterisation and inhibitory investigation of a series of tridentate [O,N,S] salicylaldiminato thiosemicarbazone Pd(II) complexes containing functionalised salicylaldehyde moieties as well as various ancillary ligands. The objectives of our study were to determine if coordination of these compounds to palladium enhances their inhibitory effects and to ascertain if variation of the ancillary ligand as well as the functional groups of the thiosemicarbazone ligand would beneficially modify biological activity.

2. Experimental

2.1. Materials and methods

All complexation reactions were performed under a nitrogen or argon atmosphere using a dual vacuum/nitrogen line and standard Schlenk-line techniques unless otherwise stated. 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 and used without further purification. $PdCl_2$ was kindly donated by Johnson-Mathey Inc. 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 signals 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. IR spectra 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 Thermo Flash 1112 Series CHNS-O 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 electrospray ionisation on a Waters API Quattro Micro instrument in the positive or negative mode.

2.2. Synthesis

The salicylaldimine thiosemicarbazone ligands **1**[44], **2**[45], **3**[41], **4**[46] and the palladium complexes, dichlorobis(4-picoline)palladium (II) [47], dichlorobis(phosphatriaza-adamantane)palladium(II) [48], **5** and **13**[43] were prepared according to the published literature procedures. The synthesis and characterisation of complexes **6**–**8** have previously been reported by us [41].

2.2.1. General procedure for preparation of palladium(II) complexes (**9**–**16**)

The appropriate thiosemicarbazone ligand (**1**–**4**, 1 mol equiv.) was added to dry ethanol (40 mL) under argon. The solution was heated to 60 °C with stirring. Triethylamine (2.1 mol equiv.) was added followed by either $Pd(PTA)_2Cl_2$ (1 mol equiv.) for complexes **9**–**12** or $Pd(4\text{-pic})_2Cl_2$ (1 mol equiv.) for complexes **14**–**16**. The mixture was refluxed over periods of time designated in the subsequent text. The products precipitated as an orange or yellow solid. The product was isolated via filtration, washed with ethanol and diethyl ether, and dried *in vacuo*. All of the products were recrystallised from DCM-Hexane.

2.2.2. Spectroscopic data for **9**

Salicylaldimine thiosemicarbazone **1** (0.055 g, 0.28 mmol) and $Pd(PTA)_2Cl_2$ (0.14 g, 0.28 mmol) were refluxed in ethanol for 10 h. The product obtained was a yellow powder (0.098 g, 77%). M.p.: 262 °C. ^{1}H NMR (300.08 MHz, DMSO–d_6, s = singlet, m = multiplet, d = doublet, dd = doublet of doublet): δ (ppm) = 4.31 (6H, s, *H*-PTA); 4.42 (3H, d, $^2J_{H-H}$ = 13.07, *H*-PTA); 4.57 (3H, d, $^2J_{H-H}$ = 12.98, *H*-PTA); 6.56 (3H, m, NH_2, H4); 6.89 (1H, d, $^3J_{H-H}$ = 8.34, H2); 7.23 (1H, t, $^3J_{H-H}$ = 8.58, H3); 7.37 (1H, dd, $^4J_{H-H}$ = 1.77, $^3J_{H-H}$ = 7.93, H5); 8.21 (1H, d, $^4J_{P-H}$ = 13.18, H7). ^{31}P NMR (121.47 MHz, DMSO–d_6): δ (ppm) = −40.70 (1P, s, PTA). ^{13}C NMR (75.46 MHz, DMSO–d_6): δ (ppm) = 49.94 (PTA); 71.87 (PTA); 114.17 (C2); 117.83 (C6), 120.59 (C4); 132.25 (C5); 134.00 (C3); 147.57 (C7); 161.70 (C1); 171.17 (C8). IR (KBr, cm^{-1}, m = medium, s = strong) υ = 3396 (m, N–H), 3285 (m, N–H), 1615 (m, C=N), 1595 (s, C=N), 1531 (s, C=C aromatics). Elemental analysis for $C_{14}H_{19}N_6SPOPd$: found C 36.77, H 4.24, N 17.97, S 6.87%; calculated C 36.81, H 4.19, N 18.40, S 7.02%. ESI–MS: *m/z* 457 (calcd. 456.82) ([M]$^+$, 100%).

2.2.3. Spectroscopic data for **10**[1]

3-Methoxy salicylaldimine thiosemicarbazone **2** (0.053 g, 0.23 mmol) and $Pd(PTA)_2Cl_2$ (0.11 g, 0.233 mmol) were refluxed in ethanol for 8 h. The product obtained was a yellow powder (0.092 g, 81%). M.p.: no melting or decomposition up to 300 °C. ^{1}H NMR (300.08 MHz, DMSO–d_6): δ (ppm) = 3.84 (3H, s, OCH_3); 4.41 (6H, s, *H*-PTA); 4.51 (3H, d, $^2J_{H-H}$ = 13.17, *H*-PTA); 4.64 (3H, d, $^2J_{H-H}$ = 12.69, *H*-PTA); 6.56 (1H, t, $^3J_{H-H}$ = 7.79, H4); 6.64 (2H, s, NH_2); 6.95 (1H, dd, $^4J_{H-H}$ = 1.53, $^3J_{H-H}$ = 7.59, H3); 7.07 (1H, dd, $^4J_{H-H}$ = 1.50, $^3J_{H-H}$ = 8.13, H5); 8.27 (1H, d, $^4J_{P-H}$ = 13.20, H7). ^{31}P NMR (121.47 MHz, DMSO–d_6): δ (ppm) = −39.37 (1P, s, PTA). ^{13}C NMR (100.58 MHz, DMSO–d_6): δ (ppm) = 50.83 (OCH_3); 57.00 (PTA); 72.68 (PTA); 114.17 (C4); 114.89 (C3); 118.50 (C6); 126.79 (C5); 148.16 (C7); 151.72 (C2); 153.74 (C1); 172.01 (C8). IR (KBr, cm^{-1}, br m = broad medium) υ = 3422 (br m, N–H), 1623 (m, C=N), 1596 (s, C=N), 1540 (s, C=C aromatics). Elemental analysis for $C_{15}H_{21}N_6SPO_2Pd$: found C 37.18, H 4.38, N 16.14, S 6.01%; calculated C 37.00, H 4.35, N 17.27, S 6.58%. ESI–MS: *m/z* 487 (calcd. 486.76) ([M]$^+$, 100%); 975 ([2 M + H]$^+$, 20%).

2.2.4. Spectroscopic data for **11**[1]

3-*tert* Butyl salicylaldimine thiosemicarbazone **3** (0.050 g, 0.20 mmol) and $Pd(PTA)_2Cl_2$ (0.096 g, 0.19 mmol) were refluxed in ethanol for 8 h. The product obtained was a yellow powder (0.080 g, 80%). M.p.: no melting or decomposition up to 300 °C. ^{1}H NMR (300.08 MHz, DMSO–d_6): δ (ppm) = 1.51 (9H, s, C(CH_3)$_3$); 4.43 (6H, s, *H*-PTA); 4.53 (3H, d, $^2J_{H-H}$ = 13.38, *H*-PTA); 4.62 (3H, d, $^2J_{H-H}$ = 12.68, *H*-PTA); 6.60 (3H, m, NH_2, H4); 7.32 (2H, d, $^3J_{H-H}$ = 7.91, H5, H3); 8.28 (1H, d, $^4J_{P-H}$ = 13.48, H7). ^{31}P NMR (121.47 MHz, DMSO–d_6): δ (ppm) = −43.77 (1P, s, PTA). ^{13}C NMR (100.58 MHz, DMSO–d_6): δ (ppm) = 30.04 (*C*(CH_3)$_3$); 35.88 (C(CH_3)$_3$); 48.92 (PTA); 72.66 (PTA); 114.46 (C4); 118.84 (C6); 131.67 (C5); 133.89 (C3); 139.36 (C2); 149.51 (C7); 161.85 (C1); 170.95 (C8). IR (KBr, cm^{-1}, br m = broad medium) υ = 3437 (br m, N–H), 3293 (br w, N–H), 1643 (m, C=N), 1593 (s, C=N), 1536 (s, C=C aromatics). Elemental analysis for $C_{18}H_{27}N_6SPOPd$: found C 42.24, H 5.35, N 17.45, S 5.65%; calculated C 42.15, H 5.30, N 16.39, S 6.25%. ESI–MS: *m/z* 513 (calcd. 512.84) ([M]$^+$, 100%).

2.2.5. Spectroscopic data for **12**[1]

5-Chlorosalicylaldimine thiosemicarbazone **4** (0.050 g, 0.22 mmol) and $Pd(PTA)_2Cl_2$ (0.11 g, 0.22 mmol) were refluxed in ethanol for 24 h.

[1] Although the elemental analyses data for "N" (compounds **10**, **11**, **12** and **16**) and "C" (compound **16**) are somewhat unsatisfactory, the ESI–MS result and other spectroscopic analysis data (^{1}H- and ^{13}C-NMR, IR), and the X-ray crystal structure determination for compound **11**, reasonably support their formula.

The product obtained was a yellow powder (0.097 g, 92%). M.p.: no melting or decomposition up to 300 °C. ^{1}H NMR (300.08 MHz, DMSO–d_6): δ (ppm) = 4.31 (6H, s, *H*-PTA); 4.42 (3H, d, $^2J_{H-H}$ = 13.12, *H*-PTA); 4.58 (3H, d, $^2J_{H-H}$ = 12.91, *H*-PTA); 6.75 (2H, s, NH_2); 6.97 (1H, d, $^3J_{H-H}$ = 9.02, H2); 7.27 (1H, dd, $^4J_{H-H}$ = 2.84, $^3J_{H-H}$ = 9.00, H3); 7.55 (2H, d, $^4J_{H-H}$ = 2.83, H5); 8.30 (1H, d, $^4J_{P-H}$ = 13.00, H7). ^{31}P NMR (121.47 MHz, DMSO–d_6): δ (ppm) = −40.06 (1P, s, PTA). ^{13}C NMR (75.46 MHz, DMSO–d_6): δ (ppm) = 49.94 (PTA); 71.86 (PTA); 117.03 (C2); 119.08 (C6); 122.39 (C4); 131.65 (C5); 132.03 (C3); 146.51 (C7); 160.36 (C1); 171.98 (C8). IR (KBr, cm^{-1}) υ = 3390 (br w, N–H), 3286 (br w, N–H), 1630 (m, C═N), 1595 (s, C═N), 1516 (s, C═C aromatics). Elemental analysis for $C_{14}H_{18}N_6SOClPdP$: found: C 34.37, H 3.77, N 17.96, S 5.92%; calculated C 34.23, H 3.69, N 17.11, S 6.53%. ESI–MS: *m/z* 493 (calcd. 491.24) ($[M+H]^+$, 100%).

2.2.6. Spectroscopic data for **14**

Dichlorobis(4-picoline)palladium(II) (0.50 g, 1.4 mmol) was reacted with 3-methoxy salicylaldimine thiosemicarbazone **2** (0.31 g, 1.4 mmol). The reaction mixture was heated to 50 °C for 2 h. The product precipitated as a crystalline orange solid (0.16 g, 27%). M.p.: 225–227 °C. ^{1}H NMR (300.08 MHz, $CDCl_3$): δ (ppm) = 8.70 (d, $^3J_{H-H}$ = 6.60 Hz, 2H, H_α-4-picoline), 7.97 (s, 1H, H7), 7.14–7.31 (m, 1H, H5), 6.95–7.02 (m, 3H, H4, H_β-4-picoline), 6.52–6.68 (m, 1H, H_3), 4.74 (s, 2H, NH_2), 3.85 (s, 3H, OCH_3), 2.43 (s, 3H, CH_3-4-picoline). ^{13}C NMR (75.46 MHz, $CDCl_3$): δ (ppm) = 170.5 (C7), 153.9 (C8), 152.2 (C2), 151.8 (C_α-4-picoline), 150.7 ($C(CH_3)$-4-picoline), 149.0 (C1), 127.4 (C_β-4-picoline), 126.2 (C5), 125.3 (C4), 119.1 (C6), 114.5 (C3), 56.7 (OCH_3), 21.2 (CH_3-4-picoline). IR (KBr, cm^{-1}) υ = 3428 (br m, N–H), 3351 (br m, N–H), 1621(s, C═N), 1603 (s, C═N), 1525 (s, C═C aromatics). Elemental analysis for $C_{15}H_{16}N_4O_2PdS \cdot \frac{1}{3}CHCl_3 \cdot \frac{1}{2}C_6H_{14}$: found C 43.81, H 4.20, N 11.88, S 6.38%; calculated C 43.54, H 4.65, N 11.88, S 6.34%. ESI–MS: *m/z* 423 (calcd. 422.80) ($[M]^+$, 100%).

2.2.7. Spectroscopic data for **15**

Dichlorobis(4-picoline)palladium(II) (0.50 g, 1.4 mmol) was reacted with 3-*tert* Butyl salicylaldimine thiosemicarbazone **3** (0.35 g, 1.4 mmol). The reaction mixture was refluxed for 5 h and the product precipitated as a fluffy yellow solid (0.29 g, 46%). M.p.: no melting or decomposition up to 300 °C. ^{1}H NMR (399.95 MHz, $CDCl_3$): δ (ppm) = 8.69 (d, $^3J_{H-H}$ = 6.50 Hz, 2H, H_α-4-picoline), 7.97 (s, 1H, H7), 7.32–7.36 (m, 1H, H5), 7.18–7.25 (m, 3H, H4, H_β-4-picoline), 6.79–6.42 (m, 1H, H3), 4.73 (s, 2H, NH_2), 2.47 (s, 3H, (CH_3-4-picoline)), 1.33 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (75.46 MHz, $CDCl_3$): δ (ppm) = 169.8 (C7), 161.8 (C8), 152.0 (C1), 151.7 (C_α-4-picoline), 150.8 ($C(CH_3)$-4-picoline), 138.9 (C2), 132.6 (C3), 130.3 (C5), 125.9 (C_β-4-picoline), 118.7 (C4), 114.3 (C6), 35.2 ($C\,C(CH_3)_3$), 29.4 ($C(CH_3)_3$), 21.2 (CH_3-4-picoline). IR (KBr, cm^{-1}) υ = 3421 (m, N–H), 3310 (s, N–H), 1621 (br w, C═N), 1609 (s, C═N), 1591 (s, C═N), 1527 (s, C═C aromatics). Elemental analysis for $C_{18}H_{22}N_4OPdS$: found C 47.81, H 5.03, N 13.01, S 6.96%; calculated C 48.16, H 4.94, N 12.48, S 7.14%. ESI–MS: *m/z* 449 (calcd. 448.88) ($[M]^+$, 100%).

2.2.8. Spectroscopic data for **16**[1]

Dichlorobis(4-picoline)palladium(II) (0.39 g, 1.1 mmol) was reacted 5-chlorosalicyl-aldimine thiosemicarbazone **4** (0.25 g, 1.1 mmol). The product precipitated out of solution as a yellow solid (0.14 g, 30%). M.p.: decomposition without melting 238–240 °C. ^{1}H NMR (300.08 MHz, $CDCl_3$): δ (ppm) = 8.59 (d, $^3J_{H-H}$ = 6.60 Hz, 2H, H_α-4-picoline), 7.83 (s, 1H, H7), 7.16–7.44 (m, 3H, H5, H_β-4-picoline), 6.94–6.96 (m, 2H, H2, H3), 4.78 (s, 2H, NH_2), 2.44 (s, 3H, CH_3-4-picoline). ^{13}C NMR (75.46 MHz, $CDCl_3$): δ (ppm) 172.71 (C7), 161.09 (C8), 152.3 (C1), 151.8 (C_α-4-picoline), 147.9 ($C(CH_3)$-4-picoline), 132.7 (C4), 132.3 (C5), 127.5 (C_β-4-picoline), 121.9 (C3), 120.5 (C6), 117.9 (C2), 21.29 (CH_3-4-picoline). IR (KBr, cm^{-1}) υ = 3403 (w, N–H), 3273 (w, N–H), 1622 (s, C═N), 1596 (s, C═N), 1518 (s, C═C aromatics). Elemental analysis for $C_{14}H_{13}N_4OPdS$: found C 40.76, H 3.40, N 13.86, S 7.43%; calculated C 39.36, H 3.07, N 13.11, S 7.51%. ESI–MS: *m/z* 429 (calcd. 427.22) ($[M+2H]^{2+}$, 100%).

2.3. X-ray crystallography

X-ray single crystal intensity data was collected on a Nonius Kappa-CCD diffractometer using graphite monochromated MoKα radiation. Temperature was controlled by an Oxford Cryostream cooling system (Oxford Cryostat). The strategy for the data collections was evaluated using the Bruker Nonius "Collect" programme Data were scaled and reduced using DENZO-SMN software [49]. An empirical absorption correction was applied using the programme SADABS [50]. The structure was solved by direct methods and refined employing full-matrix least-squares with the programme SHELXL-97 refining on F^2 [51]. Packing diagrams were produced using the programme PovRay and graphic interface X-seed [52]. All the non-hydrogen atoms were refined anisotropically. All the hydrogen atoms, except the amino hydrogens on N3A and N3B, were placed in idealised 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. The amino hydrogen atoms were located in the difference electron density maps and refined with simple bond length constraints. Crystallographic data and data collection parameters are listed in Table 1.

2.4. In vitro assay

Cultures of *T. vaginalis* T1 strain were grown in 5 mL 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, and were screened against T1 stain of *T. vaginalis*. Cells untreated and inoculated with 5 μL DMSO are used as controls. Five microlitres of 100 mM stocks of compound library were inoculated for a final concentration of 100 μM. Results were calculated based on counts utilising a hemocytometre 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.

Table 1
Selected crystallographic and refinement data for **11**.

Empirical formula	$C_{18}H_{27}N_6OSPPd$
Formula weight	512.89 g mol^{-1}
T (K)	173.2
Wavelength (Å)	0.71073
Crystal system	Triclinic
Space group	*P* 1
a (Å)	9.4573 (4)
b (Å)	12.318 (5)
c (Å)	18.679 (7)
α (°)	92.746 (2)
β (°)	104.01 (2)
γ (°)	94.480 (2)
Volume (Å^3)	2099.8 (15)
Z	4
D_{calc} (Mg/m^3)	1.622
Absorption coefficient (mm^{-1})	1.081
Crystal size (mm^3)	0.20 × 0.14 × 0.12
θ range (°)	2.07–25.37
Limiting indices	−11 ≤ h ≤ 11, −14 ≤ k ≤ 14, −22 ≤ l ≤ 22
Reflections collected	14,900
Unique reflections	7684 [*R*(int) = 0.0541]
Absorption correction	Semi-empirical from equivalents
Data/restraints/parameters	7684/4/521
Goodness-of-fit on F^2	1.018
Final *R* indices [I > 2σ(I)]	R_1 = 0.0368, wR_2 = 0.0593
R indices (all data)	R_1 = 0.0786, wR_2 = 0.0688
Max. residual density (eÅ^{-3})	0.810
Min. residual density (eÅ^{-3})	−0.662

3. Results and discussion

3.1. Synthesis and characterisation of tridentate [O,N,S] Pd(II) complexes

The thiosemicarbazone ligands, salicylaldimine thiosemicarbazone (**1**), 3-methoxy salicylaldimine thiosemicarbazone (**2**), 3-*tert*-butyl salicylaldimine thiosemicarbazone (**3**) and 5-chlorosalicylaldimine thiosemicarbazone (**4**) were prepared by Schiff base condensation reactions of the appropriate salicylaldehyde with thiosemicarbazide (Scheme 1). The ligands **1** [44], **2** [45], **3** [41] and **4** [46] are known compounds and their spectroscopic data and melting points correlate with the literature. The palladium(II) complexes **5**–**16** were prepared by reaction of the thiosemicarbazone ligand with different palladium(II) precursors of the type, $Pd(L)_2Cl_2$ (where L = triphenylphosphine (PPh_3) for **5**–**8**, phosphatriazaadamantane (PTA) for **9**–**12** and 4-picoline (4-pic) for **13**–**16**), in refluxing ethanol with triethylamine as base (Scheme 1). This weak base promotes deprotonation of the phenolic oxygen as well as the hydrazinic nitrogen thus allowing the thiosemicarbazone ligand to coordinate to palladium as a tridentate dinegative donor via the phenolate oxygen, imine nitrogen and thiolato sulphur atoms.

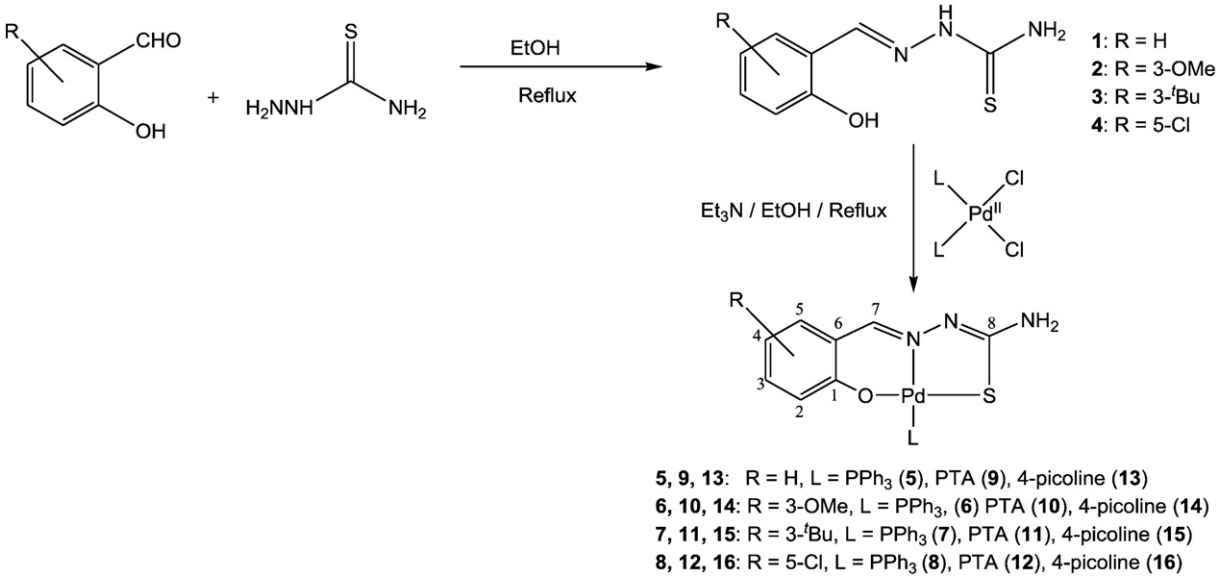
All of the palladium complexes were isolated as air, moisture and thermally stable yellow or orange solids in moderate to high yields. The synthesis and characterisation of complexes **5**, **13** [43] and **6**–**8** [41] has previously been reported.

For complexes **9**–**16**, the absence of the resonances assigned to the hydroxyl and hydrazinic protons of the free ligand in the proton NMR spectra confirms that the ligand coordinates to palladium via the thiolato sulphur and phenolic oxygen. A slight downfield shift in the resonance for the imine proton to between 8.20 and 8.30 ppm for complexes **9**–**12** and a significant upfield shift to between 7.80 and 8.00 ppm for complexes **14**–**16** compared to their corresponding free ligands suggest coordination of the imine nitrogen to the metal centre. This shielding of the imine proton in complexes **14**–**16** is expected as the 4-picoline ancillary ligand is a better sigma-donor towards palladium than PTA. For **9**–**12**, the signal for the imine proton splits to form a doublet. This splitting is attributed to coupling of the phosphorus nucleus of the PTA ligand to the nucleus of the imine proton situated four bonds away ($^4J_{PH}$ = 13.0–13.5 Hz). This phenomenon is documented in the literature for similar triphenylphosphine complexes [53,54]. The coupling constants obtained range between 13.00 and 13.48 Hz and is consistent with coordination of the phosphorus to the metal centre *trans* to the imine nitrogen.

The aliphatic protons of the PTA ancillary ligands for **9**–**12** are found in the region of 4.5 ppm. There are two types of methylene protons present in the PTA ligand. One type is assigned to the P–CH_2–N protons, which occur as a singlet in the region of 4.4 ppm. The protons of the N–CH_2–N moiety possess an AB spin system and appear as two doublets, due to geminal coupling, at 4.5 ppm. One doublet corresponds to the three N–CH_{axial}–N, while the other is observed for the N–$CH_{equitorial}$–N protons. This is consistent with other PTA transition metal complexes [55–57]. The protons of the methyl substituent of the picolyl ring resonate as a singlet at ca. 2.40 ppm for **14**–**16**. The signal of one aromatic proton of the thiosemicarbazone ligand overlaps with the signal of the protons *beta* to nitrogen in the picolyl ring of the co-ligand to occur as a multiplet in complexes **14**–**16**.

For complexes **9**–**12**, the carbon-13 NMR spectra show that the signals accounting for the phenolic carbon atoms appear to shift downfield upon coordination, from approximately 156 ppm in the free ligands to approximately 160 ppm in the complexes. This same phenomenon is observed for the azomethine carbon signals (140 ppm in the ligands *vs* 146 ppm in the complexes). In contrast, complexes **14**–**16**, where the ancillary ligand is 4-picoline, the azomethine carbon of the coordinated thiosemicarbazone ligand resonates at approximately 170.0 ppm in all of the complexes. This shift is characteristic of coordination of the imine nitrogen to palladium leading to a downfield shift of this carbon relative to the resonance observed for the free ligand [58–60]. The thiolate carbon generally occurs between 161.0 and 162.0 ppm for the complexes **15** and **16**. In the case of complex **14** the thiolate carbon resonates at 153.8 ppm. It seems that in this complex there is greater shielding of the C–S carbon due to the methoxy substituent of the thiosemicarbazone ligand. Overall, the upfield shift of the C–S carbon in all complexes compared to the thione carbon of the corresponding free ligand is evidence of coordination of sulphur to palladium in the thiolato form since shielding of the thiolate carbon is characteristic of this type of bonding [58,61–63].

In the infrared spectra for complexes **9**–**12** 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 sulphur in the thiolate form. The lower frequency band, observed between 1592 and 1598 cm^{-1}, is assigned to the



Scheme 1. Synthetic route to Pd(II) salicylaldiminato thiosemicarbazone complexes 5–16.

imine bond coordinated to palladium [44,64,65]. When the nitrogen of the imine coordinates to the metal, a loss of double bond character occurs due to stronger electron donation towards the metal, thus this bond will vibrate at a lower frequency. The absorption band observed at higher frequency, between 1615 and 1643 cm^{-1}, are thus assigned to the newly formed imine bond.

For complexes **14**–**16**, high frequency bands observed between 1625 and 1620 cm^{-1} are assigned to the new imine bond formed. The vibration of the coordinated imine for these complexes is harder to assign in 14 and 16 as the signal for this vibration may overlap with the imine vibration of the 4-picolyl ring. In complex **15** there are three distinct bands in the imine region and the coordinated imine vibration is assigned to the band observed at 1609 cm^{-1}. The C═N stretch of the 4-picolyl ring is allocated to the lowest frequency band observed. In the spectra of complexes **14** and **16**, the absorption bands observed at 1603 cm^{-1} for **14** and 1596 cm^{-1} for **16** are slightly broad and this may indicate the overlapping of two C═N vibrations resulting in only one absorption band being observed.

3.2. X-ray diffraction studies

To further confirm the integrity of these complexes, crystals suitable for X-ray structure determination were obtained for complex **11**. Crystals were grown from dimethylsulfoxide at room temperature. Table 1 (see Experimental section) summarises the crystallographic data and Table 2 lists some relevant bond parameters. The molecular structure depicts coordination of the metal centre (Pd) to the 3-*tert*-butyl salicylaldimine monothiosemicarbazone ligand via the phenolic oxygen, imine nitrogen and sulphur atom in a slightly distorted square-planar geometry. The structure confirms that the fourth coordination site is occupied by a PTA ligand (Fig. 2).

The molecular structure of **11** shows that the thiosemicarbazone ligand is coordinated to palladium in the expected tridentate (O–N–S) fashion. The structure shows the formation of a six- and a five-membered chelate ring upon coordination. The structure displays O(1A)–Pd(1A)–N(1A) and N(1A)–Pd(1A)–S(1A) bond angles of 93.40(9) and 84.45(8)°, respectively. *Trans* to N(1A), we find a PTA ligand coordinated to the metal centre. O(1A)–Pd(1A)–P(1A) and S(1A)–Pd(1A)–P(1A) bite angles of 91.85(7) and 90.19(3)°, respectively, can also be seen. These angles indicate a square planar geometry with slight distortion around the palladium centre, as these angles deviate from ideal behaviour.

In comparison to similar monomeric and dimeric palladium triphenylphosphine thiosemicarbazone complexes, Pd–O, Pd–N, Pd–S and Pd–P bond distances appear quite similar [41–43,66,67]. In addition to this, it can be seen that coordination of the thiosemicarbazone occurs via the thiolate form, rather than the thione form due to the formation of a second imine bond between C(1A) and N(2A). A value of 1.313(4) Å is assigned to this bond, further supporting the notion that the C(1A)–N(2A) bond tends towards double-bond character upon coordination. Comparison of this length to the C(1A)–N(3A) bond length, shows that the latter is slightly longer (1.349(4) Å) and therefore tends towards single-bond character. The C–S bond length is also found to be consistent with coordination of sulphur in the thiolate form, with a value of 1.754(4) Å. A previous study by Halder et al. [43], based on similar monomeric complexes, reported C–S values of approximately 1.74 Å. In simple systems it would be expected that a single C–S bond length would be approximately 1.82 Å and a double bond would exhibit a length of 1.56 Å. The length obtained, therefore appears to be an intermediate length between the two [68].

Table 2
Selected bond lengths (Å) and angles (°) for complex **11**.

Pd(1A)–O(1A)	2.028(2)	O(1A)–Pd(1A)–N(1A)	93.40(9)
Pd(1A)–N(1A)	2.012(3)	N(1A)–Pd(1A)–S(1A)	84.45(8)
Pd(1A)–S(1A)	2.2389(9)	O(1A)–Pd(1A)–P(1A)	91.85(7)
Pd(1A)–P(1A)	2.2461(10)	S(1A)–Pd(1A)–P(1A)	90.19(3)
N(1A)–C(2A)	1.293(4)	O(1A)–Pd(1A)–S(1A)	177.08(7)
N(2A)–C(1A)	1.313(4)	N(1A)–Pd(1A)–P(1A)	173.71(8)

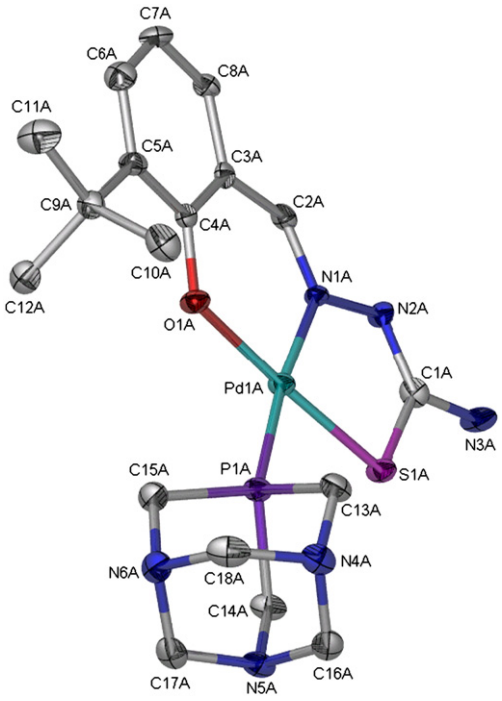


Fig. 2. Molecular structure of complex **11** at 50% probability level with all H atoms omitted for clarity.

Examination of the crystal packing arrangement revealed an extensive arrangement of hydrogen bonding between each molecule (Fig. 3). Closer inspection of this packing arrangement revealed hydrogen bonding of one molecule to two adjacent molecules via the N2 atoms, amino hydrogens and a nitrogen atom belonging to the PTA group.

3.3. Biological studies

The four thiosemicarbazone ligands (**1**–**4**) along with their corresponding palladium complexes (**5**–**16**) were tested for inhibitory activity in vitro against *T. vaginalis*. Table 3 lists the data ascertained for the initial percent inhibition screening carried at 100 μM. Two thiosemicarbazone ligands (**1** and **3**) as well as five of the Pd(II) complexes (**8** and **9**, **12**–**16**) exhibited a percent inhibition greater than 80% at 100 μM. The free ligand **1** displayed the best inhibitory activity (88.9%) out of all four thiosemicarbazone ligands. However, it's corresponding Pd(II) complex **5**, where the ancillary ligand is

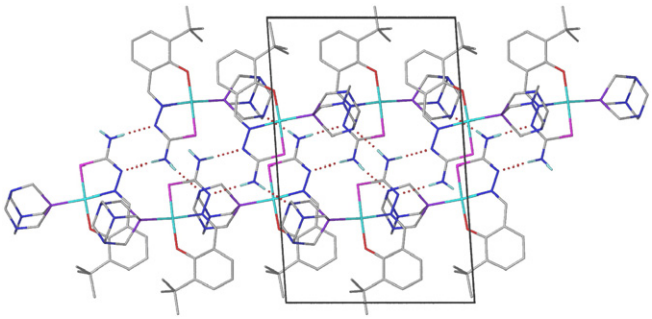


Fig. 3. Two dimensional H-bond network of complex **11** projected along [100]. All hydrogen atoms except the amino hydrogen atoms were omitted. The hydrogen bonds are shown as dotted lines.

Table 3
Average percentage inhibition determined against *Trichomonas vaginalis*.

Compound no.	Average % inhibition
Ligand **1**	89 (±6)
Ligand **2**	60 (±15)
Ligand **3**	82 (±11)
Ligand **4**	25 (±3)
Complex **5**	14 (±8)
Complex **6**	25 (±12)
Complex 7	59 (±13)
Complex **8**	81 (±7)
Complex **9**	97 (±3)
Complex **10**	35 (±15)
Complex **11**	69 (±6)
Complex **12**	95 (±4)
Complex **13**	97 (±3)
Complex **14**	96 (±4)
Complex **15**	97 (±3)
Complex **16**	92 (±8)
Metronidazole[a]	100 (±6)

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

triphenylphosphine, exhibited a value approximately six times lower. Where the ancillary ligand was PTA (**9**) or 4-picoline (**13**), a percent inhibition of 97% was observed suggesting that these co-ligands enhance the activity of these complexes.

The thiosemicarbazone Pd(II) complexes with triphenylphosphine as ancillary ligand do not show appreciable inhibition with the exception of **8** (81% inhibition) and this may be attributed to the presence of a chlorido group at the 5-position of the aromatic ring in the thiosemicarbazone ligand. Our previous studies of this ligand (**4**) and its complex (**8**) for antimalarial activity showed a similar trend in activity [41]. It is unclear if this is a steric or electronic effect and mechanistic studies into the significance of this observation are ongoing.

For the complexes where PTA is the ancillary ligand, only complexes **9** and **12** exhibited good inhibition. Complexes **13**–**16**, where 4-picoline are the co-ligand demonstrated consistently high percent inhibitions, irrespective of the substituents present on the aromatic ring of the thiosemicarbazone ligand. Overall, six complexes (**9**, **12**–**16**) were very effective at inhibiting the growth of the parasite, displaying inhibition greater than 90%. Based on this, these compounds were selected for IC_{50} determination. Table 4 lists the IC_{50} determinations along with the reference drug Metronidazole, which is the current FDA approved treatment for *T. vaginalis*.

The complexes tested displayed IC_{50} values in the low micromolar range (17–37 μM). The best activity was displayed by complex **12** (R = 5-Cl and L = PTA), where an IC_{50} value of 17 μM was observed. Complex **9** (R = H and L = PTA) also displayed good activity giving an IC_{50} value of 21 μM. It is apparent that the presence of the 5-Cl moiety enhances the activity of the complexes as complex **16** (R = 5-Cl and L = 4-picoline) also displays the best activity in the series of the 4-picoline complexes, while complex **12** shows the best activity with regards to the PTA complexes. PTA is a water soluble compound that is widely used in the preparation of metallotherapeutics as they enhance the water solubility or overall hydrophilicity of the metal complex [69,70]. The enhanced activity of these PTA complexes in comparison to some of the 4-picoline derivatives may be due to the enhanced hydrophilicity of the phosphane in comparison to the 4-picoline ancillary ligand. The PTA moiety is also able to be protonated at low pH which may increase the overall hydrophilicity of the complex which may have a favourable effect on the activity. As previously noted [18], the mechanism of action of thiosemicarbazones is complex and may be mediated through inhibition of multiple targets. Some compounds described in this paper may target *T. vaginalis* cysteine proteases while others may act against other cysteine protease targets. Since there are a number of putative cysteine proteases (as well as other proteases) in *T. vaginalis* [20,71], other proteases must be validated as potential targets for compounds described in this paper by using a proteomics approach to confirm the identity of the targets of some potent compounds.

Table 4
IC_{50} determination for Pd(II) complexes **9** and **12**–**16**.

Compound	IC_{50} [μM]
9	21 (±1)
12	17 (±1)
13	23 (±1)
14	37 (±1)
15	23 (±1)
16	21 (±3)
Metronidazole[a]	0.72 (±1)

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

4. Conclusions

Four thiosemicarbazone ligands and their corresponding tridentate Pd(II) complexes were studied for antiparasital activity against *T. vaginalis*. It was found that when the co-ligand was either PTA or 4-picoline, good inhibition was observed. The presence of a substituent in the 5-position of the aromatic ring of the thiosemicarbazone ligand clearly enhances the biological activity of these complexes and further investigation of this observation is ongoing. This preliminary study has established that a selection of these Pd(II) thiosemicarbazone complexes display inhibitory activity against *T. vaginalis*. Currently, we are investigating their antiparasitic effect against strains resistant to Metronidazole.

Abbreviations

^{t}Bu	tertiary butyl
ESI–MS	Electrospray Ionisation Mass Spectrometry
4-pic	4-picoline
PTA	phosphatriazaadamantane
saltsc	salicylaldiminato thiosemicarbazone
TMS	trimethylsilane
TSC	thiosemicarbazone

Acknowledgements

Financial support from the University of Cape Town, the National Research Foundation (NRF) of South Africa, and a donation of metal salts from the Anglo Platinum Corporation is gratefully acknowledged. KML would like to thank the Department of Biological Sciences and the Office of Sponsored Programs, the University of the Pacific for providing funding for this project. P.J.D. was supported by a Hunter-Nahas Fellowship.

Supplementary material

CCDC 794243 contains the 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, UK; fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

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