Cyclopalladated organosilane–tethered thiosemicarbazones: novel strategies for improving antiplasmodial activity

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Two series of ferrocenyl- and aryl-derived cyclopalladated organosilane thiosemicarbazone complexes were synthesised via C–H bond activation. Selected compounds were evaluated for in vitro antiplasmodial activity against the chloroquine-sensitive (NF54) and chloroquine-resistant (Dd2) strains of the human malaria parasite Plasmodium falciparum. Cyclopalladation of the thiosemicarbazones resulted in antiplasmodial activities in the low micromolar range.

Introduction

Malaria continues to be one of the most widespread parasitic infectious diseases in the world today. An estimated 198 million cases of malaria were reported in 2013, with a large percentage of people who die from the disease made up of children under the age of five.1 Most of the malaria infection cases are known to occur in sub-Saharan Africa.

Chloroquine (CQ), a quinoline-based compound, was the most successful clinical drug of choice for the treatment of malaria, but the emergence of resistant strains of the causative agent Plasmodium falciparum has rendered CQ useless in many parts of the world. The current last line of defence against resistant parasites is the artesiminin combination therapy (ACT) regimen. Unfortunately, the recent emergence of resistance to this regimen in parts of Asia, warrants the search for novel drug regimens.2,3

Thiosemicarbazones (TSCs) are thioureas known to display a large spectrum of pharmacological properties, particularly as antiparasitic agents, and are therefore exploitable in the development of new chemotherapeutics.4–9 The precise antiplasmodial mode of action of TSCs is currently unknown. Thiosemicarbazones are proposed to inhibit cysteine proteases, which are integral in several parasite functions.10

The use of transition metals in drug discovery has become a popular strategy, particularly where metals have been combined with compounds of known therapeutic value in an attempt to combat drug resistance. This has been exemplified by the bioorganometallic compound ferroquine, with ferrocene incorporated into the lateral side-chain of chloroquine, allowing for greater transmembrane interactions owing to its more lipophilic nature.11–13 The most notable pioneering work is the rhodium(1)-chloroquine derivatives synthesised by Sánchez-Delgado and co-workers.14 These derivatives showed a reduction in parasitaemia in vivo to a greater extent than that of chloroquine, endorsing the use of metal-based compounds in malaria chemotherapy. For further reports on metalloantimalarials in the literature, several excellent recent reviews abound.12,13,15–17

The application of platinum group metals (PGMs) as chemotherapeutic agents has therefore emerged as a viable area of research. There are not many examples of transition metal complexes of TSCs reported with antiplasmodial activity. Within our research group, we have evaluated transition metals from the platinum group series as antimalarials.18–21 We have explored the chemistry associated with $N,S^-$- and $O,N,S$-chelated TSC systems, and to a limited extent the preparation of $C,N,S$-chelated TSC cyclometallated complexes. Cyclometallated complexes can be prepared via the oxidative addition of aryl halides; however, within our group cyclometallated complexes prepared via C–H activation is of interest.22,23 In particular, the biological evaluation of these types of cyclopalladated complexes especially, is relatively rare.

A recent strategy that we have pursued in the search for novel antimalarial metal-based drug leads is the incorporation of organosilane moieties into our structures.24–26 This strategy has been used with great success to enhance biological activity.

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and reduce toxicity in an effort to increase the therapeutic value of drugs. Organosilane moieties also increase the lipophilicity of a drug molecule which in turn provides several physiological benefits, including improved tissue and cell penetration, which may result in an increase in permeability and ultimately bioavailability.27–32

As a follow-up to our recent studies,24–26 we were prompted to explore the effect of introducing silicon into cyclopalladated thiosemicarbazone complexes, in an attempt to identify potential new antimalarial compounds. To the best of our knowledge, there are no reports on the use of silicon-containing thiosemicarbazone cyclopalladated complexes in the field of bioorganometallic antiplasmodial research.

Herein, we report the synthesis and characterisation of a series of new cyclopalladated silicon-containing compounds based on a thiosemicarbazone scaffold along with their in vitro antiplasmodial activities.

**Results and discussion**

**Synthesis and characterisation**

The ferrocenyl- and aryl-derived TSCs (1–3) containing either a methylene (1a–3a) or propyl (1b–3b) spacer were prepared by refluxing the corresponding Schiff-base dithiocarbamate and the appropriate amine (Scheme 1). This occurred via a nucleophilic substitution of the methanethiol group of the dithiocarbamates with the amine. The dithiocarbamates and the TSCs (1a–3a) are known compounds which were prepared following published methods.36,33,34 The dithiocarbamates were prepared via the Schiff-base condensation of methyl hydrazine-carbothioate35 with either acetylferrocene or 3,4-dichloroacetophenone.33,34 The cyclopalladated complexes (4–6) were prepared via C–H activation of the cyclopentadienyl or aryl rings of the ferrocenyl- and aryl-derived TSCs, respectively, when reacted with the palladium precursor cis-[PdCl2(PTA)2] in the presence of triethylamine as the base (Scheme 1). These palladacycles are formed through an electrophilic substitution reaction. The palladium[n] metal centre inserts into the C–H bond to form a Pd–C bond with the ortho carbon atom of the ferrocenyl or aryl ring. The triethylamine facilitates the deprotonation of the ring. The incorporation of 1,3,5-triaza-7-phosphaadamantane (PTA) into complexes of biological interest has increased due to the improved solubility of PTA-containing complexes. Within the context of malaria, PTA-containing complexes have exhibited promising in vitro activities against *P. falciparum*.4,20,36–38

Synthesis of the ferrocenyl- and aryl-derived TSCs was confirmed by the absence of the methanethiol (SCH3) protons and the appearance of signals for the newly incorporated amine in the 1H NMR spectra. The absence of a signal for a proton of the substituted cyclopentadienyl ring (4) or the C-6 position on the aryl ring (5; 6) confirmed the synthesis of the cyclopalladated complexes. Formation of the palladacycle was further confirmed by the absence of the hydrazinic proton, which suggests that the TSC chelates in the thiolate form. Furthermore, in the case of the ferrocenyl-derived cyclopalladated complexes (4a–b), the 1,2-disubstitution of the cyclopentadienyl ring results in the formation of planar chiral complexes. An AB spin system splitting pattern is observed for the PTA ligand.39–41 The NCH2N protons resonate as two doublets corresponding to the different environments experienced by the axial and equatorial protons, while the PCH3N protons resonate as a singlet (ca. 4.26 ppm), as expected for these types of cyclopalladated complexes.

Formation of the complexes is further confirmed by the 13C(1H) NMR spectra. The imine carbon atom, which resonates at ca. 147 ppm for the free TSCs, shifts significantly downfield (ca. 162 ppm) for the complex. Furthermore, the imine carbon atom resonates as a doublet (J = 7.4 Hz), which is ascribed to coupling with the PTA phosphorus. A similar trend is observed for the carbon atom to which the palladium atom is bonded. The carbon atom resonates as a doublet (7.4–8.8 Hz) at 99.5 and ca. 136 ppm for the ferrocenyl- and aryl-derivatives, respectively. This further confirms C–H activation, and thus formation of the palladacycle.

**Scheme 1** Reagents and conditions: (i) EtOH, reflux [7 h (1b); 24 h (2b, 3b)]; (ii) cis-[PdCl2(PTA)2], Et3N, EtOH, reflux, 24 h.
literature values for analogous cyclopalladated compounds containing a PTA ligand.\textsuperscript{20,21} Infrared spectral analysis of the IR spectrum of the palladium-free compounds revealed an absorption band at ca. 1610 cm\(^{-1}\) which corresponds to the imine (C\(=\)N) stretching frequency.

Upon formation of the cyclopalladated complex, two absorption bands at around 1578 and 1553 cm\(^{-1}\) are observed which correspond to two C\(=\)N stretching frequencies. The absorption band observed at the lower wavenumber is assigned to the palladium-coordinated imine, while the absorption band at the higher wavenumber corresponds to the newly formed C\(=\)N bond in the cyclopalladated complexes. The formation of these complexes is further confirmed by mass spectrometry. The mass spectra for complexes 4–6 displayed peaks corresponding to either the molecular ion [M]\(^+\) or the protonated form [M + H]\(^+\). Metal-based compounds which are stable in solution are important when ensuring that the tested compound is the compound responsible for the observed biological activity. Therefore, as a model system, the stability of complex 5a was investigated and monitored by \(^1\)H NMR spectroscopy over a 72 h period at 37 °C. The \(^1\)H NMR spectra (Fig. 1) of complex 5a was recorded in DMSO-\(d_6\):D\(2\)O (9:1, v/v). The spectra remain unchanged over the time period for the assay, suggesting that compound 5a may be the compound responsible for any observed activity.

**In vitro antiplasmodial and cytotoxicity evaluation**

The dithiocarbamates, thiosemicarbazones (1–3) and the cyclopalladated complexes (4–6) were evaluated for their in vitro antimalarial activity against the CQ-sensitive (NF54) and CQ-resistant (Dd2) strains of *Plasmodium falciparum*. The cytotoxicity of selected compounds was evaluated against the Chinese Hamster Ovarian (CHO) cell-line. Chloroquine (CQDP) and artesunate were used as the control drugs for the parasite strains and emetine for the CHO cell-line. The IC\(_{50}\) values are listed in Table 1.

In general, introduction of the cyclopalladated entity resulted in an overall enhancement of activity (IC\(_{50}\) values below 2 \(\mu\)M) against the NF54 strain of *P. falciparum*, for the complexes (4–6) in comparison with the dithiocarbamates and TSCs. Comparing the three pairs of complexes (4–6), reveals that the Si-containing 3,4-dichloroacetophenone-TSC palladium complexes (5) were the most potent.

An IC\(_{50}\) value of below 5 \(\mu\)M was selected as the cut-off value for identifying potential lead compounds for further testing. Therefore, only those compounds displaying activity below 5 \(\mu\)M against the NF54 strain were further tested for their activity against the Dd2 strain. A decrease in activity was observed for the dithiocarbamates against the resistant strain, whereas the palladium-free TSCs were generally equipotent against the NF54 and Dd2 strains. However, incorporation of

<table>
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<tr>
<th>Compound</th>
<th>IC(_{50}) ((\mu)M)</th>
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<tr>
<td></td>
<td>NF54</td>
</tr>
<tr>
<td>Fc Dithiocarbamate</td>
<td>3.76 ± 1.35</td>
</tr>
<tr>
<td>3,4-Dicloro dithiocarbamate</td>
<td>2.59 ± 0.89</td>
</tr>
<tr>
<td>1a</td>
<td>7.92 ± 1.99</td>
</tr>
<tr>
<td>1b</td>
<td>1.88 ± 0.58</td>
</tr>
<tr>
<td>2a</td>
<td>2.24 ± 0.26</td>
</tr>
<tr>
<td>2b</td>
<td>14.61 ± 2.07</td>
</tr>
<tr>
<td>3a</td>
<td>175.74 ± 43.03</td>
</tr>
<tr>
<td>3b</td>
<td>8.72 ± 3.41</td>
</tr>
<tr>
<td>4a</td>
<td>1.43 ± 0.29</td>
</tr>
<tr>
<td>4b</td>
<td>1.52 ± 0.09</td>
</tr>
<tr>
<td>5a</td>
<td>0.55 ± 0.10</td>
</tr>
<tr>
<td>5b</td>
<td>0.58 ± 0.14</td>
</tr>
<tr>
<td>6a</td>
<td>0.83 ± 0.12</td>
</tr>
<tr>
<td>6b</td>
<td>1.60 ± 0.48</td>
</tr>
<tr>
<td>Ferroquine</td>
<td>0.0249 ± 0.0028</td>
</tr>
<tr>
<td>CQDP</td>
<td>0.0097 ± 0.0039</td>
</tr>
<tr>
<td>Artesunate</td>
<td>0.0104 ± 0.0026</td>
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<tr>
<td>Emetine</td>
<td>ND</td>
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</table>

\(^a\) RI = IC\(_{50}\)(Dd2)/IC\(_{50}\)(NF54).  \(^b\) SI\(_{1}\) = IC\(_{50}\)(CHO)/IC\(_{50}\)(NF54).  \(^c\) SI\(_{2}\) = IC\(_{50}\)(CHO)/IC\(_{50}\)(Dd2); ND = not determined.
the palladium-PTA fragment, generally results in a slight increase in potency against the resistant Dd2 strain. These cyclopalladated complexes were not as active as ferroquine (Table 1).

The resistance indices [RI = IC₅₀ (Dd2)/IC₅₀ (NF54)] were calculated for compounds displaying activity against the Dd2 strain. A value below or close to 1 suggests that the compounds are more likely to be active against resistant strains. The RI values for the cyclopalladated complexes were generally below 1, indicating that incorporation of the palladium-PTA fragment, delivers complexes likely to be active against resistant strains relative to chloroquine (19.59) and artemesunate (4.81). Overall, the thiosemicarbazone compounds displayed lower RI values than the controls.

The compounds containing a methylene spacer (1a–6a) were generally more potent, and thus only those compounds were further tested for their cytotoxicity against the CHO cell-line. Selectivity indices [SI = IC₅₀ (CHO)/IC₅₀ (NF54 or Dd2)] calculated for the dithiocarbamates revealed low SI values (<1) suggesting a lack of selectivity. As seen in Table 1, the thiosemicarbazone compounds (2a, 4a, 5a, 6a) are more effective at killing parasitic cells as opposed to the mammalian CHO cells, with SI values higher than that observed for the dithiocarbamates. In terms of the cyclopalladated complexes, the Si-containing 3,4-dichloroacetophenone TSC palladium complex (5a) displayed the best selectivity.

Conclusions

Two series of ferrocenyl- and aryl-derived organosilane TSCs were prepared, as well as their cyclopalladated complexes via C–H activation of the ring. The TSCs and cyclopalladated complexes were fully characterised using various spectroscopic and analytical techniques. Evaluation of the compounds against two strains of the malaria parasite Plasmodium falciparum revealed an enhancement of activity upon formation of the cyclopalladated complexes. The TSC compounds also displayed selective antiplasmodal activity. A preliminary β-haematin (synthetic haemoozin) inhibition study of the cyclopalladated complex 5b revealed that the formation of β-haematin, which is a target of antimalarial drugs, was not inhibited. Therefore, investigating other processes such as cysteine protease binding, or conducting ROS studies, could give insight into potential mechanistic routes by which these cyclopalladated complexes inhibit the parasite.

Materials and methods

Chemicals

All reagents and solvents were obtained from commercial sources (Sigma-Aldrich, Merck, Kimix, UkrOrgSyntez and FCH group) and, unless otherwise stated, were used as received. The Schiff-base dithiocarbamates,33,42 TSCs (1a, 2a, 3a)26 and PdCl₂(PtA)₉43 were prepared following literature methods. Nuclear magnetic resonance (NMR) spectra were recorded using a Varian Mercury 300 spectrometer (¹H at 300.08 MHz), a Bruker 400 Biospin GmbH spectrometer (¹H at 400.200 MHz, ¹³C(¹H) at 100.60 MHz, ³¹P(¹H) at 161.80 MHz) or a Bruker 600 FT spectrometer (¹H at 600.100 MHz, ¹³C(¹H) at 150.60 MHz) at 30.0 °C. Coupling constants are reported in Hertz. Infrared (IR) spectra were determined using a Perkin Elmer Spectrum 100 FT-IR spectrometer and was carried out using an Attenuated Total Reflectance (ATR) unit. High resolution (HR) ESI-mass spectrometry was carried out using a Waters API Quattro instrument in the positive mode. El-mass spectrometry was carried out in the positive mode using a JEOL GCmateII apparatus. Elemental analyses (C, H and N) were recorded on a Thermo Flash 1112 Series CHNS-O Analyser. Melting points were recorded using a Buchi B-540 melting point apparatus and are uncorrected.

Synthesis of thiosemicarbazones

Thiosemicarbazone 1b. Anhydrous ethanol (10.0 mL) was added to 3-aminopropyl-(trimethyl)silane (0.560 mL, 3.34 mmol) under N₂. The addition of ferrocenyl dithiocarbamate (0.457 g, 1.37 mmol) resulted in a red solution which was refluxed in the dark for 7 h. The solvent was removed in vacuo to yield a brown oil which was extracted using DCM (10.0 mL) and washed with water (3 × 20.0 mL). The crude product was further purified by column chromatography on deactivated alumina with hexane–ethyl acetate (30:70, v/v) as eluent. Compound 1b was isolated as an oily-brown solid (0.352 g, 62%). Anal. Caled (%) for C₁₅H₂₃Cl₂N₃SiS: C 54.66; H 7.27; N 9.90. IR (ATR) νmax/cm⁻¹: 1599w (C=≡N). ¹H NMR (400.22 MHz, DMSO-d₆): δ (ppm) = 9.84 (1H, s, NNH); 8.22 (1H, t, JHH = 6.0 Hz, NH); 4.79 (2H, t, JHH = 3.6 Hz, CH₂); 4.37 (2H, t, JHH = 3.6 Hz, CH₂); 4.18 (5H, s, C₅H₅); 3.52 (2H, m, CH₂); 2.19 (3H, s, CH₃); 1.58 (2H, m, CH₂); 0.48 (2H, m, CH₂); 0.01 (9H, s, Si(CH₃)₃). EI-MS (m/z) 415.03 ([M]+, 100%).

General method for the synthesis of 2b and 3b

Anhydrous ethanol (10.0 mL) was added to the amine under N₃, followed by the addition of 3,4-dichloroacetophenone dithiocarbamate (0.500 g, 1.71 mmol). Compound 2b was isolated as a white solid (0.594 g, 92%). M.p.: 110.7–111.3 °C. Anal. caled (%) for C₁₅H₂₃Cl₂N₃SiS: C 54.99; H 6.18; N 11.20. Found C 54.66; H 7.27; N 10.17. IR (ATR) νmax/cm⁻¹: 1613w (C=≡N), 1599w (C=≡N). ¹H NMR (399.95 MHz, DMSO-d₆): δ (ppm) = 10.18 (1H, s, NNH); 8.66 (1H, t, JHH = 6.0 Hz, NH); 8.18 (1H, d, JHH = 2.2 Hz, H-2); 7.90 (1H, d, JHH = 8.5, 2.2 Hz, H-5); 7.64 (1H, d, JHH = 8.5 Hz, H-6); 3.55 (2H, m, CH₂); 2.28 (3H, s, CH₃); 1.58 (2H, m, CH₂); 0.49 (2H, t, JHH = 6.4 Hz, CH₂); 0.01 (9H, s, Si(CH₃)₃).
Si(CH3)3). ¹³C[¹H] NMR (100.64 MHz, DMSO-d6): δ (ppm) = 178.4 (C=O; S)-145.7 (C=N); 138.8, 132.1, 131.8, 130.7, 128.6, 127.2 (Ar-C); 47.2, 23.7, 14.4 (CH3); 13.8 (CH3); -1.09 (Si(CH3)3). EI-MS (m/z) 377.06 [M + H⁺], 50%. Thiosemicarbazone 3b. 4,4-Dimethylpentan-1-amine (0.049 g, 0.430 mmol); 3,4-dichloroacetophenone dithiocarbamate (0.111 g, 0.380 mmol). Compound 3b was isolated as a white solid (0.103 g, 75%). M.p.: 139.0-141.0 °C. Anal. calcld (%) for C16H23Cl2N3S: C 53.33; H 6.43; N 11.66; Found C 53.23; H 6.59; N 11.70. IR (ATR) δ (cm⁻¹) = 3275 (N-H); 1622 (C=O); 1571 (C=S); 1366 (C-N); 1273 (Ar-C); 1073 (C=S); 796 (C-Cl). EI-MS (m/z) 315 [M+H⁺], 100%. Synthesis of cyclopalladated complexes General method. Excess triethylamine (0.200 mL) was added to a solution of the thiosemicarbazide dissolved in anhydrous ethanol, allowed to stir for 10 min, followed by the addition of one equivalent of the palladium precursor cis-[PdCl₂(PTA)]₂. The reaction mixture was refluxed for 24 h, and the resulting solid was collected by suction filtration. Compound 4b was synthesised in the dark under N₂. The solid was purified by washing with a minimum volume of hot methanol to remove phosphorus impurity.

Cyclopalladated complex 4a. Compound 1a (0.0738 g, 0.106 mmol); cis-[PdCl₂(PTA)]₂ (0.0525 g, 0.107 mmol). Compound 4a was isolated as a red powder (0.0434 g, 63%). M.p.: 266.8 °C (decomposition w/o melting). Anal. calcld (%) for C₂₀H₂₉Cl₂N₆Pd: C 40.45, H 4.92, N 14.15; Found C 40.31, H 4.96, N 14.27. IR (ATR) δ (cm⁻¹) = 3275 (N-H); 1622 (C=O); 1571 (C=S); 1366 (C-N); 1273 (Ar-C); 1073 (C=S); 796 (C-Cl). EI-MS (m/z) 315 [M+H⁺], 100%. Cyclopalladated complex 5a. Compound 2a (0.0767 g, 0.218 mmol); cis-[PdCl₂(PTA)]₂ (0.0995 g, 0.202 mmol). Compound 5b was isolated as a yellow solid (0.0684 g, 53%). M.p.: 247.3-248.4 °C. Anal. calcld (%) for C₂₀H₂₉Cl₂N₆Pd: C 40.45, H 4.92, N 14.15; Found C 40.31, H 4.96, N 14.27. IR (ATR) δ (cm⁻¹) = 3275 (N-H); 1622 (C=O); 1571 (C=S); 1366 (C-N); 1273 (Ar-C); 1073 (C=S); 796 (C-Cl). EI-MS (m/z) 315 [M+H⁺], 100%.

Cyclopalladated complex 5a. Compound 2a (0.0767 g, 0.218 mmol); cis-[PdCl₂(PTA)]₂ (0.0995 g, 0.202 mmol). Compound 5b was isolated as a yellow powder (0.0684 g, 53%). M.p.: 247.3-248.4 °C. Anal. calcld (%) for C₂₀H₂₉Cl₂N₆Pd: C 40.45, H 4.92, N 14.15; Found C 40.31, H 4.96, N 14.27. IR (ATR) δ (cm⁻¹) = 3275 (N-H); 1622 (C=O); 1571 (C=S); 1366 (C-N); 1273 (Ar-C); 1073 (C=S); 796 (C-Cl). EI-MS (m/z) 315 [M+H⁺], 100%.
131.7, 128.1, 127.2 (Ar–C); 72.4 (d, \(J_{\text{CP}} = 7.2\) Hz, NCH2N); 58.0 (CH3); 51.5 (d, \(J_{\text{CP}} = 15.4\) Hz, PCH2N); 32.3 (C(CH3)3); 27.3 (C(CH3)3); 13.4 (CH3). \(^{31}\)P\(^{1}\)H NMR (121.47 MHz, DMSO-d6): \(\delta\) (ppm) = −49.6. EI-MS (m/z) 592.09 ([M]+, 1.5%).

**Cyclopalladated complex 6b.** Compound 3b (0.101 g, 0.279 mmol); cis-[PdCl2(PTA)2] (0.137 g, 0.279 mmol). Compound 6b was isolated as a yellow powder (0.0586 g, 34%). M.p.: 253.0–254.9 °C. Anal. calcd (%) for C22H18Cl2N2PdPS: C 42.57, H 5.36, N 13.55; Found C 42.40, H 5.73, N 13.50. IR (ATR) \(\nu\) cm\(^{-1}\): 1584w (C=C); 1562s (C=O). \(^{1}\)H NMR (399.95 MHz, CDCl3): \(\delta\) (ppm) = 7.10 (1H, s, H-2); 7.32 (1H, d, \(J_{\text{HH}} = 4.80\) Hz, H-5); 4.98 (1H, br s, NH); 4.59 (6H, s, NCH2N); 4.43 (6H, s, PCH2N); 3.36 (2H, m, CH2); 2.28 (3H, s, CH3); 1.56 (2H, m, CH2); 1.22 (2H, m, CH2); 0.89 (9H, s, C(CH3)3). \(^{13}\)C\(^{1}\)H NMR (100.64 MHz, CDCl3): \(\delta\) (ppm) = 174.2 (C-S); 165.5 (Ar-C); 161.8 (d, \(J_{\text{CP}} = 7.0\) Hz, C–C=N); 152.4 (Ar–C); 135.8 (d, \(J_{\text{CP}} = 9.0\) Hz, C-Pd); 131.7, 128.1, 127.2 (Ar-C); 73.4 (d, \(J_{\text{CP}} = 7.0\) Hz, NCH2N); 52.4 (d, \(J_{\text{CP}} = 15.4\) Hz, PCH2N); 47.4, 41.3 (CH3); 30.2 (C(CH3)3); 29.3 (C(CH3)3); 24.8 (CH3); 13.3 (CH3). \(^{31}\)P\(^{1}\)H NMR (162.01 MHz, CDCl3): \(\delta\) (ppm) = −50.8. EI-MS (m/z) 620.13 ([M]+, 1.1%).

**DMSO : D2O stability study**

Cyclopalladated complexes 5a was dissolved in DMSO-d6 : D2O (9 : 1, v/v) and the \(^{1}\)H NMR spectrum recorded at 0 h. The solution was warmed at 37 °C, and the stability monitored by \(^{1}\)H NMR spectroscopy at 24, 48 and 72 h time intervals to confirm stability of compound during the in vitro assay time period.

**Plasmodium falciparum assay.** Continuous in vitro cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.\(^\text{44}\) Quantitative assessment of antimalarial activity in *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler.\(^\text{45}\) Antimalarial assay was conducted according to previously published methods.\(^\text{45}\) 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 ml\(^{-1}\), which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 μg ml\(^{-1}\). Reference drugs were tested at a starting concentration of 1000 ng ml\(^{-1}\), which was then serially diluted 2-fold in complete medium to give 6 concentrations, the lowest being 0.001 μg ml\(^{-1}\). The same dilution technique was applied to the all test samples. The highest concentration of solvent to which the cells were exposed had no measurable effect on the cell viability (data not shown). The IC\(_{50}\) values were obtained from full dose response curves, using a non-linear dose–response curve fitting analysis via GraphPad Prism v.4 software.

**Conflict of interest**

The authors declare no competing financial interest.

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**Notes and references**
