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Activity of phosphino palladium(II) and platinum(II) complexes against HIV-1 and *Mycobacterium tuberculosis*

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Abstract Treatment of human immunodeficiency virus (HIV) is currently complicated by increased prevalence of co-infection with *Mycobacterium tuberculosis*. The development of drug candidates that offer the simultaneous management of HIV and tuberculosis (TB) would be of great benefit in the holistic treatment of HIV/AIDS, especially in sub-Saharan Africa which has the highest global prevalence of HIV-TB coinfection. Bis(diphenylphosphino)-2-pyridylpalladium(II) chloride (**1**), bis(diphenylphosphino)-2-pyridylplatinum(II) chloride (**2**), bis(diphenylphosphino)-2-ethylpyridylpalladium(II) chloride (**3**) and bis(diphenylphosphino)-2-ethylpyridylplatinum(II) (**4**) were investigated for the inhibition of HIV-1 through interactions with the viral protease. The complexes were subsequently assessed for biological potency against *Mycobacterium tuberculosis* H37Rv by determining the minimal inhibitory concentration (MIC) using broth microdilution. Complex (**3**) showed the most significant and competitive inhibition of HIV-1 protease ($p = 0.014$ at 100 μM). Further studies on its in vitro effects on whole virus showed reduced viral infectivity by over 80 % at 63 μM ($p < 0.05$). In addition, the complex inhibited the growth of *Mycobacterium tuberculosis* at an MIC of 5 μM and was non-toxic to host cells at all active concentrations (assessed by tetrazolium dye and real time cell electronic sensing). *In vitro* evidence is provided here for the possibility of utilizing a single metal-based compound for the treatment of HIV/AIDS and TB.

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Introduction

According to the World Health Organization (WHO) HIV has been classified as one of the greatest killers in human history with an estimated 39 million deaths attributed to this single infectious agent thus far. Current treatment is associated with a number of challenges including the inability to effectively clear the infection, toxicity and the emergence of drug resistant viral variants (Montessori et al. 2004). Fatality rates are mainly due to the viral attack on the immune system resulting in the impairment of immune responses, leaving the immune system vulnerable to other bacterial infections that are otherwise treatable (Brenchley et al. 2004; Tan et al. 2012).

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB) is the leading cause of death in HIV infected patients, with Sub-Saharan Africa being the most highly affected region (WHO 2014). TB is further exacerbated by the emergence of multidrug and extensively drug resistant strains, which further complicates treatment strategies, and increases mortality of HIV-TB co-infected patients (Gandhi et al. 2006). The treatment of co-infections is associated with a number of challenges including shared drug toxicities, drug–drug interactions, high pill burdens as well as the development of immune reconstitution syndrome (Pepper et al. 2007; Narayanasamy et al. 2015).

Novel drugs that address the shortfalls of existing treatments are needed and one compound with dual-activity would intrinsically have added benefits for the holistic treatment of TB-HIV. Such an ideal treatment would ultimately offer two major advantages; firstly it would eliminate discordant temporal administration of both HIV and TB treatment, reducing the previously reported undesirable interactions between the two drug-types (e.g. interaction of protease inhibitors with rifampin). Secondly; it would reduce the number of drugs administered to patients. Reducing the high pill burdens will subsequently increase patient adherence to the treatment which could ultimately reduce the emergence of both resistant viral and bacterial strains.

The antimicrobial and antiviral abilities of metal-based complexes are well documented in literature (Sadler and Guo 1998; Haraguchi et al. 1999; Che and Siu 2010; Páez et al. 2013) and the clinical use of metal-based compounds in medicine is not a new practice, with metals like bismuth being used for over 20 decades for the treatment of ailments such as syphilis and gastrointestinal disorders (Li and Sun 2012).

In this project we investigated the activity of phosphino- palladium(II) and platinum(II) complexes against HIV-1 and *Mycobacterium tuberculosis.* Platinum drugs such as cisplatin and carboplatin have been used for the treatment of various cancers and have been classified as some of the most successful anti-cancer drugs (Abu-Surrah and Kettunen 2006). With the development of anticancer compounds containing platinum, the biomedical potential of other transition metal complexes has become a topic of interest (Best and Sadler 1996). These include palladium(II) complexes (palladium is a platinum metals that exhibits similar thermodynamic and structural properties as platinum) which have mostly been investigated for potential anti-tumor activity (De Souza et al. 2010; Rocha et al. 2010; Khan et al. 2011) due to the similarities between palladium and platinum (Mjos and Orvig 2014). Other examples include gold based compounds. Actually, gold based compounds have been in use for centuries before the advent of platinum drugs (Fricker 2007). Auranofin, the only gold-based drug in clinical use has been shown to improve the quality of life of rheumatoid arthritis patients (Kean et al. 1997). Shapiro and Masci (1996) were the first to draw attention to the fact that auranofin may be useful in the treatment of AIDS when they reported that an HIV-infected patient's CD4+ T cell count increased remarkably when the patient was treated with auranofin for psoriatic arthritis. Auranofin possesses remarkable anti-inflammatory activity and the triethylphosphino ligand in this complex is believed to facilitate cell membrane permeability (Gandin et al. 2010). Indeed, the type of ligand used in a metal complex plays a very important role in terms of thermodynamic stability and the reduction of metal toxicity without compromising activity (Rocha et al. 2010; Khan et al. 2011). For instance, palladium(II) is very labile and may undergo rapid hydrolysis resulting in non-specific activity. The use of a *N*-containing, bulky, heterocyclic ligand can circumvent this (Rocha et al. 2010).

In view of the attributes of phosphine and pyridine ligands in metal complexes as potential metallo-drugs enunciated above, we used ligands that have phosphines and pyridines to prepare palladium(II) and

platinum(II) chlorides (**1**–**4**) as potential anti-HIV and TB agents. Indeed phosphorus and nitrogen containing ligands like aminophosphines have been reported to have potential applications in various biological activities ranging from anti-cancer to anti-HIV (Lippard 1994; Fonteh and Meyer 2009). This is mainly due to their drug lipophilicity and the fact that these ligands are less labile than other ligand systems (e.g. thiolates) making them more specific in biochemical reactions (McKeage et al. 2002; Nobili et al. 2009).

In the current study, we investigated the phosphinopyridine palladium(II) and platinum(II) chlorides (**1**–**4**) against HIV-1 protease, which is an important enzyme in the viral life cycle and current clinical inhibitors against this enzyme display adverse effects. One complex (**3**) showed moderate activity. In addition, the compound was tested in whole-pathogen assays (HIV and TB) and the resulting dual activity suggests that the compound may be an ideal candidate for further drug development for co-infection treatment.

Methods

Materials and instrumentation

All manipulations were carried out under argon by standard Schlenk techniques. All Solvents were of analytical grade and were dried using a Braun MB SPS-800 drying solvent system. 2-(2-(diphenylphosphino)ethyl)pyridine (**L1**) and diphenylphosphine-2-pyridine (**L2**) were purchased from Sigma-Aldrich and used as received. Palladium dichloride $[PdCl_2]_n$ and potassium tetrachloroplatinate, $K_2[PtCl_4]$, were purchased from South Africa Precious Metals. The starting materials, $[PdCl_2(NCMe)_2]$ (Rülke et al. 1990) and $[PtCl_2(SMe_2)_2]$ (Van Asslet et al. 1994), were synthesized following literature procedures.

^{1}H NMR and ^{13}C{^{1}H} NMR spectra were recorded in chloroform-d ($CDCl_3$) on either a Varian Gemini 2000 instrument (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C{^{1}H} NMR) or a Bruker Ultrashield 400 instrument (100 MHz for ^{13}C{^{1}H} NMR) at room temperature. ^{1}H and ^{13}C{^{1}H} NMR chemical shifts were referenced to the residual signals of the protons or carbons of the NMR solvents and are quoted in δ (ppm): $CDCl_3$ at 7.24 and 77.00 ppm for ^{1}H and ^{13}C{^{1}H} NMR spectra, while it was 39.5 ppm for d^6-DMSO ^{13}C{^{1}H} NMR spectrum. Coupling constants are measured in Hertz (Hz). Elemental analyses were performed on a Vario Elementar III microcube CHNS analyser at Rhodes University, South Africa.

Syntheses of metal complexes

{Bis(diphenylphosphino)-2-pyridyl}palladium(II) chloride (1)

A dichloromethane solution (20 ml) of diphenylphosphino-2-pyridine (0.13 g, 0.50 mmol) was stirred at room temperature, to which $[PdCl_2(NCMe)_2]$ (0.06 g, 0.25 mmol) was added. Stirring continued overnight after which the solvent was removed *in vacuo*. A crude yellow powder was obtained and purified by recrystallization from a mixture of dichloromethane and hexane. ^{1}H NMR ($CDCl_3$): 8.75 (d, 1H, $J = 4.4$ Hz), 8.28 (t, 1H), 7.86 (q, 4H, $J = 7.2$ Hz), 7.47 (m, 6H), 7.37 (t, 1H). ^{31}P{^{1}H} ($CDCl_3$): 22.44 (s, 1P). ^{13}C{^{1}H} NMR ($CDCl_3$): 125.5; 128.3, 128.4, 128.5; 131.8, 131.9; 132.1, 132.2; 135.8; 150.3. Anal. Calcd. for $C_{34}H_{28}N_2P_2PdCl_2{\cdot}CH_2Cl_2$: C, 49.49; H, 3.69; N, 3.21 %. Found, C, 49.65; H, 3.50; N, 3.34 %.

{Bis(diphenylphosphino)-2-pyridyl}platinum(II) chloride (2)

To a stirring solution of diphenylphosphino-2-pyridine (0.13 g, 0.50 mmol) in dichloromethane (20 ml) was added $[PtCl_2(SMe_2)_2]$ (0.06 g, 0.25 mmol). The resultant solution was stirred at room temperature overnight. The solvent was removed *in vacuo afterwards* and the residue obtained recrystallized in a mixture of dichloromethane-hexane to afford colourless crystals of **2**. Yield = 0.14 g, 70 %. ^{1}H NMR ($CDCl_3$): 8.47 (s, 1H), 8.28 (t, 1H), 7.58 (m, 5H), 7.28 (t, 2H), 7.15 (m, 5H). ^{31}P{^{1}H} ($CDCl_3$): 11.02 (s, 1P, $J_{Pt\text{-}P} = 4540$ Hz). ^{13}C{^{1}H} NMR ($CDCl_3$): 124.8; 127.9; 128.1, 128.6; 129.5; 131.2; 135.5, 131.7, 131.8; 135.4, 135.5, 135.6, 135.7, 135.8, 135.9; 149.7, 149.8, 149.9. Anal. Calcd. for $C_{34}H_{28}N_2P_2PtCl_2{\cdot}CH_2Cl_2$: C, 47.91; H, 3.45; N, 3.19 %. Found C, 47.78; H, 3.27; N, 3.21 %.

{Bis-2-(2-(diphenylphosphino)ethyl)pyridyl}palladium(II) chloride (3)

Compound **3** was synthesized as previously described (Uhlig and Keiser 1974), using a slight modification of

the literature procedure. Solid $PdCl_2(NCMe)_2$ (0.06 g, 0.25 mmol) was added to a dichloromethane solution of 2-(2-(diphenylphosphino)ethyl)pyridine (**L1**) (0.15 g, 0.50 mmol) and stirred at room temperature overnight. The solvent was removed *in vacuo* and a yellow powder was obtained, which was recrystallized from a mixture of dichloromethane and hexane to give **3**. Yield = 0.16 g, 80 % (0.16 g). ^{1}H NMR ($CDCl_3$): 9.49 (d, 1H, J = 5.6 Hz), 7.84 (q, 4H, J = 7.6 Hz), 7.79 (t, 1H), 7.46 (m, 6H), 7.33 (d, 1H, J = 7.6 Hz), 7.25 (t, 1H), 3.46 (dt, 2H, J = 17.2 & 26.8 Hz), 2.20 (t, 2H). ^{31}P{^{1}H} ($CDCl_3$): 25.34 (s, 1P). ^{13}C{^{1}H} NMR ($CDCl_3$): 23.1, 23.3, 30.9; 35.8; 123.4; 125.2; 128,7, 128.8, 128.9; 129.3; 131.8; 133.3, 133.4; 139.9; 155.8; 158.6, 158.7. Anal. Calcd. for $C_{38}H_{36}N_2P_2$-$PdCl_2{\cdot}CH_2Cl_2$: C, 48.53; H, 4.17; N, 2.76 %. Found: C, 48.66; H, 3.77; N, 2.98 %.

{Bis-(2-(2-(diphenylphosphino) ethyl)pyridyl}platinum(II) chloride (4)

A dichloromethane solution (20 ml) of 2-(2-diphenylphosphino)ethylpyridine (0.15 g, 0.50 mmol) was stirred at room temperature to which [$PtCl_2$(-$SMe_2)_2$] (0.20 g, 0.50 mmol) was added. The solution was stirred overnight; and after removal of solvent the crude product purified by recrystallizing from dichloromethane and hexane. Yield = 0.17 g, 75 %. ^{1}H NMR ($CDCl_3$): 9.58 (d, 1H, J = 6.0 Hz), 7.82 (m, 5H), 7.45 (m, 6H), 7.30 (d, 1H, J = 7.6 Hz), 7.20 (t, 1H), 3.45 (dt, 2H, J = 14.8 & 26.8 Hz), 2.21 (t, 2H). ^{31}P{^{1}H} ($CDCl_3$): 2.06 (s, 1P, $J_{Pt\text{-}P}$ = 4668 & 4676 Hz). ^{13}C{^{1}H} NMR (d6-DMSO): 20.5; 21.0; 35.8; 124.0; 125.9; 128.5, 128.6; 129.3; 131.4, 131.5; 133.0, 133.2; 140.2; 154.6; 159.2, 159.3. Anal. Calcd. for $C_{19}H_{18}$-$NPPtCl_2$: C, 39.34; H, 3.20; N, 2.35 %. Found, C, 39.84; H, 2.89; N, 2.25 %.

X-ray data collection, structure solution and refinement

Single crystals of complexes **1**–**4** suitable for X-ray diffraction were obtained by slow diffusion of hexane into dichloromethane solution of the respective compound. The structures were determined in order to confirm the identity of all four complexes proposed from their NMR data. Some of these structures have been reported in the literature, although in our hands some crystalized with solvents, as such the structures are reported as supplementary materials.

A crystal of **1** (as a representative procedure) of dimensions 0.18 × 0.17 × 0.06 mm^{-3} was selected and glued on to the tip of a glass fibre. The crystal was then mounted in a stream of cold nitrogen at 100(1) K and centred in the X-ray beam by using a video camera. The crystal evaluation and data collection was performed on a Bruker APEXII diffractometer with Mo Kα (λ = 0.71073 Å) radiation and diffractometer to crystal distance of 4.00 cm. The initial cell matrix was obtained from three series of scans at different starting angles. Each series consisted of 12 frames collected at intervals of 0.5° in a 6° range with the exposure time of 10 s per frame. The reflections were successfully indexed by an automated indexing routine built in the APEXII program suite (Bruker-AXS 2009). The final cell constants were calculated from a set of strong reflections from the actual data collection.

The data was collected by using the full sphere data collection routine to survey the reciprocal space to the extent of a full sphere to a resolution of 0.75 Å. A total of 4286 data points were harvested by collecting 1991 frames at intervals of 0.5° scans in ω and φ with exposure times of 10 s per frame. These highly redundant data sets were corrected for Lorentz and polarization effects. The absorption correction was based on fitting a function to the empirical transmission surface as sampled by multiple equivalent measurements (Bruker-AXS 2009).

The systematic absences in the diffraction data were uniquely consistent for the space group *P*1 that yielded chemically reasonable and computationally stable results of refinement. A successful solution by the direct methods of SIR92 provided all non-hydrogen atoms from the E-map (Altomore et al. 1993). All non-hydrogen atoms were refined with anisotropic displacement coefficients. All hydrogen atoms except those on the solvent water molecules were included in the structure factor calculation at idealized positions and were allowed to ride on the neighbouring atoms with relative isotropic displacement coefficients. The final least-squares refinement of 205 parameters against 4286 data points resulted in residuals R (based on F2 for $I \geq 2\sigma$) and wR (based on F2 for all data) of 0.0198 and 0.0515, respectively. The final difference Fourier map was featureless. The molecular diagrams

are drawn with 50 % probability ellipsoids (Farrugia 1997; Farrugia 1999; Palatinus and Chapuis 2007; Sheldrick 2008).

Cytotoxicity

The compound concentration required for the death of 50 % of cells in vitro (CC_{50}) was determined using TZM-bl cells (obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: TZM-bl from Dr. John C. Kappes, Dr. Xiaoyun Wu and Tranzyme Inc.) (Platt et al. 1998; Derdeyn et al. 2000; Wei et al. 2002; Takeuchi et al. 2008; Platt et al. 2009). The cells were cultured to confluence in DMEM media (Sigma Aldrich, USA) with 3.7 g/l $NaHCO_2$, 0.05 % (v/v) Gentamycin, 1 % (v/v) Antibiotic/Antimitotic and 10 % (v/v) Foetal Bovine Serum and were plated in 96-well plate (1.0×10^5 cells/ml incubated overnight). Serial dilutions of each complex were prepared (6.25–200 μM) and the treated cells were incubated for three days (37 °C, 5 % CO_2, 95 % humidity) after which the plate was centrifuged ($250\times g$; 10 min) and the supernatant removed. MTT working solution (5.0 mg/ml MTT in DMEM, 1:5 ratios, Sigma Aldrich, Germany) was added to each well (100 μl) and the plate was incubated for 2 h. Produced formazan crystals were solubilized with acidified isopropanol (1 ml of 1 M HCl: 9 ml propanol) and the absorbance was measured at 550 nm using a microtiter plate reader (Multiskan Ascent, Thermo Labsystems, USA).

HIV-1 protease assay

The complexes were investigated for inhibitory effects on the HIV-1 protease enzyme using a fluorescent substrate (Sigma-Aldrich, Missouri, USA). The assay was performed as described by Fonteh et al. (2009) (Fonteh et al. 2009) with slight modifications. A stock solution (1 mM) of substrate dissolved in DMSO was further diluted in protease buffer (0.1 M sodium acetate, 1 M NaCl, 1 mM EDTA, 1 mM dithiothreitol and 1 mg/ml bovine serum albumin; pH 4.7) into a working stock of 10 μM. Each complex was diluted in a 96-well black plate resulting in final test concentrations of 10, 25 and 100 μM (49 μl per well). An aliquot of the substrate (10 μM, 49 μl) and 2 μl of the Protease enzyme (1 μg/ml; Bachem, Switzerland) was added to the diluted complex, resulting in final volume of 100 μl per well. Acetyl-pepstatin (AP) (Bachem, Switzerland) a known inhibitor of the enzyme was used as a positive control (Li et al. 2012). The plate was incubated for 1 h at 37 °C and thereafter, the fluorescence readings were obtained at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. Complexes with percentage inhibition values greater than 50 % were considered to be active against the enzyme (Ellithey et al. 2014).

Kinetic studies were performed for the active complex using substrate concentrations ranging from 4.8 to 23 μM. For inhibited reactions, a concentration of twice the IC_{50} of **3** was used and the reactions were monitored in 30 s intervals over 390 s at the wavelengths specified above.

Pseudotyped HIV-1 virus neutralization assay

Complex **3,** which was the only complex active against protease was screened for its ability to inhibit the infection of TZM-bl cells by a pseudotyped HIV-1 subtype C virus, ZM53. TZM-bl cells are a HeLa cell line derivatives expressing high levels of CD4 and CCR5 as well as endogenously expressed CXCR4 (Platt et al. 1998; Derdeyn et al. 2000; Wei et al. 2002; Takeuchi et al. 2008; Platt et al. 2009). The assay was performed as described by Mufhandu et al. (2012) with slight modifications. The complex was tested at three different concentrations 25, 50 and 63 μM. The complex was diluted in DMEM media supplemented with 5 % FBS (v/v) and 60 μl was added to a black 96-well plate. Diluted virus (1:1 dilution, 25 μl) was added to each well and the plate was incubated for 45 min at room temperature to allow interaction of the complex with the virus. TZM-bl cells were cultured to confluence as described above. Post trypsinization, the cells were re-suspended in DMEM media containing 5 % FBS (v/v) and DEA-Dextran to a final concentration of 20 μg/ml. The cells were added to the plate containing complex and virus (10,000 cells/well, 50 μl) and the plate was incubated for 48 h (37 °C, 5 % CO_2, 90 % humidity). The supernatant was then removed (85 μl) and BrightGlo™ Luciferase Assay Substrate (Promega, USA) was added at 50 μl/well. The plate was incubated for 2 min in order to allow for cell lysis, and luminescence was read using the GloMax® Multidetection System (Promega, USA).The percentage virus neutralization was calculated using the formula:

$$\% \text{ Neutralization} = \{100 - [(\text{RLU}_{\text{Complex}} - \text{RLU}_{\text{Cells}}) / (\text{RLU}_{\text{virus}} - \text{RLU}_{\text{Cells}})] \times 100\}.$$

Percentage inhibition of greater than 50 % was regarded as activity against the virus (Mufhandu et al. 2012).

Real-time cell electronic sensing

A real time cell electronic sensing (RT-CES) device (xCELLigence, Roche Germany), which monitors the effects of compounds on the viability/proliferation of adherent cells in real time (Atienzar et al. 2011; Fonteh et al. 2011) was used to confirm the MTT-determined CC_{50} information for the compound. The assay was performed as previously described by Fonteh et al. (2011), with slight modifications. Briefly, a pre-titrated cell concentration of 20,000 cells/well were plated into e-plates and allowed to attach for ~24 h at which point the cell index (CI) was 1 ± 0.5 (37 °C, 5 % CO_2, 95 % humidity). Four different concentrations of compound **3** (25, 50, 100 and 200 µM) were tested, and the cells were monitored for three days. Changes in the cell index values indicating cell responses were used to calculate the area under each curve utilizing GraphPad Prism, and a decision tree by Kustermann et al. (2013) was used to determine whether the compound had significant toxicity or not (Fig. S1).

Determination of minimal inhibitory concentration (MIC) of complexes against *Mycobacterium tuberculosis*

MICs were determined by the broth microdilution method using a twofold dilution series of each complex in 96-well microtitre plates (Domenech et al. 2005). Briefly, *M. tuberculosis* H37Rv cells were grown in Middlebrook 7H9 media supplemented with 0.2 % glycerol, Middlebook OADC enrichment and 0.05 % Tween 80 (7H9) to OD_{600nm} of 0.2–0.3 (~10^6 CFU/ml) and diluted 1:500 in 7H9 media to be used as inoculum. The complexes were dissolved in DMSO and diluted to a concentration of 640 µM in 7H9 media for testing. For the assay, 50 µl of media was dispensed in a 96 well round-bottom plate in each well except in the top row, in which 100 µl of media was added to the first well (control), the appropriate concentration of the DMSO diluted in 7H9 to the second well (control) and each complex (640 µM) in the next wells in triplicate. Two fold dilutions were carried out using a multichannel pipette from the top row to the last row from which 50 µL was discarded. All the wells in the plate were inoculated with 50 µl of the diluted H37Rv culture. The plates were incubated (37 °C, 95 % humidity) and read visually using an inverted mirror after 7 and 14 days. The standard drugs, rifampicin, isoniazid and streptomycin were used as positive controls. The MIC was determined as the lowest concentration that prevented growth of the bacterial cells.

Statistical analysis

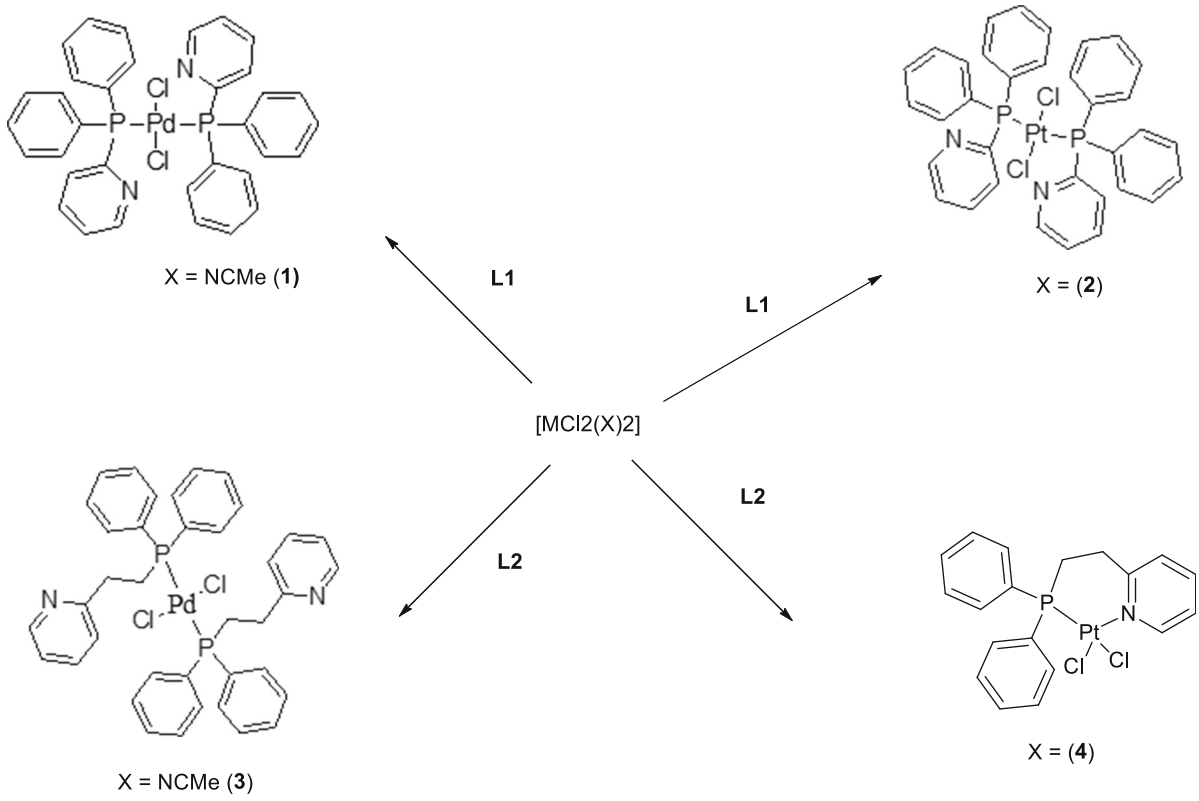
GraphPad Prism (version 5.00 for Windows, GraphPad Software, San Diego,California, USA) and Microsoft Excel (version 14.00, 2010, Microsoft Corporation by Impressa Systems, Santa Rosa, California USA) were used for data analysis. Data was analyzed using the *f* test and *t* test for $n = 3$, $P < 0.05$. Error bars represent % standard error of the mean (SEM).

Results and discussion

Synthesis of palladium and platinum complexes

Complexes **1–4** (Scheme 1) were synthesized in good to excellent yields under mild conditions, using either modifications of reported literature procedures or new procedures. In all cases complexes were characterized by NMR spectroscopy and X-ray crystallography in order to confirm the structures of compounds and the purities of compounds were established by microanalysis.

Crystallographic data for all four complexes are shown in Table S1 and the molecular structure of **1–4** are shown in Figs. 1, 2, 3, and 4. All four compounds have distorted square-planar geometries around the metal and do not have any unusual bond distances and angles. The crystal structure of **1** (Fig. S2) was first determined by Xinxin et al. (1992) as structure that has unit cell parameters of: *a*, 8.05(1) Å; *b*, 20.152(4) Å; *c*, 13.067(9) Å; $\alpha = \beta = \delta = 90°$; while in the current structure, **1** crystallizes with a solvent in the unit cell and has cell parameters of: *a*, 20.04(3) Å; *b*, 7.9176(10) Å′ *c*, 22.944(4) Å; $\alpha = \beta = \delta = 90°$. In



Scheme 1 Structures of the synthesized phosphino metal complexes

the structure two **L2** molecules are coordinated to Pd through the phosphorous atoms only and are *trans* with two terminal Cl atoms. The average Pd–P bond length is 2.3140(5) Å for both phosphorous bonds and is comparable to the average Pd–P bond length found in *trans*-[Pd(κ^1-Ph_2PpyH)[$MeSO_3$] (Flapper et al. 2009). The P1–Pd1–Cl1 angle is 93.023(18)° which deviates from 90°. The bond angle Cl–Pd1–Cl angle is 180°; the palladium atom is in the mean plane passing through the two phosphorous and two chlorine atoms.

The structure of complex **2** (Fig. 1) has Pt–P bond length of 2.2160(5) (P1) Å and is in the expected range for Pt–P reported by Liu et al. (Liu et al. 2010). The P–Pt–Cl2 angle (91.70(3)°) and the Cl(1)–Pt–Cl(2) angle (90.043(3)°) are similar to the respective angles in the complex [Pt(triphos)Cl]Cl (84.69(7)°, 84.92(7)° reported in literature (Sevillano et al. 1999). In complex **3** (Fig. 2) the Pd–P bond length was found to be 2.3319(5) Å; which is longer than the Pd–P bond length of 2.189(2) Å reported by Uhlig et al. (1974) (Uhlig and Keiser 1974). The bond angles for P–Pd–P and Cl–Pd–Cl are both 180°; and the Pd atom is in the plane passing through the two phosphorous and two chlorine atoms.

Interestingly, in spite of complexes **1**–**3** featuring potential bidentate ligands (**L1** and **L2**) none of these three compounds has its ligand bonded in a bidentate fashion. However, **L2** binds platinum in a bidentate fashion to form [Pt(κ^2 − **L2**)Cl_2]. The structure of this complex, **4**, (Fig. 3) has Pt–P = 2.2160(5) Å; Pt–N = 2.0403(17) Å and Pt–Cl (2.3722(5) Å bond lengths that are in the expected range as reported for a similar platinum complex by Farr et al. (Pt–P = 2.232(6) Å; Pt–Cl = 2.340(3) Å; Pt–N = 2.07(2) Å) (Farr et al. 1983). The P–Pt–Cl2 angle of 91.70(3)° and N1–Pt1–Cl1 angle of 88.42(2)° are indicative of a distorted square-planar environment around the platinum atom.

HIV-1 inhibition

Adverse effects associated with current Protease inhibitors (PIs) as well as the emergence of drug resistant viral strains necessitate development of novel

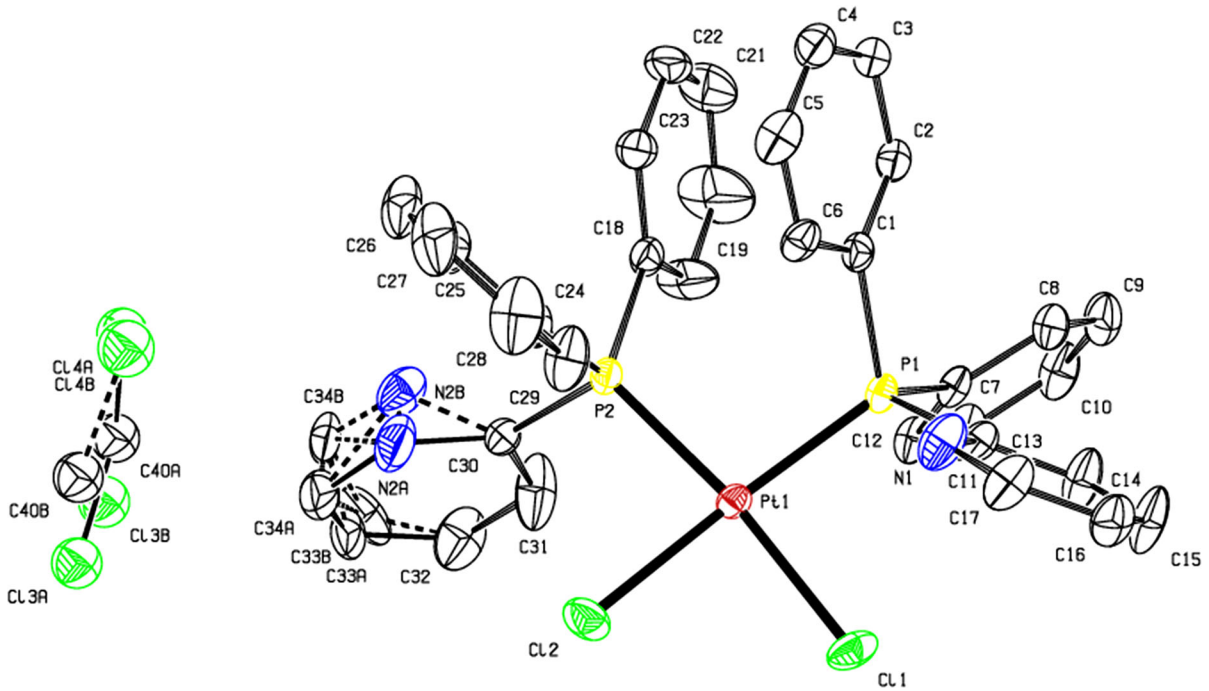


Fig. 1 Molecular structure of **2** with 50 % probability ellipsoids. The ORTEP diagram is presented for the purposes of showing the connectivity of the atoms. Hydrogen atoms are omitted for clarity. Selected bond distances (Å) and angles (°): Pt1–P1, 2.2418(9); Pt1–P2, 2.2449(10); Pt1–Cl1, 2.3510(9); Pt1–Cl2, 2.3458(10); P1–Pt1–P2, 98.97(3); P1–Pt1–Cl1, 86.977(18); Cl1–Pt1–Cl2, 88.58(4)

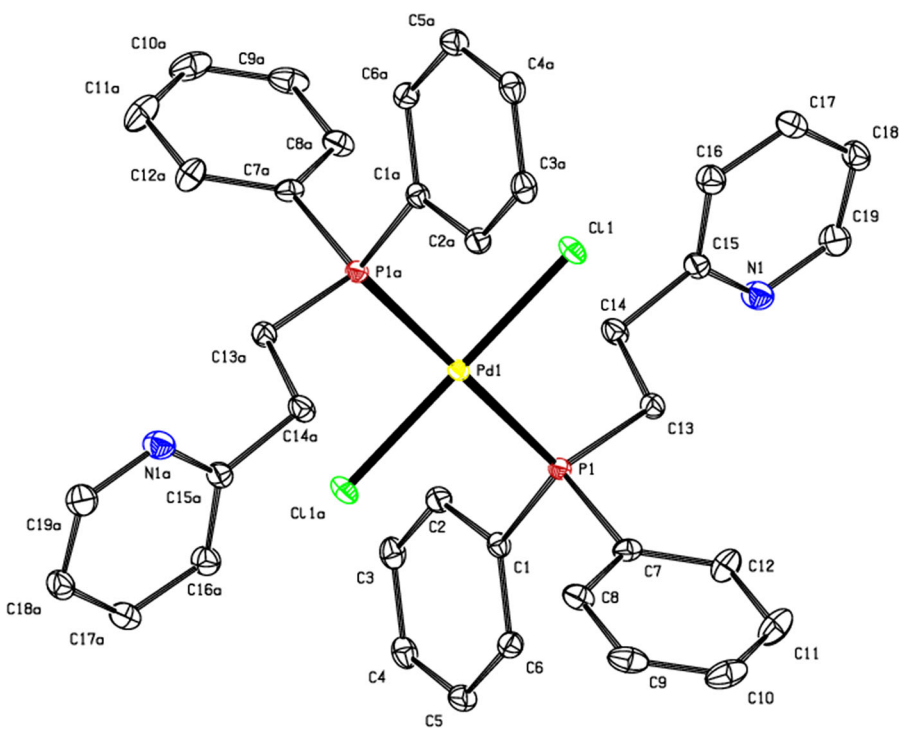


Fig. 2 Molecular structure of **3** with 50 % probability ellipsoids. The ORTEP diagram is presented for the purposes of showing the connectivity of the atoms. Hydrogen atoms are omitted for clarity. Selected bond distances (Å) and angles (°): Pd1–P1, 2.3319(5); Pd1–Cl1, 2.2995(3); P1–Pd1–Cl1, 88.981(12); P1–Pd1–Cl2, 91.019(12)

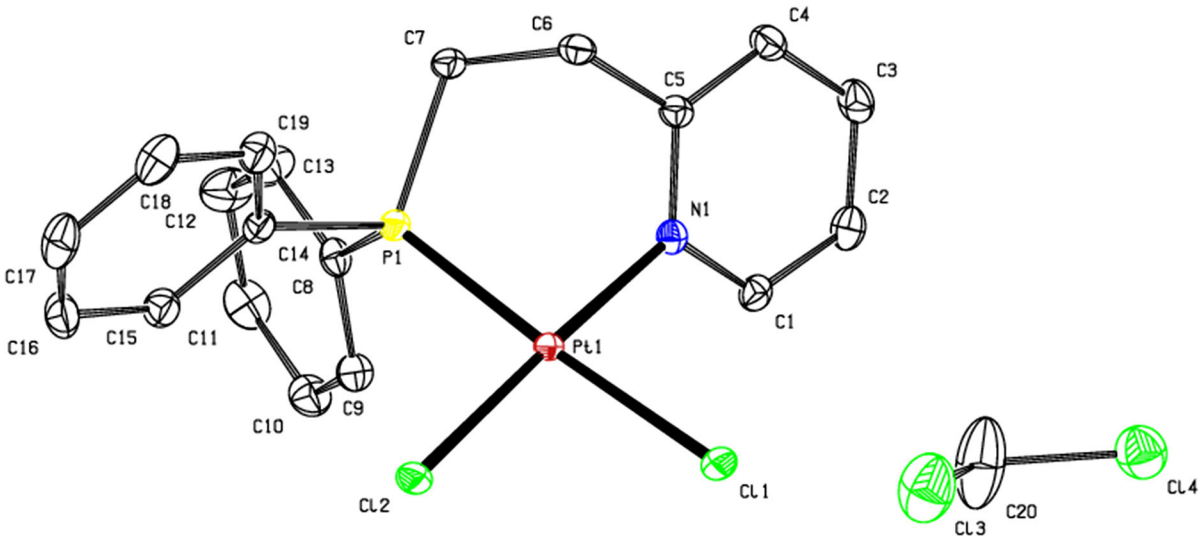


Fig. 3 Molecular structure of **4** with 50 % probability ellipsoids. The ORTEP diagram is presented for the purposes of showing the connectivity of the atoms. Hydrogen atoms are omitted for clarity. Selected bond distances (Å) and angles (°): Pt1–P1, 2.2160(5); Pt1–N1, 2.0403(17); Pt1–Cl1, 2.3722(5); P1–Cl2, 2.2970(5); N1–Pt1–P1, 90.00(5); P1–Pt1–Cl2, 91.70(2); N1–Pt1–Cl1, 88.42(5); Cl1–Pt1–Cl2; 90.043(19)

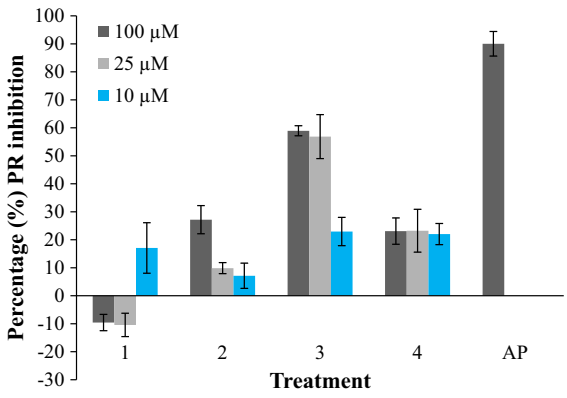


Fig. 4 HIV-1 protease inhibition by the complexes. As expected, AP exhibited high enzyme inhibition while **3** was the only complex with inhibition values greater than 50 %, indicative of activity. $n = 3$, *error bars* represent the standard error of the mean (SEM)

PIs. The inhibition of HIV-1 protease by metal based complexes has previously been reported (Karlstrom and Levine 1991; Fonteh and Meyer 2009; Meggers 2009) with the exception of palladium(II) complexes. The phosphino palladium and platinum complexes were investigated for their effect on the HIV-1 protease enzyme at three different concentrations and dose dependant inhibition was observed (Fig. 4).

Table 1 Kinetic properties of complex **3** in comparison to the uninhibited HIV-1 Protease. $n = 3$, ± the standard error of the mean (SEM)

	Km (µM)	Vmax (µmol·s^{-1})
Complex 3	63.15 ± 8.95	0.0107 ± 0.0051
Uninhibited enzyme	6.99 ± 3.22	0.0103 ± 0.007

The palladium complex **3** was the only complex with inhibition values against the enzyme with an IC_{50} value of 85.59 ± 3.77 µM. Kinetic data for the complex are shown in Table 1. An increase in the K_m value was observed in the presence of complex **3** with the V_{max} remaining relatively the same in the presence and absence of the complex. This is suggestive of competitive inhibition of the enzyme by the complex.

In order to prove that the observed activity was facilitated by the presence of the palladium, the ligand used in the synthesis of complex **3** was also assessed (Fig. S3) which resulted in little or no activity at all tested concentrations, supporting the concept that a metal ion is necessary for activity. Although there have been no previous reports of any palladium-based inhibitors for the HIV enzyme, reports of metal-based inhibitors include gold(III) compounds which were

speculated to function through ligand-exchange reactions with the enzyme (Fonteh et al. 2009). The thiol group of Cys 95 found in the dimerization interface of the enzyme could be facilitating the possible ligand-exchange reactions (Zutshi and Chmielewski 2000).

Kinetic data indicated that the complex inhibited the enzyme competitively (Table 1) and therefore, interacting directly with active site of the enzyme which consists of two aspartic acid residues (Asp25 and Asp25′), one from each monomer of the homodimer, and any interactions with these residues could result in the dissociation of the monomers, rendering the enzyme inactive (Camarasa et al. 2006). The activity of HIV-1 protease is to catalyse the hydrolysis of peptide bonds using an activated water molecule to attack the amide bond carbonyl of the substrate, and the activation of this water molecule is through the action of the two aspartyl-β-carboxy groups (Brik and Wong 2003). There have been reports of non-peptide, cyclic structures with anti-HIV activity in the μM ranges that form hydrogen bonds with the flap residues and subsequently displace this water molecule (Lucca et al. 1997). Pyridyl copper(II) complexes have been reported for the inhibition of HIV-1 protease, and molecular modelling suggested that the S2/S2′ pocket of the enzyme is occupied by the pyridyl group. The metal ion then coordinates with the structural catalytic water molecule in the active site (Meggers 2009). Complex **3** could be acting in a similar manner. In addition to the palladium(II) ion, activity could also be attributed to the presence of a lone pair of electrons on the ligand (**L1**) attached to **3**; which could then be forming hydrogen interactions with these active site Asp residues, allowing for a ligand exchange reaction. Furthermore, the absence of this lone pair of electrons, as is observed in compound **4**, as a result of the coordination of the platinum(II) ion with this lone pair of electrons, could be the cause of the inactivity of compound **4**. In addition, the coordination of the Pt ion to the pyridyl nitrogen could be affecting the flexibility of this compound, restricting interactions with active site residues. The need for the accessibility of this lone pair of electrons is further emphasized by the inactivity of compound **1** which is also a palladium(II) compound, however, ligand **L2** lacks the linker CH_2 groups, meaning that the structural flexibility and the distance between the metal ion and the nitrogen could potentially play a major role in the activity of these metal complexes.

Complex **3** was investigated for its inhibition of a pseudovirus, ZM53 on TZM-bl cells and the observed virus neutralization values are shown in Fig. 5. The complex showed approximately 80 % virus neutralization at 63 μM which is half the 50 % cell cytotoxicity (CC_{50}) of this complex on these cells as revealed by the MTT cytotoxicity assay (Table 3).

As stated before, the inhibition of PR by phosphino palladium(II) complexes has never been reported, however, there has been reports of polysulfonates of palladium(II) complexes with activity against both HIV-1 and HIV-2 whole virus particles (Bergstrom et al. 2002). Although the mode of action of these complexes still needs to be elucidated, they displayed little cytotoxicity in vitro, similarly to complex **3**. In fact, concentrations as high as 100 μM were shown to be non-toxic for complex **3** (Table 2) which had an estimated CC_{50} of 126 ± 0.95 μM when using MTT detection. This is quite surprising as palladium(II) complexes have mainly been investigated for anti-cancer activity with IC_{50} values ranging between 1.5

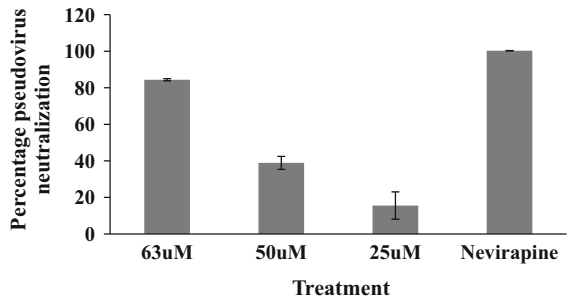
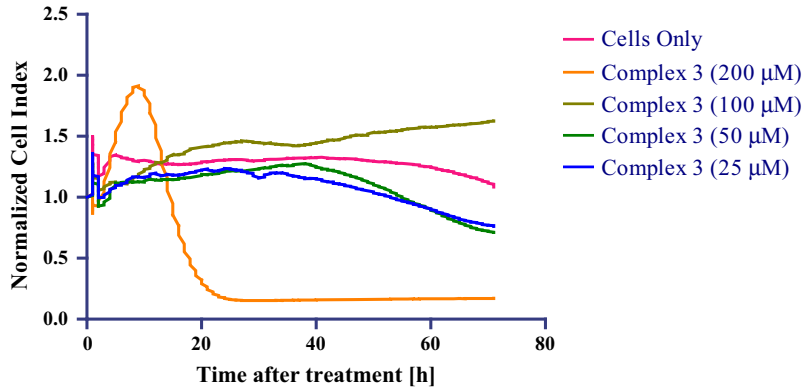


Fig. 5 The neutralization of a pseudo typed HIV-1 virus by complex **3**. $n = 3$, *error bars* represent the standard error of the mean (SEM)

Table 2 Complex cell cytotoxicity values that result with 50 % cell death values as determined by the MTT assay. $n = 3$, ± the standard error of the mean (SEM)

Complex	CC_{50} (μM)
1	N/T
2	92.59 ± 4.83
3	126 ± 0.95
4	N/T
L1	N/T
L2	N/T

N/T = the complex was non-toxic at all tested concentrations



	Cells Only	Complex 3 (200µM)	Complex 3 (100µM)	Complex 3 (50µM)	Complex 3 (25µM)
Area of Positive Peaks	19.84	6.631	30.28	9.248	8.020
Area of Negative Peaks	0.0	44.74	0.0	2.569	2.285

Fig. 6 The effects of complex **3** on TZM-bl cells as analyzed by RT-CES. Concentrations of 100 µM and below showed cell responses similar to untreated cells complementing the MTT findings. Three data sets where done and this graph is a representative graph (See Fig. S4 for the additional data sets)

Table 3 Susceptibility of H37RV to the metal complexes and the ligands used in the synthesis 1

Treatment	MIC_{99} at 7 days	MIC_{99} at 14 days
1	10	20
2	20	20
3	2.5	5
4	80	160
L1	160	160
L2	160	320

MICs are reported in µM

and 47 µM (Quiroga and Navarro Ranninger 2004; Khan et al. 2011). As the ligand used in the synthesis of the metal complex plays a very important role in the activity of the complex, the phosphine ligand complexed to compound **3** may be responsible for reduced toxicity of compound **3**.

The non-toxicity of compound **3** was confirmed by real-time cell analysis (Fig. 6). Profiles exhibited that compound treated cells succumb to toxicity during uptake but recover and even show better growth compared to untreated cells.

Notably, complex **3** at 200 µM first showed a sharp increase in cell index which could be thought of as cell proliferation however, we have shown in our lab that uptake of the complex results in the swelling of cells and a successive increase in the cell index (Fonteh et al. 2015). Based on a decision tree by Kusterman et al. (2013) (Fig. S1), concentrations below 100 µM where predicted to have no significant negative effect on the growth of TZM-bl cells while 200 µM was toxic, supporting our MTT data.

Inhibition of *Mycobacterium tuberculosis*

Treatment of log phase cells of *M. tuberculosis* strain H37Rv with complex **3** resulted in death of the bacteria at a concentration of 2.5–5 µM after 14 days of treatment (Table 3) while the other complexes and the ligands showed an MIC of ≥20 µM. To the best of our knowledge, this is the first report of a palladium(II) complex with an MIC of below 10 µM (Moro et al. 2009; De Souza et al. 2010). A previous study on the antimycobacterial activity of palladium(II) isonicotineamide derivatives indicated the inability of these complexes to inhibit the bacterium (MICs ranging between 125 and 15.6 µg/ml), however, the metal produced compounds where more active than the uncomplexed isonicotineamide (De Souza et al. 2010). This was similar in our study, where the ligand system (**L1** and **L2**) was less active than the metal complexes for all the complexes, indicating the necessity of the metal ions for possible activity.

Using the MIC and the CC_{50} of **3** on TZM-bl cells, the selective index (SI) was calculated to be 25.45. According to recommendations by Pavan et al. (2012),

for a compound to be considered for in vitro preclinical studies, the MIC must be ≤10 μg/ml or the molar equivalent and have a SI value ≥10 (Pavan et al. 2012). Based on this, complex **3** can be considered for preclinical investigations.

Conclusion

The ability of {*bis*(2-(2-(diphenylphosphino)ethyl) pyridyl}palladium(II) chloride (**3**) to inhibit HIV-1 protease and an HIV-1 pseudo virus was shown here. Although activities were in the micromolar ranges, these concentrations were non-toxic in vitro (Table 3, Fig. 4). In addition to that, the complex showed MIC and SI values that warrant further in vitro preclinical investigation. These studies could include testing of complex **3** against clinical *M. tuberculosis* isolates as well as against mono (rifampicin) and multi drug resistant strains (rifampicin and isoniazid) to evaluate the full potential of this compound to treat both susceptible and drug resistant TB strains (Pavan et al. 2012). This research serves as proof of concept for the possible use of a single complex for the management of both HIV-1 and tuberculosis co-infections.

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