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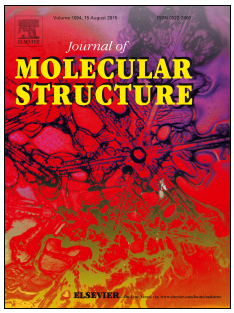

Schiff base triphenylphosphine palladium (II) complexes: synthesis, structural elucidation, electrochemical and biological evaluation

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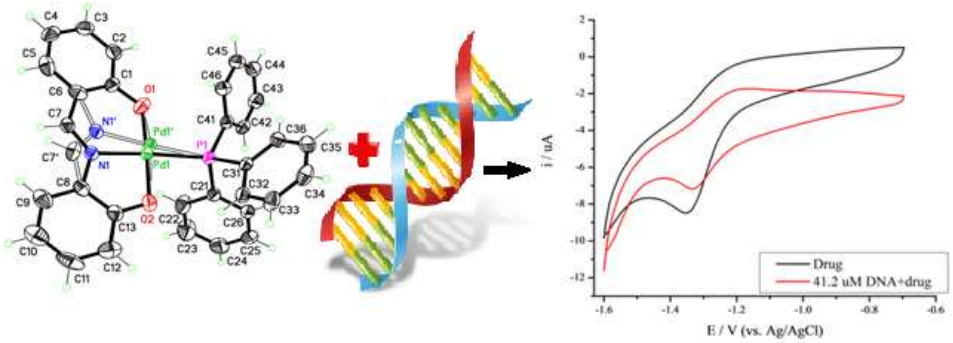

Schiff base triphenylphosphine palladium (II) complexes: synthesis, structural elucidation , electrochemical and biological evaluation

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Abstract

The complexes N-(2-oxidophenyl)salicylideneiminatotriphenylphosphine palladium(II)(**1**) and N-(2-sulfidophenyl)salicylideneiminato triphenylphosphine palladium(II)(**2**) of tridentate Schiff bases derived from salicylaldehyde and an amino- or thiophenol, have been synthesized and characterized by various spectroscopic, analytical and electro-analytical techniques. X-ray single crystal analysis of complex **1** has revealed its square planar geometry. The thermal analysis has shown the absence of coordinated water and final degradation product is PdO. The alkaline phosphatase studies have indicated that enzymatic activity is concentration dependent which is inversely proportional to the concentration of the compounds. The biological assays (brine shrimp cytotoxicity, DPPH) have reflected their biologically active and mild antioxidant nature. However, results of DNA protection assay have shown that they possess moderate protective activity against hydroxyl free radicals (·OH). The voltammetric studies ascertain two-electron reduction of the compounds through purely diffusion controlled process and reveal intercalative mode of drug DNA interactions.

Key Words**:** Palladium (II) complexes; structural elucidation; Drug-DNA intercalation

1. Introduction

Schiff bases, the condensation products of primary amines with either aldehydes or ketones, are versatile compounds capable of coordinating with metals through imino nitrogen and another donor atom (O&O/S) [1]. Such ligands having functional groups like -OH and/or -SH present ortho to the azomethine group form five or six membered stable chelates. The metal complexes having O, N, S donor Schiff bases possess unusual configuration, structural lability and are sensitive to molecular environment. The environment around the metal center (coordination geometry, number of coordinating ligands and their donor groups) is the key factor for metallo proteins in order to carry out specific physiological functions. S-ligated complexes may mimic the ligation of certain biomolecules in proteins. The presence of oxygen moiety can enhance antitumor activity of compounds. Similarly, antibacterial and antifungal activities of the complexes appear due to the chelating behavior of the ligands with most of the metal ions coordinated through N and S donor atoms [2].

Schiff base transition metal complexes are widely used for organic synthesis, drug design and catalysis [3, 4] .The redox behavior of palladium is dominated by electrochemical conversions between palladium(0), palladium(II), and palladium(IV) species. Palladium(I) complexes are rarely found due to their instability and disproportionation whereas Palladium(II) complexes are comparatively stable [5,6]. The interest in the electrochemical investigation of palladium and platinum based complexes has emerged in last few years because of their higher cytotoxic activity and less side effects in comparison to the established drug molecules like Cisplatin. Palladium(II) complexes have been found to be potential inhibitors of enzyme alkaline phosphatase (ALP). They interact with the negatively charged functional groups of ALP that are necessary for regulating the structural and catalytic activities of enzymes [7]. Alkaline phosphatases (EC 3.1), catalyze the transfer of phosphate groups to water (hydrolysis) or alcohol (transphosphorylation) using a wide variety of phosphomonoesters [8, 9]. Palladium complexes with nitrogen and sulfur containing ligands are known to exhibit antibacterial and antitumor activity. They play a vital role in controlling gene expression, inhibiting cell replication and hence are used as valuable drugs. They show various modes of interactions, for example electrostatic, intercalative and groove binding that can be monitored by cyclic voltammetry, UV–Vis., fluorescence, Raman and NMR spectroscopies [10-12]. However, problem associated with such complexes is their ready dissociation in solution leading to very reactive species that are unable to reach their pharmacological targets such as DNA. This rapid aquation and formation of very reactive species could be overcome if palladium(II) complexes are stabilized by bulky ligands such as triphenylphosphine [13].

Inspired by the wide applications of such compounds in various fields, palladium(II) triphenylphosphine complexes (**1&2**), containing N-(2-oxidophenyl)salicylideneiminate) (H_2L_1) and N-(2-sulfidophenyl)salicylideneiminate) (H_2L_2) Schiff bases were synthesized. Contrary to their previous studies [14, 15] we have explored them for enzyme inhibition towards ALP, biological (brine shrimp cytotoxicity, potato disc antitumor and hydroxyl free radical ($^{\bullet}OH$) scavenging assays) and voltammetric studies to investigate their DNA binding mode.

2. Experimental

2.1 Materials and methods

All chemicals were of reagent grade and solvents were purified by standard techniques and drying methods [16]. Triphenylphosphine, palladium(II) chloride and ALP buffer solution were used as received from Aldrich. $[PdCl_2(PPh_3)_2]$ was prepared by mixing anhydrous $PdCl_2$ dissolved in acidified methanol and triphenylphosphine in acetone with constant stirring for an hour in molar ratio 2:1 [17]. The Schiff bases H_2L_1 and H_2L_2 were synthesized by condensation reactions of salicylaldehyde with 2-aminophenol and 2-aminobenezenethiol in ethanol following the reported method [18]. Elemental analysis was carried out on a CHNS 932 (Leco-USA) elemental analyzer. Melting points were determined, using a MPD Mitamura Riken Kogyo (Japan) electrothermal melting point apparatus. FTIR spectra were recorded on a Thermoscientific (USA) Nicolet 6700 spectrometer, number of resolutions 4 cm^{-1}, the scan's numbers16, detector DTGS-KBr and the frequency range of 4000-400 cm^{-1}. ^1H NMR (300 MHz), ^{13}C NMR (75 MHz) and ^{31}P NMR (122 MHz) spectra were recorded on a Bruker NMR Spectrometer in deuterated chloroform using tetramethylsilane as internal reference. Thermogravimetric analyses of the complexes was performed on TGA/SDTA 851e Mettler Toledo at a heating rate of 20 ^oC /min over a temperature range of 40-800 ^oC under nitrogen flow.

A single crystal of complex **1** was obtained by the slow evaporation from the ethanol mother liquor at room temperature. X-ray data were collected at 150(2) K on a Bruker Apex II CCD diffractometer using MoK_α radiation (λ = 0.71073Å). The structure was solved by direct methods and refined on F^2 using all the reflections [19]. All the non-hydrogen atoms were refined using anisotropic atomic displacement parameters and hydrogen atoms were inserted at calculated positions using a riding model. There was a disorder in the structure arising from a 180° rotation of the ligand (positions of O1 and O2 exchanged) and this also affected the position of the metal atom. This was modeled for the Pd ion and the "linker" atoms C7 and N1 but no attempt was made to model the resultant alight disorder of the phenolate groups (Fig. 1). The relative occupancy of the two sites refined to 82:18. Parameters for data collection and refinement are summarized in Table 1.

Activity of the enzyme ALP was determined at room temperature by following the increasing absorbance at 405 nm due to para-nitrophenol formed by the hydrolysis of the substrate para-nitrophenyl phosphate (pNPP). In this method, 100 µL of ALP was added to 5 mL of an assay system containing pNPP (2 mM) in 0.01 M Na_2CO_3-$NaHCO_3$ buffer (pH=10.1) containing different concentrations of palladium(II) complexes [20,21].

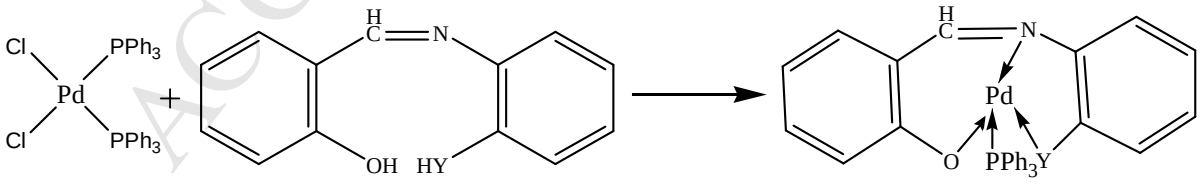
The biologically active nature of compounds was assessed by brine shrimp cytotoxic assay. Seawater (34 g/L) was used for hatching of brine shrimp (*Artemia salina*) eggs (Ocean Star Inc., USA) under constant aeration at room temperature. After 24 h of hatching, shrimps were gathered via pipette from lightened side of the dish and were transferred to marked vials.Ten shrimps were transferred to each vial using a Pasteur pipette and the volume was raised up to 5 mL with artificial seawater having four different concentrations 200, 66.6, 22.2 and 7.4 µg mL^{-1} for each compound. Three replicates were prepared for each concentration.Vials were kept at room temperature under illumination for 24 h and then survivors were counted by the aid of 3× magnifying glass. Percentage death was calculated via Abbott's formula and the LD_{50} value was calculated by using Finney (1971) software [22]. Antitumor activity was screened by using potato disc antitumor assay [23]. *Agrobacterium tumefacians*, refreshed AT-10 culture was used to prepare inoculum with three different concentrations (1000, 100, 10 &1µg mL^{-1}). Surface sterilized potatoes were then loaded with 50 µL of inoculum. DMSO was used as negative control while positive control was doxorubicin. Tumor inhibition ability of compounds was

calculated by following formula, (Percentage inhibition = 100 – average number of tumours of sample/ average number of tumours of negative control × 100). Antioxidant activity of the compounds (**1&2**) was evaluated by DPPH free radical scavenging assay [24] employing ascorbic acid and DMSO as positive and negative controls respectively. Five different concentrations of both compounds (200, 66.6, 22.2, 7.4, 2.7 µg mL^{-1}) were tested in triplicate. The reaction mixture was incubated for 30 min at 37°C and absorbance at 515 nm was determined by spectrophotometer. DNA damaging activity was assessed by inhibition of hydroxyl (·OH) free radical induced DNA protection assay [25]. Compounds were analyzed at four different concentrations (1000, 100, 10 and 1µg mL^{-1}). Reaction mixture of 15 µL (5 µL compound, 3 µL $FeSO_4$, 4 µL H_2O_2 and 3 µL plasmid) was prepared, using DMSO as positive control. For negative control 3 µL of plasmid and 12 µL of phosphate buffer were taken. Damaging effect of compound on DNA (control compound) was checked in the absence of H_2O_2. Reaction mixture along with controls was incubated for an hour in dark at 37°C and then it was subjected to gel electrophoresis after staining with ethidium bromide. Ladder consisting of 1 kb was employed as marker. Gel was then analyzed *via* Gel- Doc (Bio Rad) software.

Voltammetric (cyclic, differential pulse and square wave voltammetry) experiments were performed employing Eco Chemie Autolab PGSTAT 302 potentiostat/galvanostat (Utrecht, The Netherlands) using software version GPES 4.9. All the voltammetric experiments were performed with conventional three-electrode cell with Ag/AgCl as a reference, home-made thin platinum wire as a counter and a glassy carbon electrode (GCE) with surface area of 0.09 cm^2 as a working electrode. Prior to use the active surface of GCE was polished with 0.25 m alumina paste on a nylon buffing pad, followed by washing with distilled water and finally rinsing with DMSO. Tetrabutylammonium tetrafluoroborate (TBATFB) was used as supporting electrolyte. For compound-DNA interaction studies solutions of the compounds were prepared in aqueous-DMSO mixture (2:8, v:v) and subjected to CV analysis at a scan rate of 100 mVs^{-1} with progressive addition of DNA.

2.2 General Procedure for the Synthesis of Pd (II) Complexes

Complexes **1** and **2** were prepared by refluxing equimolar ethanolic solutions of Schiff base (H_2L_1 or H_2L_2) and $PdCl_2(PPh_3)_2$ in 250 mL two neck flask under nitrogen for 24 h. The red solid was filtered off, washed with ethanol, dried in air and stored in vacuum desiccator (Scheme 1).



where PPh_3 = triphenylphosphine, Y = O for **1** ,S for **2**

Scheme 1: Synthesis of complexes**1**and **2**.

2.2.1 N-(2-oxidophenyl)salicylideneiminato triphenylphosphiepalladium(II)(1)

Yield: 83%. mp 254 ^{o}C, $\upsilon_{max/}$ cm^{-1} 1595 (-CH=N), 1338 (C - O), 545 (Pd-O), 412 (Pd-N) δ_H 7.9-6.6 (m, 23 H, Ar–H), 8.8 (s, ^{1}H, –CH= N–). δ_C 169.0 (C–O),163.5 (C=N),147.6 (C–P), 138.1-114.5(Ar-C), δ_P 22.8. Calc. for $C_{31}H_{24}NO_2$ PPd: C 64.2, H 4.1, N 2.4.Found: C 64.3, H 4.1, N 2.3%.

2.2.2 N-(2-sulfidophenyl)salicylideneiminato triphenylphosphine palladium(II)(2)

Yield: 79%. mp 242 ^{o}C, $\upsilon_{max/}$ cm^{-1} 1612 (-CH=N), 1327 (C - O), 744 (C-S), 538 (Pd-O),408 (Pd-N) δ_H 7.7–6.5 (m, 23H, Ar–H), 8.7 (s, 1H,–CH= N–). δ_C 169.4 (C–O),159.9 (C= N), 145.3 (C–P), 135.1-117.9 (Ar-C), δ_P 23.3. Calc for $C_{31}H_{24}NOS$ PPd: C 62.4, H, 4.0, N 2.4, S 5.4.Found: C 62.4, H, 4.0, N 2.4, S 5.3%.

3 Results and Discussion

3.1 Synthesis and characterization

The complexes **1** and **2** were prepared from equimolar ethanolic solutions of Schiff base (H_2L_1 or H_2L_2) and $PdCl_2(PPh_3)_2$.The synthesized complexes(**1&2)** were characterized by various spectral techniques(FTIR, ^{1}H NMR, ^{13}C NMR, ^{13}P NMR) and single crystal X-ray analysis.

All the characteristic bands observed for the complexes in FTIR spectra are in close agreement with their structures. The bands at 1595, 1612 ν(C= N), 1338, 1327 ν(C–O) vibrations are for **1** and **2** respectively. The C=N bond vibrations are shifted to lower frequency due to reduction in electron density which shows coordination of the ligand with palladium. For complex 2 a band at 744 was shown due to ν(C–S) vibration [26, 27]. The bands at 545 and 538 cm^{-1} in the complexes (**1 & 2**) were assigned to ν (Pd–O) vibrations. The bands observed at 412 and 408cm^{-1} were due to ν (Pd–N) mode [15]. Further, the compounds showed strong bands near 1425, 1108, and 659 cm^{-1}characteristic of triphenylphoshine indicating the coordination of this ligand with palladium metal [28] .The FTIR spectra of complexes (**1 & 2**) is presented in Fig. S1.

In the ^{13}C NMR spectra of the complexes, azomethine carbon resonances were observed at 163.5 and 159.9 ppm. The resonances at 169.0(C–O), 147.6 (C–P) and 169.4 (C–O), 145.3 (C–P) ppm, respectively, were observed for both the complexes. The signals at 138.1-114.5 and 135.1-117.9 ppm were due to the aromatic rings of Schiff bases and triphenylphosphine for the complexes **1** and **2**, respectively as revealed in Fig. S3.

^{31}P NMR spectra exhibited singlets at 22.8 and 23.3 ppm suggesting coordinated triphenylphosphine in each case [29] as displayed in Fig. S4.

3.2 X-ray structure analysis of complex 1

A perspective diagram of neutral complex Pd(L1)PPh_3 (**1**) is shown in Fig. 1. Data collection and refinement parameters are given in Table 1 while selected bond lengths and angles are given in Table 2.The palladium ion has approximate square planar coordination (rms deviation from the PdO_2NP mean plane, 0.22Å) bound to the phenolate and imine donors of L1 and to the PPh_3 ligand. The two Pd – O distances are distinctly different, presumably reflecting the different chelate ring size. There is a disorder in the structure arising from a 180° rotation of the ligand (positions of O1 and O2 exchanged) and this also affects the position of the metal atom. The disorder was modeled for the palladium ion and the "linker" atoms C7 and N1 but no attempt was made to model the resultant slight disorder of the phenolate groups. The relative occupancy

of the two sites refined to 82:18.The complexes are arranged into interdigitated, π-stacked columns.

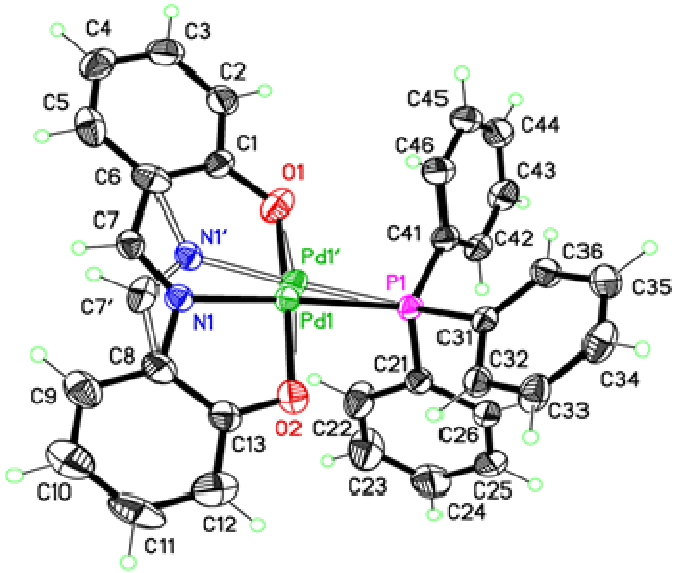


Fig.1. Perspective view of Pd(L1)PPh_3 (**1**). Ellipsoids are drawn at the 50% probability level. White bonds and primed labels show the minor (18%) component of the disorder.

The principal π interaction is between the imine of one L1 ligand and neighboring C1-C6 and C8-C13 phenol rings (under symmetry operations 1-x, 1-y, -z and -1x, -y, -x respectively); the imine centroid - ring centroid distances are 3.340 and 3.665Å (Fig. 2).

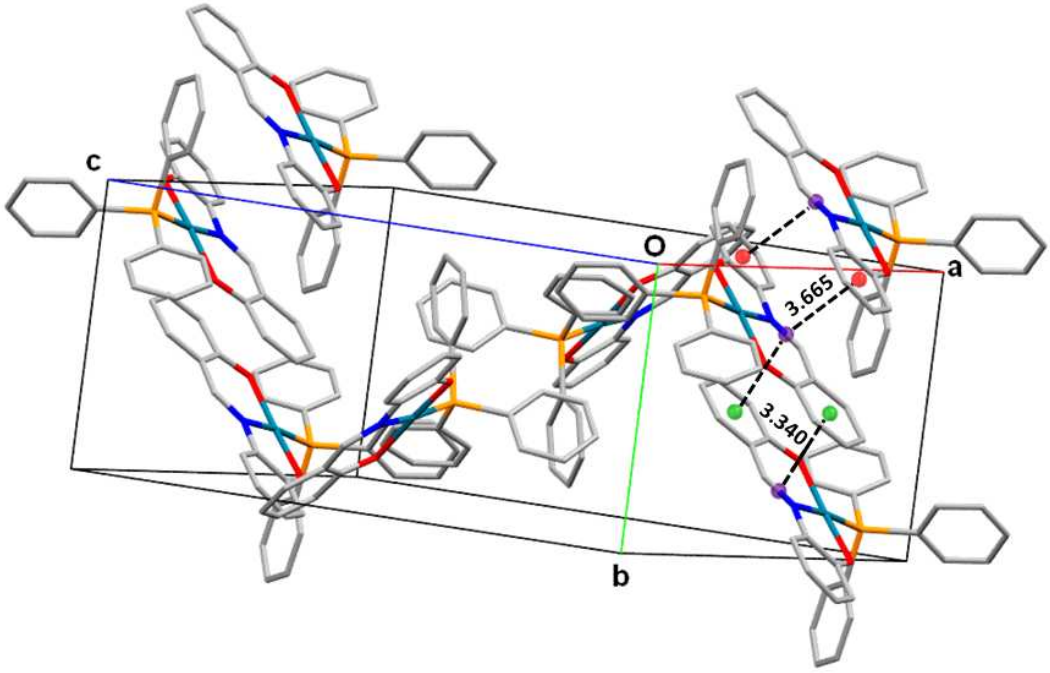


Fig. 2. Packing diagram for **1** showing imine-phenol π-stacking. Purple dots indicate the centroid of the imine; green and red dots indicate the centroids of the C1-C6 and C8-C13 phenolate rings respectively.

The only significant link between stacks is an (aromatic) C-H ···π interaction between C34-H34 to the centroid of the phenyl ring containing C41 (H34–centriod 2.594 Å under symmetry operation x, y-1, z (Fig.S5).

Table 1. Data collection and refinement parameters.

$C_{31}H_{24}NO_2PPd$	V = 2486.4 (3) Å^3
M_r = 579.88	Z = 4
Monoclinic, $P2_1/n$	Mo $K\alpha$ radiation, λ = 0.71073 Å
a = 15.4856 (12) Å	μ = 0.84 mm^{-1}
b = 9.0872 (7) Å	T = 150 K
c = 18.3498 (14) Å	0.37 × 0.23 × 0.05 mm
β = 105.655 (1)°	
Bruker APEX 2 CCD diffractometer	6196 independent reflections
Absorption correction: multi-scan *SADABS* v2012/1, Sheldrick, G.M., (2012)	5310 reflections with $I > 2\sigma(I)$
T_{min} = 0.339, T_{max} = 0.431	R_{int} = 0.031
measured reflections	24586
$R[F^2 > 2\sigma(F^2)]$ = 0.037	5 restraints
$wR(F^2)$ = 0.085	H-atom parameters constrained
S = 1.05	$\Delta\rangle_{max}$ = 0.78 e Å^{-3}
6196 reflections	$\Delta\rangle_{min}$ = -0.62 e Å^{-3}
parameters	353

Table 2. Selected bond lengths [Å] and angles [°] for **1** (major component)

Pd1—O1	2.019 (3)	Pd1—N1	2.006 (4)
Pd1—O2	1.948 (3)	Pd1—P1	2.2980 (13)
O2—Pd1—N1	83.66 (12)	O2—Pd1—P1	91.55 (7)
O2—Pd1—O1	176.57 (10)	N1—Pd1—P1	174.48 (12)
N1—Pd1—O1	95.08 (11)	O1—Pd1—P1	89.85 (9)

3.3 Thermal analysis

Thermal analysis was carried out in an inert atmosphere of argon at a heating rate of 20 °C/min over a temperature range of 25-800 °C to check the presence of coordinated water molecules. The weight loss for each complex was calculated within corresponding temperature range. The decomposition of both the complexes occurred in a single step starting above 140 °C, showing absence of coordinated water [30] and completed at 706 °C with the loss of organic moiety (found/calc. 79.59/79.82 %) leaving behind the residue, PdO, (found/calc. 20.40/20.17 %).

3.4 Electrochemical studies

Electrochemical behavior of the complexes was investigated by employing advanced electrochemistry techniques namely cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV). The objective of these studies was to assess the redox mechanism of the complexes, both qualitatively and quantitatively, the former tells about the characteristic redox potential and the nature of electron transfer (ET) process while latter gives the number of such ET processes and the electrons involved in each step. The kinetics across the electrode solution interface was also investigated while determining the diffusion coefficient value which in turn is applied to compare the ET process of complex alone and in presence of DNA- a medicinal application through in vitro study.

3.4.1 Cyclic Voltammetry

The CV of 2 mM each of **1** and **2** in their argon saturated aqueous/DMSO solution (2:8, v: v) containing 0.1M tetrabutylammonium tetrafluoroborate as a supporting electrolyte was performed at glassy carbon electrode first in the potential range of -1.5 V to +1.5 V at scan rate of 100 mVs^{-1} at 25^{o} C (Fig. 3). During anodic scan no well-defined oxidation signal (except a hump) was observed for both the compounds while scanning cathodically a reduction peak centered at E_{pc} = -1.34 V (vs. Ag/AgCl) for **1** and for **2** two reduction signals centered at E_{pc} = -1.40 V and -1.26 V (vs. Ag/AgCl) were observed represented as 1_c and 2_c, respectively. Hence further experiments were performed in potential window of -1.6 to -0.5 V. The absence of oxidation wave clearly indicates the highly reactive nature of the reduced form of the complexes and thus an electron transfer followed by a chemical reaction i.e. EC mechanism was followed. The higher reduction values (beyond -1.0V) can be correlated to the lower electron affinity of the electrophore having electron donating ligands of PPh_3. Relatively easier reduction in **2** can be ascribed to the less electronegative character of S(2.58) in comparison to **1**(3.44). The very weak anodic signal, as a hump around -1.3V, particularly in complex **2**, indicates the outer reorganization phenomenon commonly present in metal complexes [31]. The appearance of two explicit reduction peaks in complex 2 and one reduction peak in complex 1 could be attributed to the presence of less electronegative S in complex 2 which not only facilitates the first reduction (anion formation) but also allows the dianion formation. It is notable that there is no structural difference other than replacement of oxygen in complex 1 by sulphur in complex 2. Thus there is high probability of intramolecular rearrangement that makes the PPh_3 more available to induce e-donating character in presence of less electronegative S in comparison to that of more electronegative O.

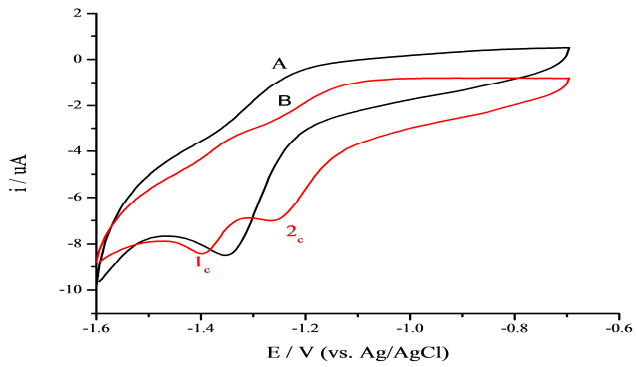


Fig. 3. Cyclic voltammograms of (2 mM each) **1** (A) and **2** (B) at GCE (0.09 cm^2) in aqueous-DMSO (2:8, v: v) solution containing 0.1M TBATFB at scan rate of 100 mVs^{-1} at 25°C.

Assignment of number of electrons involved in the reduction step was confirmed by DPV discussed later. The reduction process involving two electrons might be attributed to the formation of a palladium(0) center with coordination number between 2 and 4 (or to an equilibrium mixture of several palladium(0) species) [32]. There are many examples of stable Pd(0) complexes available in literature, the most relevant is $[Pd(diars)_2]^0$ (diars = 1,2-bis(dimethylarsino)benzene), which is sufficiently stable to be isolated in the solid form [33]. The absence of an oxidation peak during anodic scan for both the complexes indicates that the complexes undergo apparently irreversible reduction.

To further investigate the redox behavior of complexes CV studies were performed at different potential scan rates ranging from 20 mVs^{-1} to 140 m Vs^{-1} which are shown in Fig. S6.

By increasing the scan rate, a shift in cathodic peak potential towards more negative potential was observed for both the complexes establishing irreversible reductive behavior. It was observed that cathodic peak 2 of complex **2** disappeared at higher scan rate (≥ 1000mVs^{-1}).

For irreversible processes peak current is given by Randles-Sevcik equation given below [34]

$$i_p = (2.99 \times 10^5) n(\alpha n)^{1/2} AC^*(D_o v)^{1/2} \quad (1)$$

Where i_p is peak current, n is number of electrons involved in the redox process, α is the charge transfer coefficient, A is the surface area of working electrode, C* is the bulk concentration of analyte, *v* is the potential scan rate and D_o is the diffusion coefficient.

It was observed that with the increase of scan rate the cathodic peak current increases linearly following the equation 1 for both of the complexes. Plots of cathodic peak currents (i_{pc}) vs. $v^{1/2}$ (Fig. S7) give a straight line with slope values of 5.91×10^{-6} and 3.47×10^{-6} for **1** and **2**, respectively. By substituting the values of α as 0.5 (supposed), n as 2 (calculated from DPV), A and C*in the slope (Slope = (2.99×10^5) n $(\alpha n)^{1/2} AC^* D_o^{1/2}$) of equation 1, the values of diffusion coefficient were calculated to be 3.02×10^{-9} cm^2s^{-1} and $1.04 \times 10^{-9} cm^2s^{-1}$ which reveal that the electrochemical reduction of **1** and **2** is diffusion controlled.

3.4.2 Differential pulse voltammetry

DPV is an excellent technique to determine the number of electrons involved in the electron transfer process. In DPV the number of electrons involved in the electrochemical process can be

calculated from the value of peak width at half of the maximum height by employing equation [35]

$$W_{1/2} = \frac{3.52RT}{nF} = \frac{90.4}{n}\,mV \qquad (2)$$

Where $W_{1/2}$ is the peak width at half height, n is the number of electrons involved in electrochemical process and F is the Faraday constant.

The DPVs of **1** and **2** (2 mM each) in their argon saturated aqueous-DMSO (2:8, v: v) solutions containing 0.1M TBATFB as a supporting electrolyte at GCE are presented in Fig. 4 at 25° C. The values of $W_{1/2}$ are 62 mV and 60 mV (peak 1) for **1** and **2**, respectively. These values are close to the theoretical value of 45 mV for a redox process involving two electrons. Hence the two electron redox process is proposed for the electrochemical reduction of the complexes.

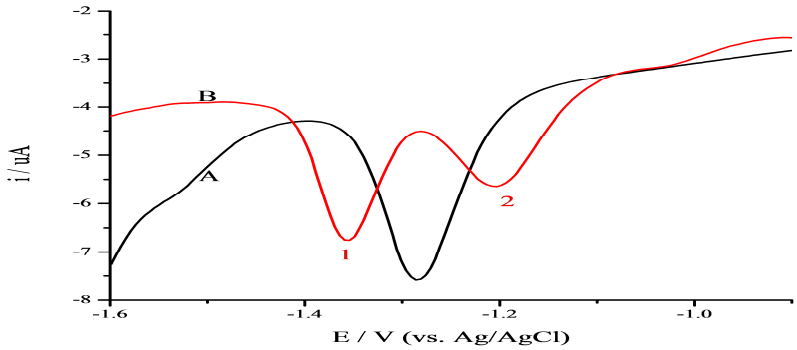


Fig. 4. DP voltammograms of 2 mM complex **1**(A) and 2mM complex**2** (B) in aqueous-DMSO (2:8, v: v) solution containing 0.1M TBATFB as a supporting electrolyte at GCE at 25° C.

3.4.3 Square wave voltammetry

The advantages of square wave voltammetry (SWV) on DPV are greater speed of analysis, lower consumption of electroactive species and reduced problems with the poisoning of electrode surface [36]. Further SWV study is the best criterion to diagnose the reversibility of redox process. The reversibility of the obtained peaks for the complexes was evaluated by plotting the forward and backward components of the total current obtained in 2mM solution of the complexes. SWVs of **1** and **2** recorded at 25° C are shown in Fig. S8. It is clear that the reduction process of both the complexes are quasi-reversible instead of completely irreversible as apparently observed in case of CV. The steric hindrance of triphenylphosphine group may be one of the reasons to cause electrochemical irreversibility; as geometrical influence affects the electron transfer process both in terms of its thermodynamic feasibility and kinetics. Moreover, the observed broad redox waves complement the inference of structural rearrangements (both of inner and outer sphere) as conferred above. This can be further validated by computational studies such as DFT calculations.

3.5 Enzymatic activity

The synthesized Schiff base ligands and their corresponding complexes (**1** & **2**) were screened for the study of inhibitory effects on ALP. Both ligands were found to be inactive but

palladium(II) complexes inhibit the enzyme in a concentration dependent manner (Fig. S9). It was observed that enzyme loses its more than 80% of activity at the concentration of 0.73 mM. The Pd may replace Zn or Mg of the enzyme and hence the enzyme fails to bind with the substrate. This affect is similar to earlier studies that more than 50% inhibition of the enzyme was observed when the concentration of Pd complex was 0.25 mM [37]

3.6 Biological studies

3.6.1 Brine shrimp cytotoxic assay

Brine shrimp cytotoxic assay was carried out in order to prescreen biologically active nature of the compounds. Results were compared with standard drug doxorubicin (LD_{50} 25.2 µg mL^{-1}) and it was observed that the compound **1** showed promising cytotoxic potential with LD_{50} value of 64.2 µg mL^{-1}. Results indicate that the compounds were toxic even at low dose which is consistent with the study of 1-indanone thiosemicarbazones coordinated to palladium (II) [38].

3.6.2 Potato disc antitumor assay

Cytotoxic potential of compounds is predictive of their antitumor potential. The data revealed that complex **1** had promising antitumor activity as compared with doxorubicin showing IC_{50} value 24.9 µg mL^{-1} while **2** did not show any antitumor activity.

Table 3. Results of cytotoxicity, potato disc antitumor, DPPH free radical scavenging antioxidant and inhibition of hydroxyl free radical induced DNA damage assays

complex	Cytotoxic activity	antitumor activity				IC_{50} (µg mL^{-1})	% scavenging of DPPH at different conc.(µg mL^{-1})					IC_{50} (µg mL^{-1})	DNA protection activity at different conc.(µg mL^{-1})			
	LD_{50} value (µg mL^{-1})	Percentage inhibition ± SD(µg mL^{-1})														
		1000	100	10	1		200	66.6	22.2	7.4	2.7		1000	100	10	1
1	64.2	69	62	39	27	18.5	76	42.2	26.4	1.0	0	116.9	-	+	++	++
2	724.8	36	20	6	0	nil	90	40.3	14.7	10.4	5.57	101.8	-	+	++	++
Standard	25.2	80	69	40	37	24.9	82.4	76.4	58.6	39.2	19.9	20.2				

Doxorubicin was used as standard drug for cytotoxic and antitumour activity while ascorbic acid for % scavenging of DPPH (-) = no protection. (+) = slight protection. (++) = good protection.

These results are consistent with the findings of Butour *et al* [39] that most of the palladium compounds displayed antitumor activity. Moreover the presence of oxygen moiety (mostly at ortho position in ring structure) can also enhance antitumor activity of palladium compounds [40]. This might seems to be the reason for non antitumoral activity of **2** which contains sulphur.

3.6.3 DPPH free radical scavenging antioxidant assay

DPPH free radical scavenging activity of complexes was determined spectrophotometrically by measuring absorbance at 515 nm. Both the compounds show mild antioxidant potential with IC_{50}

values of 116.9 and 101.8 µg mL^{-1} (Table 3) by using ascorbic acid (IC_{50} value = 20.2 µg mL^{-1}) as standard and these are in agreement as already reported [41]. This antioxidant potential may be due to ligand associated electronic factors, ligand complexation with palladium and extended π configuration of aromatic ligands [42].

3.6.4 DNA protection assay

Inhibition of hydroxyl (•OH) free radical induced DNA damage assay was performed in vitro in order to check the antioxidant and prooxidant behaviors of compounds at four different concentrations (1 µg/mL, 10 µg/mL, 100 µg/mL and 1000 µg/mL) listed in Table 3. It was observed that complex **1** and **2** showed no protection at 1000 µg mL^{-1} while DNA protection activity was observed for rest of tested concentrations (Fig. 5).

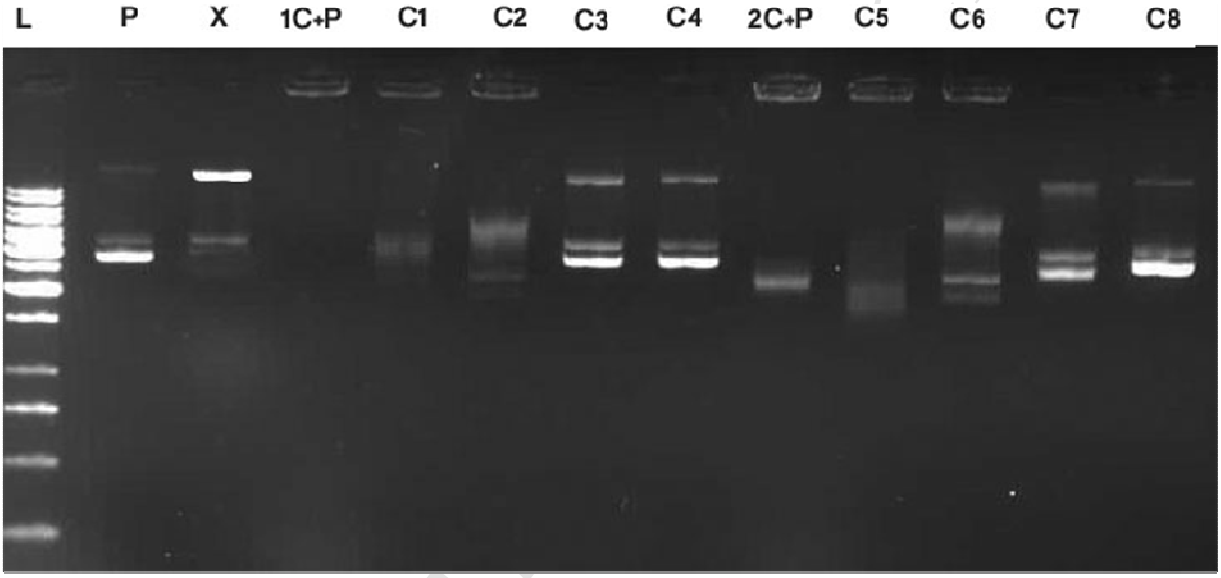


Fig. 5. Effect of compounds **1** and **2** on pBR322 plasmid DNA[L=1Kb ladder , P=pBR322 plasmid, , X = Plasmid DNA + $FeSO_4$ + H_2O_2, 1(C+P) =Plasmid + phosphate buffer + 1000 µg mL^{-1} of **1**, C1 =Plasmid + phosphate buffer + 1000 µgmL^{-1} of **1** + $FeSO_4$ + H_2O_2,C2=Plasmid + phosphate buffer +100 µg mL^{-1} of **1** + $FeSO_4$ + H_2O_2, C3 = Plasmid + phosphate buffer + 10 µg mL^{-1} of **1** + $FeSO_4$ + H_2O_2, C4=Plasmid + phosphate buffer + 1 µg mL^{-1} of **1** + $FeSO_4$ + H_2O_2, 2(C+P)=Plasmid + phosphate buffer + 1000 µg $mL^{-1}$of **2**, C5 =Plasmid + phosphate buffer + 1000 µg $mL^{-1}$of 2 + $FeSO_4$+H_2O_2, C6 = Plasmid + phosphate buffer +100 µg mL^{-1} of **2** + $FeSO_4$ + H_2O_2, C7 = Plasmid + phosphate buffer + 10 µg mL^{-1} of **2** + $FeSO_4$ + H_2O_2, C8 = Plasmid + phosphate buffer + 1 µg mL^{-1} of **2** + $FeSO_4$ + H_2O_2].

3.7 Drug DNA Interaction Study

The cyclic voltammetric method was employed to study the interaction of palladium complexes with the DNA. The amounts of 21, 41.2, 61.2, 81, 100, 119 and 137 µM DNA were added into the 2 mM solution each of **1** and **2** separately and corresponding CV response was recorded at glassy carbon electrode under the same conditions as were performed for pure complexes. It was noted that the cathodic peak currents for both the complexes decreased substantially along with a

positive shift of reduction peak potentials on DNA addition (Fig. 6 & Table 4). The regular anodic shift accompanying the decline in peak current is noticeably showing the decrease in the concentration of free drug molecules (Pd complex used) while rest of the drug intercalates with the added DNA molecules [43].

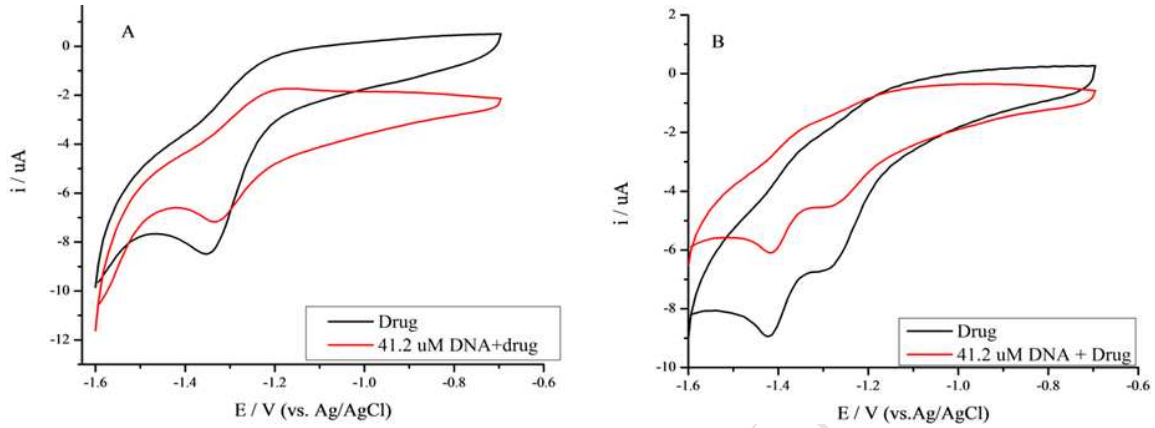


Fig. 6. Cyclic voltammograms of 2 mM each of 1 (A) and **2**(B) (—) without DNA and (—) in the presence of 41.2 µM DNA on GCE at 100 mVs^{-1} in aqueous-DMSO (2:8, v: v).

To further evaluate and quantify the interaction of complexes with DNA, CV studies were executed at different potential scan rates. The relationship of cathodic peak current with square root of scan rate ($v^{1/2}$) for both the complexes was found to be linear in the presence of DNA (drug-DNA adduct) (Fig. S10) and directed that the redox process of complexes persisted mainly diffusion controlled as observed for pure complexes. However, the slope of the linear line declined substantially in presence of DNA as shown in Fig. S10. The diffusion coefficient values of these adducts were considerably smaller than that of free complexes, showing that DNA-bound complexes diffused more slowly as compared to free complexes (Table 4). The observed behavior could be ascribed to the diffusion of an equilibrium mixture of the free and DNA-bound complexes to the surface of working electrode [44]. To promote the stated argument, binding constant values were evaluated by employing the following equation [45]

$$1/[DNA] = \{K(1-A)/(1-I/I_0)\} - K \qquad (3)$$

Where A is proportionality constant and K is the binding constant calculated from the intercept of the plots of equation 3 as shown in Fig. S11. The K values given in Table 4, define the binding strength of a complex to the DNA molecule. The value of binding constant of complex **2** is close to (1.74×10^4 M^{-1}) the typical intercalator lumazine-DNA complex [46].The binding site size in terms of concentration of base pairs was calculated by employing equation 4 [47,48].

$$Cb/Cf = K\{[DNA]/2s\} \qquad (4)$$

Where 's' is the size of binding site, C_f and C_b are the concentrations of free and DNA-bound species, respectively. The Cb/Cf can be given by equation 5.

$$Cb/Cf = (Io - I)/I \qquad (5)$$

The values of binding site size (s) were determined from the slope of plots of Cb/Cf vs. [DNA] (Fig. S12) and are presented in Table 4. The values of binding site size show that the complex 2 includes ≈ 1 base pair of the DNA and thus has stronger interaction than 1 as depicted from the values of binding constant for the complexes. Relatively a bit stronger interaction of complex 2 was as per expectation due to its easy reduction and thus more susceptible for interaction through intercalation into staked base pairs of with DNA molecules.

Table 4. The drug-DNA interaction electrochemical parameters of complexes on glassy carbon electrode vs. Ag/AgCl in aqueous DMSO (2: 8, v: v) solution at 100 m Vs^{-1} scan rate at 25^{o}C

	In the absence of DNA		In the presence of DNA			
Complex	E^{o} (V)	$D_o \times 10^{9}$(cm^2s^{-1})	E^{o} (V)	$D_o \times 10^{10}$ (cm^2s^{-1})	$K \times 10^{-4}$(M^{-1})	s(bp)
1	-1.39	3.02	-1.36	3.89	0.38	0.27
2	-1.45	1.04	-1.44	5.42	1.02	0.64

4 **Conclusion**

Palladium (II) complexes derived from the condensation of N-(2-oxidophenyl)salicylideneiminate) and N-(2-sulfidophenyl)salicylideneiminate) with salicyaldehyde were synthesized and characterized by physio-chemical, spectroscopic and electrochemical methods. The results suggested the coordination of the ligand to the metal through O, N, S donors and attachment of one coordinated triphenylphosphine with palladium. Structure of complex **1** was determined by X-crystallography which is stabilized by intermolecular π-π stacking between the ligand and by various C-H....π interactions. Based on the spectroscopic studies and single crystal analysis, the new complexes possess square planar geometry. Thermal analysis has shown that decomposition of both the complexes occurred in a single step above 140 ^{o}C, verifying that no water was physically or chemically bonded with the complexes. A rapid weight loss above 140 ^{o}C indicated the decomposition of coordinated ligands. The broad band above 706 ^{o}C is resulted the formation of stable PdO.The enzymatic activity exhibited the inactivation of the enzymes by increasing the concentration of the compounds. These complexes demonstrated cytotoxic and antitumor activity due to which they can be used for therapeutic purposes. They unveiled quasi-reversible redox behavior on glassy carbon electrode involving two-electron reduction of palladium(II) to palladium(0). Diffusion coefficients of the complexes have divulged that the electron transfer process is diffusion controlled. Voltammetric and biological studies explored DNA damaging nature of these compounds suggesting their anticancerous potential. Although our work is preliminary but in future it can serve as a ground to study their mechanistic action as well as structure activity relationship.

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Disclosure Statement

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Highlights

- Synthesis and characterization of palladium(II)complexes.
- The enzymatic activity declines by increasing concentration of the compounds.
- Biological assays (brine shrimp cytotoxicity, DPPH) reflect mild antioxidant nature.
- Voltammetric studies ascertained two-electron reduction.
- Electrochemical findings illustrate intercalative mode of drug DNA interactions.