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SYNTHESIS, SPECTRAL CHARACTERIZATION, AND BIOLOGICAL ACTIVITIES OF NOVEL PALLADIUM(II) AND PLATINUM(II) COMPLEXES OF ACTIVE SCHIFF BASE LIGANDS

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ABSTRACT. A new series of palladium(II) and platinum(II) Schiff base complexes have been synthesized by the interaction of ligands N-(2-fluoro benzylidene)isonicotinohydrazone (L^1H) and N-(2-fluorophenylethanone) isonicotinohydrazone (L^2H) with $PdCl_2$ and $PtCl_2$. Elemental investigations, melting point determinations, molecular weight determinations, IR, ^1H NMR, and UV-Visible spectral studies were used to describe the structure and bonding pattern of ligands and their metal complexes. These analyses revealed that the ligands coordinate with the metal ions in a monobasic bidentate manner and that the complexes have a square planar geometry. The antimicrobial activities of both the ligands and their palladium(II) and platinum(II) complexes have been tested against various bacterial and fungal strains and showed considerable antifungal and antibacterial characteristics. The *in vitro* cytotoxic activity of $[Pt(L^2H)]Cl_2$ complex was assessed by examining its potential to inhibit cell proliferation against the human HeLa cell line(cervical cancer cell line) using MTT assay and the antioxidant activity of $[Pt(L^1H)_2]Cl_2$ compound was performed against DPPH. The results showed a dose-dependent cytotoxic and radical scavenging activity thus pointing towards the biological significance of Pt(II) complexes.

KEY WORDS: Schiff base ligands, Antimicrobial activity, Scavenging activity, Cytotoxic activity

INTRODUCTION

Transition metal complexes and their massive applications in medicine have been extensively investigated since the discovery of cisplatin by Rosenberg in 1960 [1, 2] which is highly effective for the treatment of various types of tumors. However, the use of cisplatin is limited because of severe toxic side effects and acquired resistance exhibited in various types of cancers [3, 4]. Therefore, there is a need for new approaches that are purposefully designed to circumvent these drawbacks. Efforts are focused to develop novel platinum [5] and non-platinum [6, 7] based antitumor drugs to improve clinical effectiveness to reduce general toxicity and broaden the spectra of activity. Schiff base ligands and their metal complexes have sparked a lot of attention due to their ease of synthesis and a vast variety of uses. These molecules are crucial in the development of coordination chemistry for catalysis, organic synthesis [8, 9], and anticancer, antibacterial, and cytotoxic activity [10-12]. In recent years, isoniazid and its derivatives have received attention and are being widely studied. The Schiff bases of isoniazid and their derivatives exhibited antitubercular [13-15], antibacterial, antifungal, and cytotoxic activities [16]. They also exhibiturease inhibitory activity [17], and antidepressant and analgesic properties [18]. Pt(II) and Pd(II) coordination compounds have played a significant role in medicinal chemistry by exhibiting remarkable antitumor properties against a wide range of cancers such as adrenocortical cancer, breast and colon cancer, small-cell and non-small-cell lung cancer, melanoma and head/neck cancers, non-Hodgkin lymphoma, ovarian cancer [19-23]. The primary mechanism of antitumor activity of cisplatin and subsequent generations of Pt medicines, according to popular belief, comprises entrance into the cell, equation, and activation, creationintra strands and cross-links with DNA, transcription inhibition, and induction of programmed cell death [24]. Many platinum(II) and palladium(II) complexes have been described as exhibiting a wide range of

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biological activities, including antibacterial and anti-inflammatory properties [25]. Various research has been conducted on the effects of various palladium complexes on the development and metabolism of various bacteria. There have been several reports of antiviral, antibacterial, and antifungal activity of palladium(II) complexes with various kinds of ligands [26, 27].

Keeping in view the diversified significance of palladium(II) and platinum(II) complexes in mind, we are describing the preparation, characterization, and biological potential of these complexes with isoniazid Schiff base ligands. Cytotoxic studies of $[Pt(L^2H)]Cl_2$ complex was carried out to examine its potential against HeLa cell lines to evaluate its pharmacological significance. The studied platinum(II) complex exhibits significant cytotoxicity against HeLa cancer cell lines. Furthermore, the antioxidant activity of the platinum(II) complex was investigated against 2,2-diphenyl-1-picrylhydrazyl(DPPH) which was found to be dosage dependent. In addition, the ligands and their platinum(II) and palladium(II) complexes showed significant *in vitro* antimicrobial activity when tested against *Aspergillus fumigates*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* microbes.

EXPEIMENTAL

Analytical methods and physical measurements

All of the chemicals and reactants utilized were of analytical quality and the solvents were dried, distilled, and purified according to industry standards. The Kjeldahl [28] and Messenger [29] methods were used to determine nitrogen and sulfur, respectively. Palladium(II) and platinum(II) were calculated gravimetrically, while chlorine was estimated volumetrically using Volhard's method [30]. The molecular weights were determined using the Rast Camphor technique. The SHIMADZU-1800 UV Spectrophotometer was used to record ultraviolet spectra.

The FT-IR Spectrum (Perkin-Elmer) spectrophotometer was used to record the infrared spectra of the ligands and their metal complexes. At MNIT, Jaipur, ^1H and ^{13}C NMR spectra were obtained in DMSO-d_6 using an ECS 400 MHz (JEOL) NMR spectrometer (the internal standard used was TMS).

Preparation of the ligands

To prepare Schiff base ligands (L^1H and L^2H), isonicotinic acid hydrazide (2.0 g, 14.58 mmol) and 2-fluorobenzaldehyde (1.80 g, 14.58 mmol) (L^1H) and 2-flouroacetophenone (L^2H) (2.0 g, 14.58 mmol) were dissolved in ethanol (~100 mL) in 1:1 molar ratio. For around 3-5 hours, the contents were heated under reflux. The resulting residue was separated, filtered, recrystallized from ethanol, and then vacuum dried on fused calcium chloride. The yield of isoniazid Schiff bases (L^1H and L^2H) was 89% and 85%, respectively. The synthetic process for the synthesis of ligands is depicted in Figure 1.

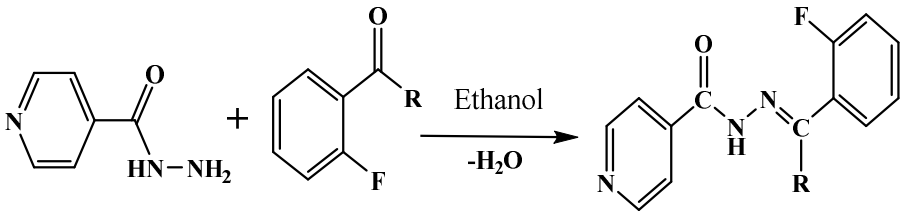


Figure 1. Synthetic route of the ligands, where R = H (L^1) and R = CH_3(L^2).

Preparation of the complexes

The metal chlorides, $PtCl_2$ (in 1:1 water-ethanol solution) and $PdCl_2$ (in methanol) were heated under reflux to dissolve the respective metal chlorides and then reacted with the ligands (L^1H and L^2H) in 1:2 (M:L) molar ratios.

Addition complexes $[M(L^nH)_2]Cl_2$

Pt(II) complexes were made by mixing a 1:1 water-ethanol solution of $PtCl_2$ with an ethanolic solution of the ligands (L^nH n = 1 and 2), whereas Pd(II) complexes were made by mixing a methanolic solution of $PdCl_2$ with a methanolic solution of the ligands in 1:2 molar ratios. The reaction mixture was then stirred on a magnetic stirrer for 2-3 hours in the presence of a few drops of concentrated HCl to create $[M(L^nH)_2]Cl_2$ complexes.

Substitution complexes $[M(L^n)2]$

Pt(II) complexes were generated by combining a 1:1 water-ethanol solution of $PtCl_2$ with an ethanolic solution of the ligands, while Pd(II) complexes were formed by mixing a 1:2 molar ratio of $PdCl_2$ with a methanolic solution of the ligands. To create $[M(L^n)_2]$ type complexes, dropwise addition of aqueous ammonia to the reaction mixture until it was weakly alkaline was done, and the reaction mixture was then refluxed for 1-2 h. After cooling, the complexes were separated, filtered, washed with ethanol, and dried under a vacuum.

Antimicrobial studies

Antifungal studies. Using Sabouraud's "well diffusion approach" [31] the antifungal activity of the synthesized palladium(II) and platinum(II) complexes against the pathogenic fungus *Aspergillus fumigatus* was evaluated in vitro using dextrose agar as a selective medium.The medium is made up of the following ingredients: 40 g dextrose, 10 g mycological peptone, 15 g agar, and 1000 mL distilled water; pH was adjusted to 5.6-5.9 at 28±2 °C. The test sample was then immersed in dimethylsulphoxide (DMSO) to achieve concentrations of 50 and 100 ppm. Through an inoculum needle, the fungi's spores were injected into the medium in the Petri plates. The Petri plates were covered in polythene bags containing a few drops of alcohol and then placed in an incubator at 25±4 °C. The antifungal activity, or linear growth, was measured after 96 hours of incubation by measuring the fungal colony's diameter. The % inhibition was estimated using the following equation (1):

$$\%\text{Inhibition} = \frac{(C - T)}{C} \times 100 \quad (1)$$

where C = After 96 hours, the diameter of the fungal colony in the control plate, and T = after the same duration, the diameter of the fungal colony in the test plates. Antifungal screening results of tested compounds were compared with the industry standard (Itraconazole).

Antibacterial activity. The "Kirby Bauer Disc Diffusion Method" with the inhibition zone technique [32] was used to measure the antibacterial activity of the ligands and their associated palladium(II) and platinum(II) complexes against various microorganisms. Gram-positive microorganisms like *Staphylococcus aureus* and Gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* were tested for antibacterial activity.

The ligands and their metal complexes were evaluated against bacteria using Müeller-Hinton agar for this purpose (HiMedia, Laboratories, Mumbai, India) [33, 34]. The agar medium was made up of 1.5 g starch, 300 g beef infusion, 17.5 g casein hydrolysate, 17 g agar, and 1000 mL

distilled water; the pH of the agar was kept between 7.2 and 7.4 at room temperature. The test chemicals were dissolved in DMSO at concentrations of 50 and 100 ppm and soaked on Whatmann no. 1 filter paper discs with a diameter of 5 mm. The dry discs were put on seeded petri plates and cultured for 24-48 hours at 37 °C. The diameter of the inhibition zone surrounding each disc was measured precisely in millimeters and compared to that of the reference antibiotic (Streptomycin), whose antibacterial activity was also assessed using the same method. The inhibitory zone surrounding each disc containing the test chemicals was measured in mm.
The Equation (2) for calculating the % Activity Index for the complex is as follows:

$$\%\ \text{Activity index} = \frac{\text{Zone of inhibition by the test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100 \qquad (2)$$

Antioxidant activity

DPPH free radical scavenging activity was used to test the antioxidant activity of the chemical $[Pt(L^1H)_2]Cl_2$. The radical scavenging activity of antioxidant substances is commonly measured using 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical and a trap ("scavenger") for other radicals. The essential principle in this approach is the production of the non-radical form DPPH-H by reducing DPPH in a methanol solution in the presence of a hydrogen–donating antioxidant. A strong absorption band at about 520 nm marks the deep violet color of the DPPH radical in the solution, which changes to colorless or light yellow when neutralized. This propensity enables visual monitoring of the reaction, which is monitored in a UV-Visible spectrophotometer at 517 nm.

Procedure. Extract of compound $[Pt(L^1H)_2]Cl_2$ of concentration 1 mg/mL was prepared with DMSO solution. The uniform mixture was made and working solutions of 100–500 µg/mL concentrations were prepared. Further 0.1 mM solution of DPPH in methanol was added to the working solutions. The mixture was shaken and incubated for 60 min in the dark at Room Temperature. The absorbance was measured at 517 nm against blank (methanol). The absorbance of ascorbic acid as positive control was recorded using same concentration of dilution series as taken for the test compound i.e. 100–500 µg/mL.

The DPPH scavenging activity was calculated using Equation (3) as follows:

$$\text{Scavenging effect}(\%) = 1 - (\text{sample } OD/\text{control } OD) \times 100 \qquad (3)$$

where; sample *OD* is the absorbance of test compound and control *OD* is the absorbance of control.

Anticancer activity

The "MTT cell proliferation assay" [35] was used to assess the anticancer activity of the produced platinum(II) complex $[Pt(L^2H)]Cl_2$. The MTT cell proliferation test is a colorimetric method for determining the metabolic activity of cells. Under some situations, NAD(P)H-dependent cellular oxidoreductase enzymes may reflect the number of live cells present. The tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide may be reduced by these enzymes to its insoluble formazan, which has a purple color. Cytotoxicity can also be measured using tetrazolium dye tests (loss of viable cells).

Materials. DMEM (Dulbecco's modified Eagles medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS), and Fetal

Bovine Serum (FBS) were all acquired from Sigma Chemicals Co. (St. Louis, MO). Eppendorf India provided 25 cm^2 and 75 cm^2 flasks, as well as 96 well-plated flasks.

Maintenance of cell line. NCCS, Pune, provided the HeLa Cell Line (Cervical Cancer Cell Line). The cells were kept at 37ºC in DMEM supplemented with 10% FBS and antibiotics penicillin/streptomycin (0.5 mL^{-1}), in a 5%CO_2/95% air environment.

Preparation of test compound. Each test chemical was weighed individually and dissolved in DMSO for the MTT experiment. The cells were plated at 5.0 x 10^3 cells per well in culture media in a 96-well plate and incubated overnight at 37 °C. The cells were given a series of test drug doses ranging from 5 to 100 µg/ mL.

Procedure. The MTT Assay was used to assess cell viability in three separate triplicate trials with six different chemical doses (5, 10, 25, 50, 75, and 100 µg). Each treatment was withdrawn after 24 hours of incubation, and MTT solution (0.5 mg/mL) was added to each well, followed by 3 hours of incubation at 37 °C. Precipitates were generated after the incubation period as a result of the cells with metabolically active mitochondria reducing the MTT salt to chromophore formazan crystals. On a microplate reader, the optical density of solubilized crystals in DMSO was determined at 560 nm.

RESULTS AND DISCUSSION

The ligands appear to react with metal salts in a 1:2 (M:L) molar ratio to create complexes with the typical compositions $[M(LH)_2]Cl_2$ and $[M(L)_2]$, according to the analytical results. In the presence of a few drops of concentrated HCl, the metal chlorides combine with the ligands to create $[M(LH)_2]Cl_2$. When reactions were carried out in the presence of aqueous NH_4OH, however, complexes of the type $[M(L)_2]$ were formed. The reactions might be written as follows:

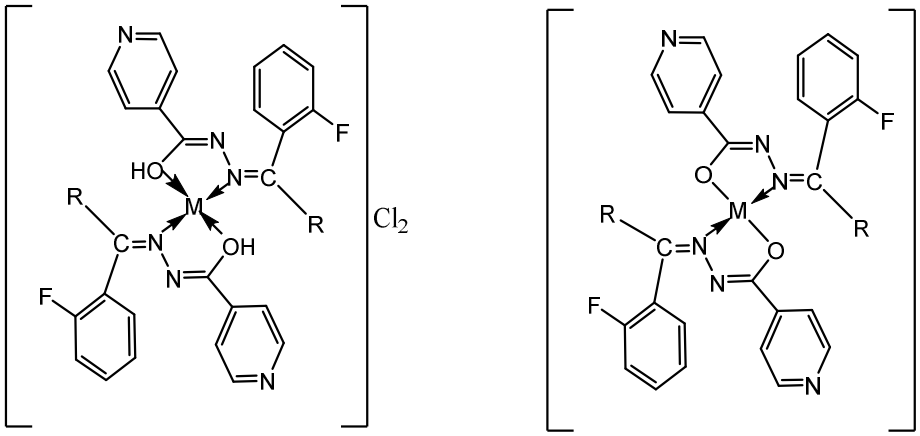
Addition reaction

$$MCl_2+2LH \xrightarrow{HCl} [M(LH)_2]Cl_2$$

Substitution reaction

$$MCl_2+2LH+NH_4OH \longrightarrow [M(L)_2]+2NH_4Cl+2H_2O$$

In these reactions, the metal derivative is obtained as the precipitate, whereas ammonium chloride formed during the reaction remains soluble. The resulting compounds are colored, stable, and insoluble in many organic solvents, although their solubility in DMF and DMSO is noticeable. In dry DMF, the molar conductance values of 10^{-3} mol L^{-1} solutions of $[M(L)_2]$ complexes vary from 19 to 23 Ohm^{-1} cm^2 mol^{-1}, suggesting that they are non-electrolytes. $[M(LH)_2]Cl_2$ complexes, on the other hand, are 1:2 electrolytes with conductance of 210-230 Ohm^{-1} cm^2 mol^{-1}. As predicted for square planar d^8 metal complexes, all of the complexes are diamagnetic. The molecular weight measurements demonstrate that the complexes are monomers. The Proposed structures of the addition and substitution complexes are shown in Figure 2.



2a 2b

Where, M=Pd(II) and Pt(II); R=H(L^1H) and R= CH_3(L^2H)

Figure 2. Proposed structure for addition complexes (**2a**) and Substitution complexes (**2b**).

Spectroscopic characterization

Electronic spectra

The electronic spectra of the ligands (L^1H-L^2H) and their Pt(II) and Pd(II) complexes have been recorded in distilled DMSO to better understand the nature of the M-L bond. Due to three d–d spin permitted transitions, the complexes spectra display three bands. Transitions from the three lower-lying d orbitals to the empty dx^2-y^2orbital are allocated to these. The ground state is ^1Ag, while the excited states corresponding to the aforementioned transitions are ^1A_2g, ^1B_1g, and ^1E_1g in order of increasing energy. Three different orbital parameters Δ_1, Δ_2, and Δ_3 were calculated using a value of $F_2 = 10F_4 = 600$ cm^{-1} for Slater Condon inter electronic repulsion parameter proposed by Gray and Ballhausen[36].Three d–d transition bands appear in the ranges 19,120-22,222, 23,529-24,385 and 26,664-28,984 $cm^{-1}$for Pd(II) and Pt(II) complexes, which may be assigned to $^1A_1g,\rightarrow{}^1A_2g(\nu_1)$, $^1A_1g,\rightarrow{}^1B_1g(\nu_2)$, and $^1A_1g,\rightarrow{}^1E_1g(\nu_3)$ transitions, respectively (Table 1).

The electronic spectra of these complexes reveal their square planar geometry, and the results match those previously reported for square planar complexes [37].

IR spectra

When the IR spectra of metal complexes and ligands are compared, it is clear that the ligands (C=O) at 1665-1675 cm^{-1} and (NH) at 3240-3246 cm^{-1} are not present in the spectra of the corresponding complexes. This is most likely due to amide–imidol tautomerism and the two compounds' subsequent coordination via the imidol oxygen.The presence of a new band in the spectra of the complexes at 1520-1550 cm^{-1}, attributable to the azine group >C=N-N=C, which is missing in the spectra of the ligands, supports this hypothesis. Bands at 1600-1614 cm^{-1} shift to lower wavelengths due to (C=N) of the hydrazones, suggesting azomethine nitrogen coordination. (M-O) and (M-N) have been attributed to non-ligand bands at 415-425 cm^{-1} and 325-456 cm^{-1}, respectively. The pyridine ring nitrogen in both ligands remains unchanged on complexation,

indicating that the ring nitrogen is not involved in complex formation. The overall IR spectral evidence suggests that both ligands are bidentate [38, 39], coordinating through amide oxygen and azomethine nitrogen to form a five-membered chelate ring. The IR spectrum data of the ligands and their metal complexes are shown in Table 2.

Table 1. Electronic spectral data (cm^{-1}) of the palladium(II) and platinum(II) complexes.

Complexes	Transition	Spectral bands (cm^{-1})	Δ_1 cm^{-1}	Δ_2 cm^{-1}	Δ_3 cm^{-1}	v_2/v_1
$[Pd(L^1H)_2]Cl_2$	$^1A_{1g}\rightarrow{}^1A_{2g}(\nu_1)$	22,222	24322	3363	1979	1.09
	$^1A_{1g}\rightarrow{}^1B_{1g}(\nu_2)$	24,385				
	$^1A_{1g}\rightarrow{}^1E_{1g}(\nu_3)$	26,664				
$[Pd(L^2)_2]$	$^1A_{1g}\rightarrow{}^1A_{2g}(\nu_1)$	21,596	23696	3525	2659	1.10
	$^1A_{1g}\rightarrow{}^1B_{1g}(\nu_2)$	23,921				
	$^1A_{1g}\rightarrow{}^1E_{1g}(\nu_3)$	26,880				
$[Pt(L^1H)_2]Cl_2$	$^1A_{1g}\rightarrow{}^1A_{2g}(\nu_1)$	19,120	21220	5609	4901	1.23
	$^1A_{1g}\rightarrow{}^1B_{1g}(\nu_2)$	23,529				
	$^1A_{1g}\rightarrow{}^1E_{1g}(\nu_3)$	28,730				
$[Pt(L^2)_2]$	$^1A_{1g}\rightarrow{}^1A_{2g}(\nu_1)$	19,230	21330	5778	4876	1.24
	$^1A_{1g}\rightarrow{}^1B_{1g}(\nu_2)$	23,808				
	$^1A_{1g}\rightarrow{}^1E_{1g}(\nu_3)$	28,984				

Table 2. IR (cm^{-1}) spectral data of the ligands and their metal complexes.

Compound	ν(NH)	ν(C=O)/ν(C-O)	ν(C=N)	>C=N-N=C<	ν(M─O)/ν(M-N)
L^1H	3240	1665	1600	-----	----
L^2H	3246	1675	1612	-----	----
$[Pd(L^1H)_2]Cl_2$	-----	1290	1590	1520	415
$[Pd(L^1)_2]$	-----	1293	1585	1526	418
$[Pd(L^2H)_2]Cl_2$	-----	1320	1595	1537	422
$[Pd(L^2)_2]$	-----	1322	1588	1540	425
$[Pt(L^1H)_2]Cl_2$	-----	1298	1592	1542	423
$[Pt(L^1)_2]$	-----	1300	1589	1546	424
$[Pt(L^2H)]Cl_2$	-----	1309	1597	1548	423
$[Pt(L^2)_2]$	-----	1312	1594	1550	428

^1H NMR spectra

In DMSO-d_6 using TMS as an internal standard, the proton NMR spectrum data of the ligands and their metal complexes were collected. When the ^1H NMR spectra of metal complexes and their ligands are compared, the –NH proton signal disappears at 12.0-13.0 ppm in the complexes,

indicating that the ligand is enolized during complexation. A singlet at 8.88-9.07 ppm may be assigned to α-protons of the pyridine ring. The multiplet at 8.03-8.87 ppm may be assigned to β-protons of the pyridine ring and protons of the aromatic ring. A sharp singlet at 4.31-4.35 ppm due to the –OH proton of the addition complexes and another sharp singlet at 2.45-2.46 ppm and 0.9-1.3 ppm are due to the methyl protons and protons respectively which are attached to azomethine of the ligand and these undergo a downfield shift due to the coordination of the azomethine nitrogen in the complexes.

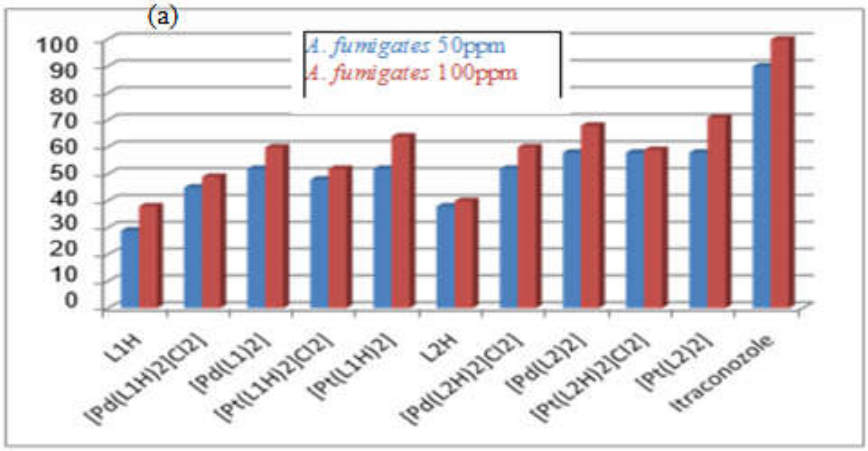
Biological results

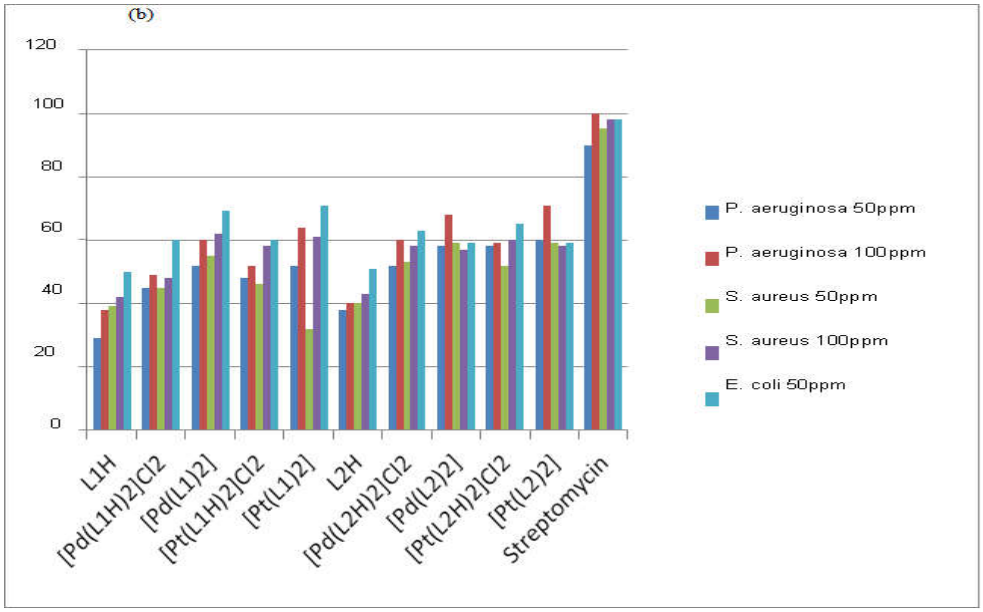
Antimicrobial assay

The antimicrobial screening results demonstrate that both the ligands and their complexes independently inhibited the growth of the tested bacterial and fungal species to various degrees. The activity of the metal complexes against microbial strains is much higher than that of the ligands, as shown in Graph 1, suggesting that metal chelates are more active than their respective ligands. Overtone's notion and Tweedy's chelation theory [40] can explain the higher activity of the produced complexes relative to the activity of the ligands. A lipid membrane enclosing the cell, according to Overtone's theory, allows the transit of molecules that dissolve in lipids. As a result, liposolubility is a critical determinant in determining antimicrobial efficacy. Due to ligand orbital overlaps and a partial sharing of the positive charge of the metal ion with the charge of the donor groups, the polarity of the metal ion is lowered to a greater extent during chelation. Furthermore, the delocalization ofelectrons across the whole chelate ring is increased, and the complexes' lipophilicity is improved. The complexes may easily cross a lipid membrane and inhibit metal binding sites in microorganism enzymes due to their enhanced lipophilicity. With increasing concentration, the antifungal and antibacterial properties rise. As a result, concentration is important in increasing the degree of inhibition. Inhibition is less severe at lower concentrations because the organisms' activities are hindered, however, at greater concentrations, more enzymes are stopped, resulting in a faster death of the organisms.The results of antibacterial and antifungal activity have been given in Table 3.

Table 3. Antibacterial activity and antifungal activity of the ligands and their metal complexes.

Compound	*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Eschericha coli*	*Aspergillus fumigatus*	
	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	50 ppm	100 ppm
L^1H	30±0.16	38±0.18	39±0.08	42±0.06	52±0.11	29±0.02	38±0.21
$[Pd(L^1H)_2]Cl_2$	45±0.06	50±0.09	45±0.14	48±0.12	60±0.08	44±0.13	48±0.06
$[Pd(L^1)_2]$	52±0.20	60±0.23	57±0.17	62±0.07	70±0.22	50±0.08	60±0.18
$[Pd(L^2H)_2]Cl_2$	48±0.07	52±0.06	46±0.05	58±0.27	60±0.24	48±0.22	50±0.11
$[Pd(L^2)_2]$	52±0.13	64±0.15	32±0.09	61±0.11	72±0.09	50±0.07	62±0.08
L^2H	38±0.06	40±0.08	40±0.11	43±0.14	52±0.11	37±0.33	39±0.28
$[Pt(L^1H)_2]Cl_2$	54±0.09	60±0.12	55±0.23	58±0.25	63±0.21	50±0.26	59±0.17
$[Pt(L^1)_2]$	58±0.24	70±0.25	59±0.18	57±0.16	59±0.20	57±0.08	67±0.09
$[Pt(L^2H)]Cl_2$	58±0.24	59±0.21	54±0.06	60±0.10	65±0.08	58±0.15	59±0.13
$[Pt(L^2)_2]$	60±0.11	77±0.14	59±0.10	58±0.08	59±0.14	57±0.18	70±0.22
Streptomycin	90	100	96	98	98	-	-
Itraconazole	-	-	-	-	-	79	89





Graph 1. Antifungal (a) and antibacterial (b) screening data for the ligands (L^1H and L^2H) and their palladium(II) and platinum(II) complexes [diameter of inhibition zone in mm (concentration in ppm)].

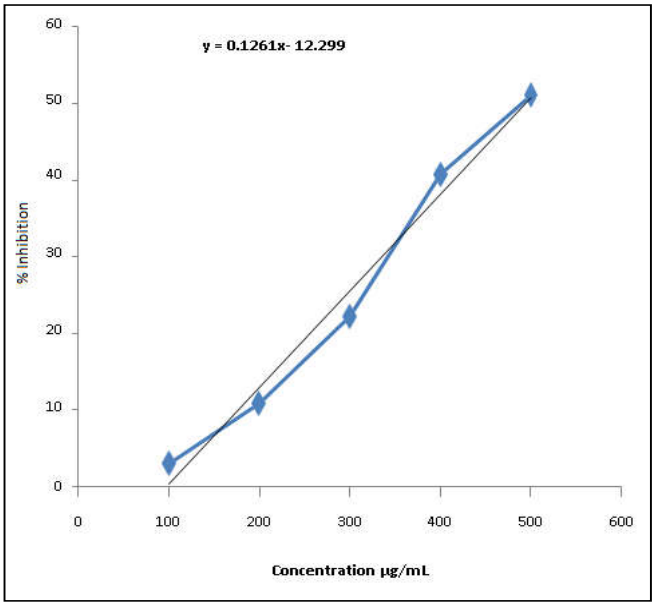
Antioxidant activity

Different concentrations of the sample were tested to check the dose-dependent response. It was found that the concentration plays an important role in DPPH % inhibition as the scavenging activity of the test compound against DPPH was dosage dependent. The test compound with the highest DPPH % inhibition was found at a 500 µg/mL concentration. The test compound's DPPH

antioxidant activity was measured using an IC_{50} value. The number of antioxidants necessary to reduce the initial DPPH concentration by 50% was recorded as the IC_{50} (half maximal inhibitory concentration). Table 4 shows the IC_{50} value of the test chemical, which was determined to be 493.48 µg/mL, and the IC_{50} value of the positive control, which was calculated to be 113.21 µg/mL (vitamin C). As shown in Graph 2, the percentage inhibition of DPPH was plotted against the concentration of the test drug.

Table 4. Percentage inhibition and IC_{50} value of $[Pt(L^1H)_2]\ Cl_2$ and positive control(vitamin C).

Concentration	Test compound		Positive control	
	% Inhibition	IC_{50}	% Inhibition	IC_{50}
100 µg/mL	2.93	493.48 µg/mL	51.21	113.21 µg/mL
200 µg/mL	10.81		55.85	
300 µg/mL	22.15		71.38	
400 µg/mL	40.74		82.86	
500 µg/mL	51.01		94.19	



Graph 2. DPPH Scavenging activity of $[Pt(L^1H)_2]Cl_2$.

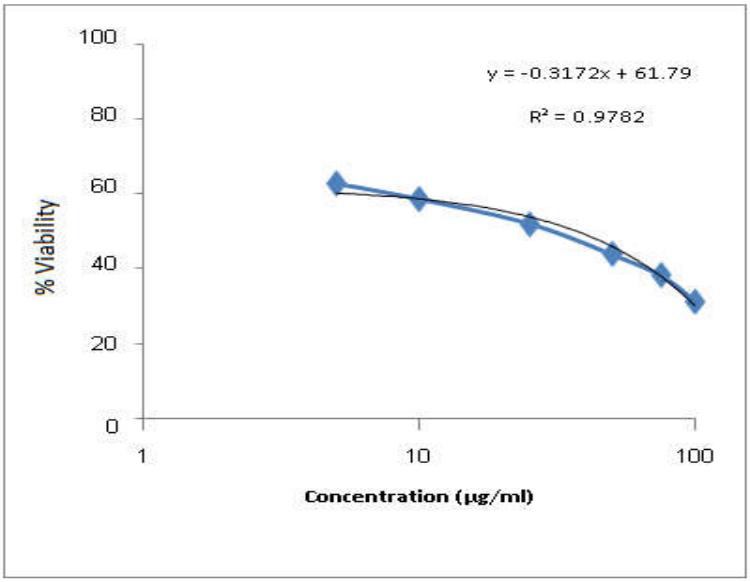
Anti-cancer activity

Cellular viability assays are ubiquitously used to assess the effect of cytotoxic agents.Cell viability is the number of healthy cells in a sample. Cell viability assay can determine the effect of drug candidates on cells and be used to optimize the cell culture conditions.MTT assay, which is very sensitive and very applicable to measure cytotoxicity (loss of viable cells). MTT test was used to assess the effects of $[Pt(L^2H)]Cl_2$ compound on the viability of the HeLa cell line (Cervical Cancer Cell line). For 24 hours, the cells were exposed to chemicals at increasing concentrations. The researchers discovered that raising the concentration of the new platinum(II) complex lowers cell viability, resulting in increased cytotoxicity of the test compound. It is often feasible to kill cells

much faster by using a higher concentration of drugs. Higher drug concentrations may kill cells. An increase in cell death is the desired goal. The IC_{50}value for $[Pt(L^2H)]Cl_2$ in cervical cancer cells was 37.192 µg/mL, as shown in Table 5. The cytotoxic impact of $[Pt(L^2H)]Cl_2$ complex on the HeLa cell line is shown in Graph 3.The findings demonstrate that complexes can slow tumor development and might be examined further as anticancer agents [41].

Table 5. Cytotoxic properties of $[Pt(L^2H)]Cl_2$ complex against HeLa cell line.

Concentration (µg/mL)	Absorbance at 570 nm			Average	Average-Blank	% Viability	IC_{50} (µg/mL)
100	0.405	0.407	0.408	0.406	0.398	31.338	37.192
75	0.492	0.493	0.495	0.493	0.485	38.189	
50	0.563	0.564	0.566	0.564	0.556	43.779	
25	0.668	0.67	0.671	0.669	0.661	52.047	
10	0.751	0.752	0.754	0.752	0.744	58.582	
5	0.804	0.805	0.807	0.805	0.797	62.755	
Untreated	1.278	1.279	1.278	1.278	1.270	100	
Blank	0.008	0.009	0.008	0.008	0		



Graph 3. Cytotoxic effect of $[Pt(L^2H)]Cl_2$ complex on HeLa cell line.

CONCLUSION

We have successfully synthesized and characterized a novel class of Pd(II) and Pt(II) Schiff base complexes. Based on analytical and spectral data a square planar geometry has been proposed for these complexes.The complexes exhibited significant antimicrobial activity, higher than the parent ligands when screened against *Aspergillus fumigates*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* microbial species. The studied $[Pt(L^1H)_2]Cl_2$ complex demonstrates dose-dependent DPPH scavenging activity. The *in vitro* cytotoxic activity of

$[Pt(L^2H)]Cl_2$ complex was assessed by screening their ability to inhibit cancer growth in the human cervical cancer cell line (HeLa). The results are indeed positive.

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