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RESEARCH PAPER

Synthesis, Spectroscopic Characterization and Biological Studies as an Anticancer of a Novel Schiff Base Ligand (LH) and Its Palladium (II) Complex Derived from Thiazole and Salicylaldehyde

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ABSTRACT

A distinct approach in this study was the synthesis of a novel nano heterocyclic Schiff base ligand (LH) by the reaction of 1, 2-diphenyl-2-(thiazol-2-ylimino) ethan-1-one with 1-(4-((2-hydroxybenzylidene) amino) phenyl) ethenone and 4,4'-methylene dianiline. A chelate complex is formed when Schiff base ligand (LH) reacts with palladium (Pd (II)) ions. Synthesized ligand and its complex were characterized by different characterization techniques including UV-Visible, Fourier-transform infrared (FTIR) spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR), molar conductivity, melting point, atomic absorption, magnetic susceptibility, CHNS elemental analysis, Field emission scanning electron microscopy (FESEM) and X-ray diffraction (XRD). Results of the characterization study suggested that the Pd (II) complex have a square-planar geometry with metal to ligand ratio of 1:1. For exploring the potential of the synthesized complex against a breast cancer cell line (MCF-7) and normal HEK cell line (for comparison study), the *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT)* cytotoxicity assay was employed for evaluating the anticancer activities of the synthesized materials. Overall, the results of the study revealed that synthesized novel nano Schiff base ligand (LH) and its Pd (II) complex possess the remarkable anticancer properties and can be used as an anti-cancer drug in the field of medicine.

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INTRODUCTION

Cancer and degenerative diseases pose serious threats to human health and is mainly caused by free radicals. These highly reactive and unstable free radicals are generated at the time when oxygen is used by the cells in energy yielding process. Low concentration of these radicals is beneficial for the body, however, when their concentration increases to the higher level, they are responsible for causing cancer, cardiovascular, and neurodegenerative diseases by inducing oxidative stress (OS) in the body. This leads to the development of some

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agents called as antioxidants for the elimination of these free radicals from the human body [1]. In medicine field, uses of transition metals and their complexes is gaining popularity since the discovery of cisplatin by Rosenberg in 1960 [2] for the treatment of different types of tumors. However, it is observed that with the passage of time, cancerous cells develop resistance against cisplatin that limit its uses for a prolonged time [3]. Keeping in view the magnitude of ever-growing anticancer resistance, production of novel Schiff base and its transition metal based chelating compounds having resistance improved medicinal profile, particularly for cancer treatment, should be the ultimate aim.

Schiff bases mainly comprised of azomethine linkage and are obtained through the condensation reaction between carbonyl compounds (aldehydes or ketones) with primary aliphatic/ aromatic/ heteroaromatic amines [4]. The general formula for these compounds is C=NR'R"R, where R' and R" represent an aromatic or aliphatic group, or a hydrogen atom. When R is a substituted or unsubstituted benzene ring, these Schiff bases are referred to as Aniles or Benzaniles [5]. Schiff bases find wide range of applications for organic synthesis and catalysis [6], antitumor [7, 8] anticancer [9, 10], antibacterial [11-14], antifungal [13, 15], cytotoxic activity [16], antiviral [17] and others [18-20]. Schiff base ligands draw's researcher's attentions due to their easy synthesis [6] and are used to synthesize the complexes based on the transition metal. Chelating complex compounds produced from the Schiff bases and transition metals find wide range of applications as insecticides, pesticides, bactericides and fungicides [4]. Presence of some heteroatoms i.e., nitrogen (N), Sulphur (S), oxygen (O) etc. in Schiff base ligands results in improving the properties of the their metal complexes making them effective for uses as antitumor [21, 22], antioxidant [23], antibacterial [24], antifungal [25], anticancer [26], antiviral [27], anti-inflammatory [28] and anti-HIV [29]. This study mainly deals with the synthesis of the novel Schiff base ligand that was mainly derived from thiazole and salicylaldehyde.

Thiazole, a heterocyclic compound featuring sulfur and nitrogen at the 1,3-positions, is significant and versatility in various chemical applications as a constituent of Vitamin B1, as an antiviral, anticancer, antimicrobial, antidiabetic, antihypertensive, and anti-inflammatory agents. Thiazole was first synthesized by Hantzsch, a method that remains the most commonly used for the preparation of thiazole and its derivatives. Established in 1887, this method involves the reaction of alpha-halocarbonyl compounds with thiourea or thioamides in the presence of bromine/iodine and silicon chloride, or utilizing various homogeneous and heterogeneous catalysts [30, 31].

Owing to the distinctive antitumor activities, the palladium (Pd (II)) chelate complex compounds are widely used in medicinal chemistry [1] for cancer treatment of adrenocortical, breast colon, head/neck, ovarian and melanoma [32-34]. In view of the diversified significance of Pd (II) complexes in medicine and biological field, primary intention of the study is to synthesize a novel heterocyclic Schiff base ligand (LH) and its palladium (Pd (II)) chelate complex based anticancer drug in the hope of treating cancer cells particularly breast cancer cells with greater efficiency. Within the frameworks presented, the objectives of this research are: (*i*) synthesize a novel heterocyclic Schiff base ligand (LH), by the reaction of 1, 2-diphenyl-2-(thiazol-2-ylimino) ethan-1-one with 1-(4-((2-hydroxybenzylidene) amino) phenyl) ethenone, and its palladium (Pd (II)) chelate complex. (*ii*) characterize the synthesized novel ligand and its complex by different techniques including UV-Visible, FTIR, ^{1}H-NMR, molar conductivity, melting point, atomic absorption, magnetic susceptibility, elemental analysis (CHNS), FESEM and additionally by XRD and (*iii*) explore the biological applications of the synthesized novel Pd (II) complex with a focus on its anticancer activity against breast cancer cells (MCF-7) by comparing the results of the treatment with normal HEK cell line.

MATERIALS AND METHODS

Reagents and instruments

The chemical reagents used in this study were of analytical grade and used as procured from Al-Drich, Merck, HIMEDIA, and BDH without any additional purification. UV –Vis. measurements were performed using a Shimadzu U.V-165PCS Spectrophotometer within the range of 200-1000 nm. FTIR spectra was recorded with a Shimadzu FTIR 8400S Spectrophotometer within the range of 400-4000 cm^{-1}. NMR spectra was obtained on a Varian Fourier transform spectrometer operating at 300 MHz, using tetramethylsilane (TMS) as an

internal standard in DMSO-d_6 solvent. Further, melting points of all compounds were determined using a Stuart melting point apparatus while a balanced magnetic susceptibility meter, model MSB-MKI was used for the measurement of the magnetic susceptibility at room temperature. Flame atomic absorption spectroscopy was performed using a Shimadzu AA-6300 spectrometer for determining the metal content in the synthesized complex. Using the EURO 2012EA 300 CHN Element analysis device, the element ratios of carbon, hydrogen, nitrogen, and sulfur (C.H.N.S) of the prepared ligand and its complex are determined. Field emission scanning electron microscopy (FESEM) analysis was performed for all prepared compounds using a ZEISS EM 3200

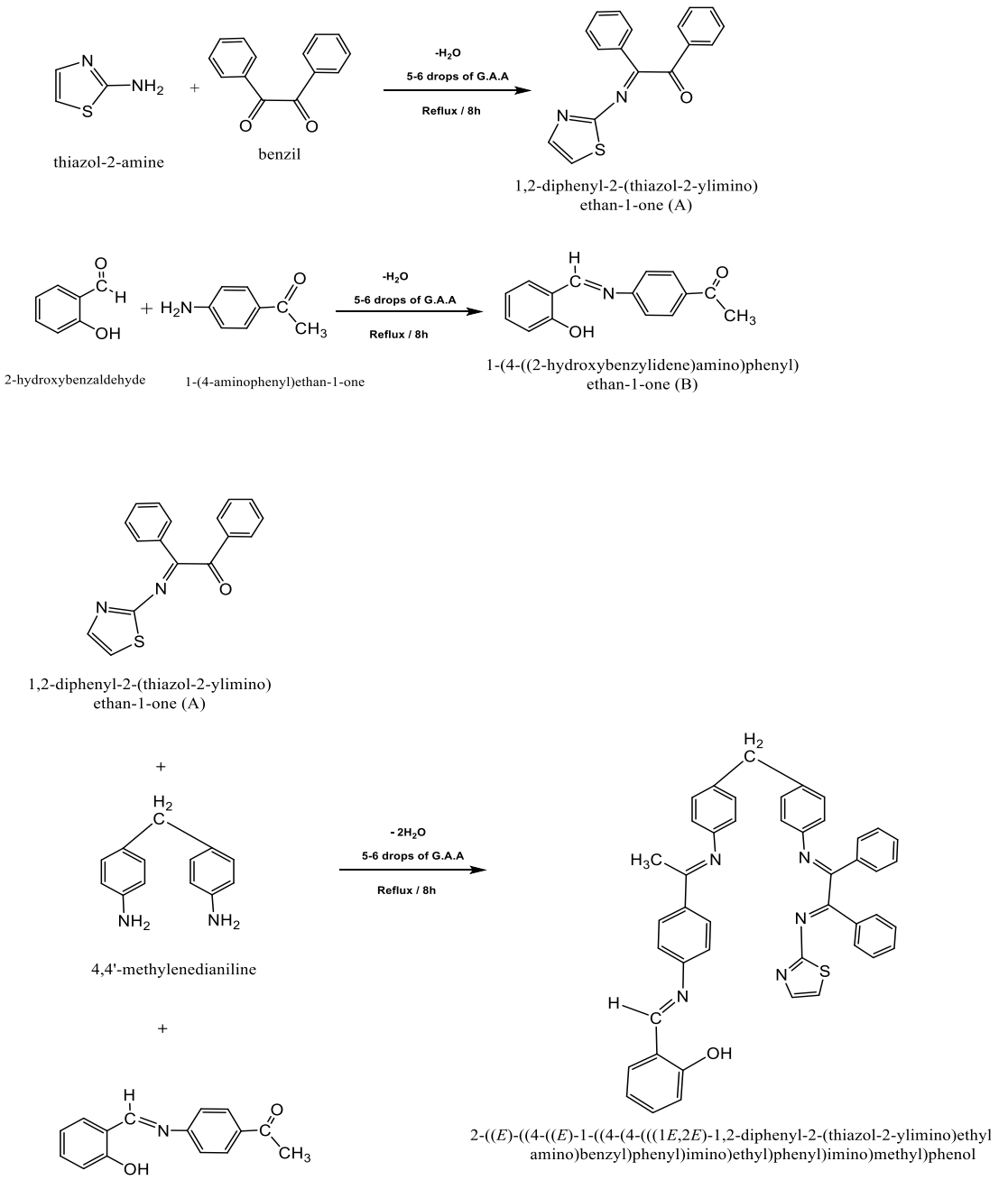


Fig. 1. Scheme for the synthesis of the novel nano Schiff base ligand (LH) derived from thiazole and salicylaldehyde.

device , while the device (Bestic Aluminum anode) was used to measure X-ray diffraction (XRD) of the prepared compounds with an angular range of 2θ (20-80°).

Synthesis Procedure

Synthesis of the heterocyclic nano Schiff base ligand (LH)

Synthesis of a novel nano tridentate Schiff base ligand (LH) can be carried out in three steps as summarized below:

Step 1: Synthesis of Compound A, 1,2-diphenyl-2-(thiazol-2-ylimino) ethan-1-one

For the synthesis of compound A, initially two different solutions were prepared by dissolving accurately weighed amount of 1.00 g (0.01 mol) of 2-aminothiazole and 2.1 g (0.01 mol) benzil in 25 mL of absolute ethanol separately. This was followed by the addition of 5-6 drops of glacial acetic acid to the benzil solution. The solutions were then mixed and refluxed for 8h followed by cooling, filtration and drying to yield precipitate. The resulting precipitates were then recrystallized from hot ethanol followed by filtration and drying. The resulting compound was the compound A i.e., 1,2-diphenyl-2-(thiazol-2-ylimino) ethan-1-one and its melting point and yield percentage was determined.

Step 2: Synthesis of Compound B, (E)-1-(4-((2-hydroxybenzylidene) amino) phenylethan-1-one

For the synthesis of compound B, two different solutions were prepared by dissolving accurately weighed amount of 1.22 g (0.01 mol) of salicylaldehyde and 1.35 g (0.01 mol) of 4-aminoacetophenone in 25 mL of absolute ethanol separately. These solutions were then processed according to the same method as described in step 1. The resulting compound was the compound B i.e., (E)-1-(4-((2-hydroxybenzylidene) amino) phenylethan-1-one and its melting point and yield percentage was determined.

Step 3: Synthesis of nano ligand (LH)

The novel nano Schiff base ligand (LH) was prepared by dissolving 2.92 g (0.01 mol) of Compound A and 1.98 g (0.01 mol) of 4,4'-methylene dianiline, each in 25 mL of absolute ethanol, along with a solution of 2.39 g (0.01 mol) of Compound B in 25 mL ethanol. To each solution, five to six drops of glacial acetic acid were added. The solutions were then mixed thoroughly, refluxed for 8h followed by cooling to form precipitate. These precipitates were then filtered, dried, and recrystallized from absolute ethanol to yield a nano Schiff base ligand (LH) having a melting point of 100-102°C and a yield of 79.85%. Synthesis of novel nano Schiff base

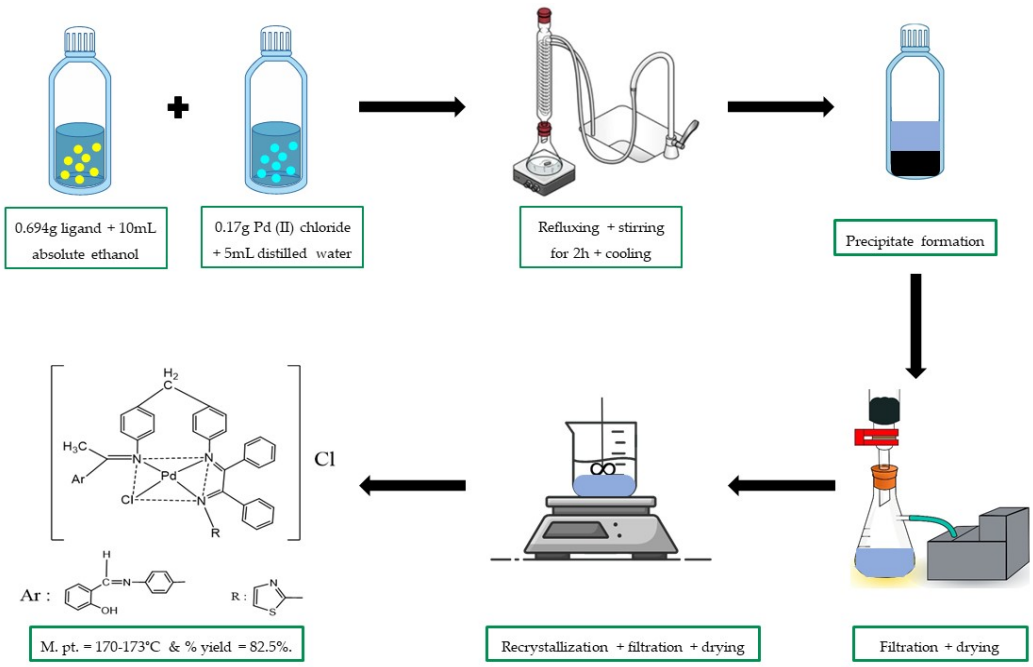


Fig. 2. Schematic illustration for the synthesis of Pd (II) complex of novel nano Schiff base ligand (LH).

ligand (LH) thiazole and salicylaldehyde has been summarized in Fig. 1.

Synthesis of the Complex

For the synthesis of Pd (II) complex of novel nano Schiff base ligand (LH), 0.694 g (1 mmol) of the nano ligand (LH) was dissolved in 10 mL of absolute ethanol. This solution then mixed with palladium (II) chloride solution (prepared by dissolving 0.17 g (1 mmol) of Pd (II) chloride in 5 mL water. Resulting mixture was thereafter refluxed with continuous stirring for 2h followed by cooling. The precipitate thus formed was filtered, dried followed by recrystallization from absolute ethanol for yielding a colored and pure precipitate as shown in Fig. 2. The resulting product exhibits the melting point and a yield of 170-173°C 82.5% respectively.

Biological activity analysis

Cell Toxicity Tests: Cell lines and cultivation of MCF-7 breast cancer cell line

For conducting biological activity analysis, the MCF-7 breast cancer cell line and a normal cell line was obtained from the Pasteur Institute, University of Tehran, Iran. The maintenance and development of these cancer cell lines, along with necessary tests (wherever applicable), were conducted at the aforementioned university. For the growth of the MCF-7 breast cancer cells, the Freshney method was used. In this method, cells were dissolved in water at a temperature of 37°C which was then put in a 25 cm^2 animal cell culture vessel with Dulbecco's Modified Eagle Medium (DMEM) media and 10% bovine calf serum. These vessels were then incubated in 5% CO_2 at 37°C for one day. For investigating cell viability and contamination, an inverted microscope was used and the cell count was adjusted to 500–800 thousand cells/ mL. These cells were placed in a growth cabinet and the media was discarded. This is followed by washing of cells twice with Phosphate buffered saline (PBS) for 10 minutes. The monolayer cells were treated with trypsin and incubated at 37°C for 30-60 seconds to obtain individual cells. It is observed that the enzyme activity stopped when fresh bovine calf serum medium was added. For the removal of the trypsin and media, the cells were centrifuged at a speed of 2000 rpm for 10 minutes at room temperature. Fresh media with 10% bovine calf serum was added to the cells after discarding the supernatant. For counting the cells, determining the cell viability and % cell viability, Hemocytometer slide was employed and calculations are done with the help of expressions 1-2 correspondingly.

$$C = N \times 10^4 \times F\ /ml \quad (1)$$

$$Viability\ percentage = (live\ cells\ /\ dead\ cells) \times 100 \quad (2)$$

Where cell count per mL, dilution factor and the cells number on the slide are represented by C, F and N respectively. Whereas, the term 10^4 signifies the dimensions of the slide used. The prepared cell suspension was then distributed into new vessels and incubated in a 5% CO_2 incubator at 37°C for one day.

MTT dye test for cell viability

The cytotoxic activity of the nano Schiff base ligand (LH) and its palladium complex against MCF-7 breast cancer cells and normal HEK cell line was determined for comparison analysis and to evaluate their potential as anticancer drugs. For 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT*)* cytotoxicity assay, the cancer cell lines were prepared and the cell suspension was placed in a 96-well plate with a flat base and incubated in a 5% CO_2 incubator at 37°C for one day. Afterwards, a 100 µL of the cell suspension added to each well, afterwards addition of the prepared concentrations of the compounds under study (50, 100, 200,400,800 and 1600 µg/ml) into the wells (for each concentration, the number of wells is three). The plate was

Table 1. Some physico-chemical properties of the prepared novel ligand (LH) and Pd (II) complex.

Compound	M. wt. g/mol	Color	MP (°C)	Yield %	Calc. (Found)%				
					C	H	N	S	M
Ligand, $C_{45}H_{35}N_5OS$	693.87	yellow	100-102	79.85	77.90 (78.81)	5.08 (5.19)	10.09 (10.31)	4.62 (4.69)	-
[Pd(LH)Cl]Cl	871.19	brown	170-173	82.5	62.04 (62.97)	4.05 (4.14)	8.04 (8.23)	3.68 (3.63)	12.22 (12.46)

incubated again for another next one day at 37°C. Afterwards, 10 mL of MTT solution (at 0.5 mg/mL) was added to each well and incubated for 4 hours at 37°C. A 100 µL of Solubilization Solution was added for dissolving the Formazan crystals. The absorbance of the samples was measured at 570 nm using a DNM-9602G plate reader. Cell viability was then presented as a percentage of the viability of treated cells in comparison to its to untreated cell counterparts.

RESULTS AND DISCUSSION

Physio-chemical characteristics of the nano ligand (LH) and its Pd (II) complex

Newly prepared nano Schiff base ligand (LH) was yellow crystal, however, its color changes on the reaction with metal ion i.e., palladium ion. Its Pd (II) complex was stable at room temperature and is brown in color. Some other key features of the prepared nano ligand and its complex have been summarized in Table 1.

NMR Spectrum of the novel nano Schiff base ligand (LH)

The ^{1}H-NMR spectrum of the newly prepared nano Schiff base ligand (LH) was identified with the help of DMSO-d_6 as the solvent and TMS as the internal standard at laboratory temperature. The spectrum showed a singlet at 2.264 ppm (3H, S) that mainly assigned to the protons of the CH_3 group [32]. A singlet at 3.792 ppm (2H, S) correspond to the methylene group protons [35]. Further, multiple signals were detected within the range of 7.215-7.272 ppm (4H, m) and 7.318-8.161 ppm (4H, m) that are attributable to the phenyl ring protons connected to the methylene group and to the phenyl ring attached to the azomethine group [36], respectively. Additionally, the multiple signals of phenyl rings in the benzil compound (10H, m, 7.566 -8.317 ppm) [37], thiazole ring protons (4H, m, 7.521-7.929 ppm) [38], and salicylaldehyde ring protons (4H, m, 6.942-7.659 ppm) were also observed [35]. Proton group (C=NH) showed a clear signal at (H, s, 8.947ppm) [39]. As for the hydroxyl group, it gave a signal at (1H, s, 11.12 ppm) [35].

The ^{1}H-NMR spectrum of the nano ligand (LH) is shown in Fig. 3.

Functional group study of the nano ligand (LH) and its Pd (II) complex

FTIR analysis of both the newly prepared nano Schiff base ligand (LH) and its Pd (II) complex was carried out with 16 scans per sample revealing the presence of several functionalities. FTIR spectra of the free nano ligand (LH) (Fig. 4a) showed bands

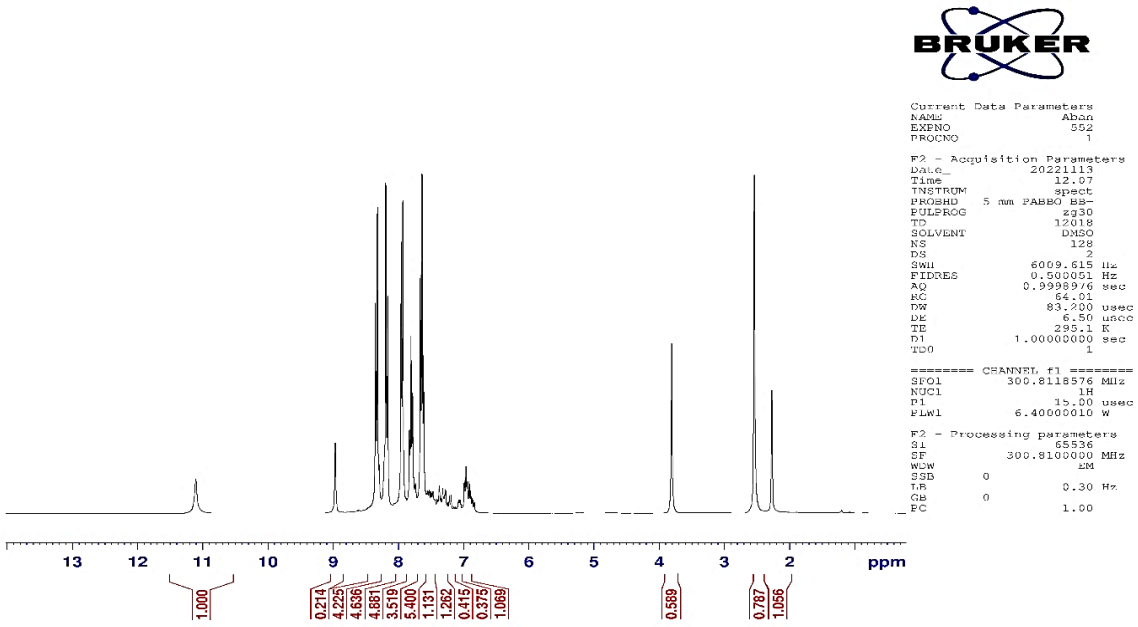


Fig. 3. The ^{1}H-NMR spectrum of the newly prepared Schiff base ligand (LH).

at 3417 cm^{-1} and 3062 cm^{-1} that were mainly assigned to the and aromatic groups, respectively. Vibrations of the aliphatic group showed peaks at nearly 2923 cm^{-1} and 2854 cm^{-1}, while a prominent new band at 1674 cm^{-1} indicates presence of azomethine group formation in the prepared nano ligand. Bands appeared at 1596 cm^{-1} and 1519 cm^{-1}, 1442 cm^{-1} attributed to the azomethine in the thiazole ring and aromatic groups [38, 39], respectively. Additionally, bands at 1026 cm^{-1}, 1172 cm^{-1} and 1211 cm^{-1} reveals the vibrations of the and groups respectively [40, 41]. Comparing the FTIR spectrum of the free nano ligand (Fig. 4a) with its complex (Fig. 4b), it is observed that there exist some variations in the intensity and position of some bands along with the appearance of some new bands thus confirming the coordination between the metal and the nano ligand [38]. There was shifting of the band associated with the azomethine group from a higher frequency i.e., 1674 cm^{-1} to a lower frequency at 1662 cm^{-1} in the complex spectrum. This confirms the involvement of azomethine group in coordination of metal with nano ligand [42]. Additionally, no significant variation in the bands associated with the hydroxyl and azomethine bands in the thiazole ring was observed revealing non contribution of these functionalities in coordination. When it comes to the new bands, it is revealed that the vibrations of group appear at 586 cm^{-1}. This functional group is an important indicator of nitrogen coordination between prepared nano ligand and metal to form metal complex of nano ligand [38]. Some key functionalities of the nano ligand (LH) and its Pd (II) complex along with their respective wavenumbers on FTIR spectra have been summarized in Table 2.

Electronic spectra and molar conductivity measurements of the newly prepared nano ligand (LH) and its Pd (II) complex

Electronic spectra analysis of the prepared nano ligand (LH) exhibited two absorption peaks at 204 nm (49020 cm^{-1}) and 260 nm (38462 cm^{-1}). The first peak at 204 nm was assigned to the π-π* transition, while the second peak corresponds to the n-π* transition of the azomethine (C=N) group [43, 44] as shown in Fig. 5a. The electronic spectrum of the Pd (II) complex shown in Fig. 5b exhibiting the peaks at 212 nm (47170 cm^{-1}) and 289 nm (34602 cm^{-1}), These peaks reveal the intra-ligand transitions in the complex. Further, absorption peaks observed at 439 nm (22779 cm^{-1}), 498 nm (34602 cm^{-1}), and 558 nm (17921 cm^{-1}) in complex correspond to the electronic transitions i.e., $^1A_{1g} \rightarrow {}^1E_g$, $^1A_{1g} \rightarrow {}^1B_{1g}$, and $^1A_{1g} \rightarrow {}^1A^{2g}$ respectively [42]. all the data informing Fig. 5 (a,b)

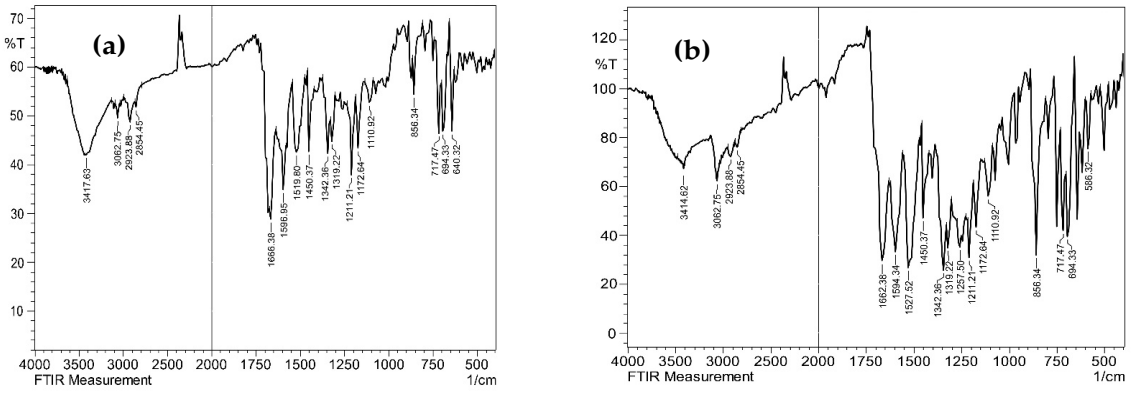


Fig. 4. The FTIR spectra of prepared nano ligand (LH) (a) and its Pd (II) complex (b) 400-4000 cm^{-1}.

Table 2. Infrared absorption bands of prepared nano ligand (LH) and its Pd (II) complex.

	υ (cm^{-1})						
Compound	O-H	C-H, aromatic	C-H, aliphatic	C=N, Imine	C=N, Thiazole ring	C=C, aromatic ring	M-N
Ligand (LH)	3417	3062	2923 2854	1674	1596	1519 1442	-
Pd (II) complex	3414	3062	2923 2854	1662	1594	1527 1450	586

data has been summarized in Table 3. Results of the magnetic sensitivity suggested that the complex demonstrated near-zero magnetic moment due to the pairing of all electrons in d orbitals. This confirms the diamagnetic properties of the complex and suggesting a square planar geometry of the complex with dsp^2 hybridization [45] as shown in Fig. 6. For the molar conductivity measurements, solvent used was the absolute ethanol having the concentration of 10^{-3} M at room temperature. The synthesized complex showed a molar conductivity value of 43.5 $\Omega^{-1}cm^2mol^{-1}$, suggesting the ionic characteristics with ratio a 1:1 [46].

Morphological and elemental analysis of the prepared nano ligand (LH) and its Pd (II) complex

Field emission scanning electron microscopy (FESEM) analysis provided insights into the surface morphology, particle size, shape, and crystalline structure [47] of both the newly prepared nano ligand (LH) and its Pd (II) complex. FESEM results revealed the spherical and the heterogeneous nature of the newly prepared nano ligand (LH), as shown in Fig. 7a, with an average particle size of the 94.3 nm [48]. However, FESEM results of the Pd (II) complex showed grainy and spherical nature of the surface (Fig. 7b) with an average particle size of 87.9 nm [49].

For determination of the elemental composition or the ratios of carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) in the prepared nano ligand (LH) and its Pd (II) complex, elemental microanalysis was conducted. Results of the study revealed the chemical formulae of both the newly prepared nano ligand (LH) and its Pd (II) complex. The empirical results of study show a close correlation with the theoretical calculations, validating the composition and proposed structures of ligand (LH) and its Pd (II) complex as shown in Fig. 8a-b respectively.

Crystallography analysis of the newly prepared nano ligand (LH) and its Pd (II) complex

X-ray diffraction (XRD) studies of crystalline structures of both the newly prepared nano ligand

Table 3. UV-Vis absorption peaks, magnetic momentum μeff (B.M) and predicted geometries for the prepared nano ligand (LH) and its Pd (II) complex.

Compounds	λ (nm)	υ (cm^{-1})	Transitions	μeff (B.M)	Geometry
Ligand (LH)	204	49020	π-π*	–	–
	260	38462	n-π*		
Pd (II) complex	212	47170	Intra-ligand	(Dia.)	Square planar dsp^2
	289	34602	Intra-ligand		
	439	22779	$^1A_{1g} \rightarrow {}^1Eg$		
	498	20080	$^1A_{1g} \rightarrow {}^1B_1g$		
	558	17921	$^1A_1g \rightarrow {}^1A_2g$		

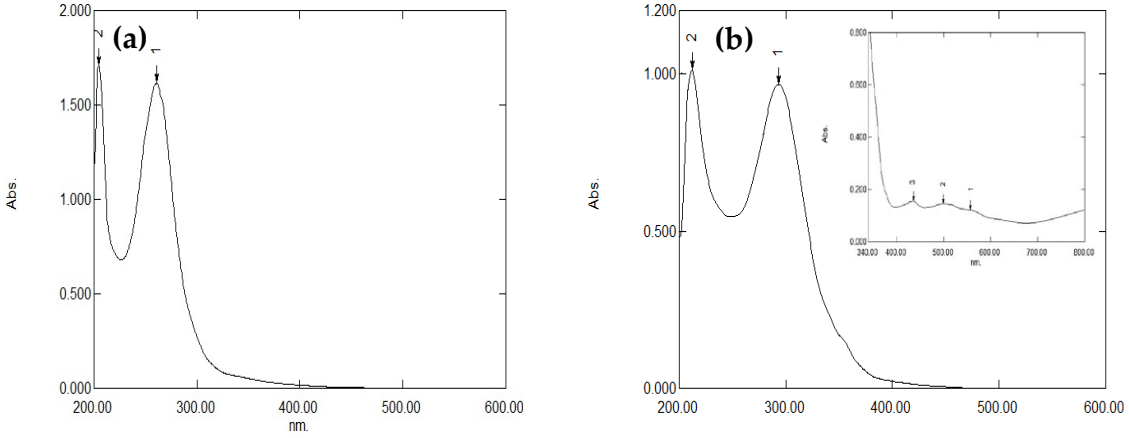


Fig. 5. The electronic spectra of the prepared nano ligand (LH) (a) and its Pd (II) complex (b) suggesting a square planar geometry of the complex with dsp^2 hybridization.

(LH) and its Pd (II) complex conducted in solid state, covering an angular range (2θ) of 10° to 80°. Results of the XRD study revealed that the diffraction peaks sometimes appeared broad due to micro-strains such as lattice deformation, crystalline faulting, domain size and distribution of crystal [50]. However, the prepared LH ligand spectrum (Fig. 9a) displayed sharp crystalline peaks, as did the Pd (II) complex (Fig. 9b). The study confirmed that both the newly prepared nano ligand (LH) and its Pd (II) complex were crystalline in nature [51]. Results of XRD study with diffraction angles

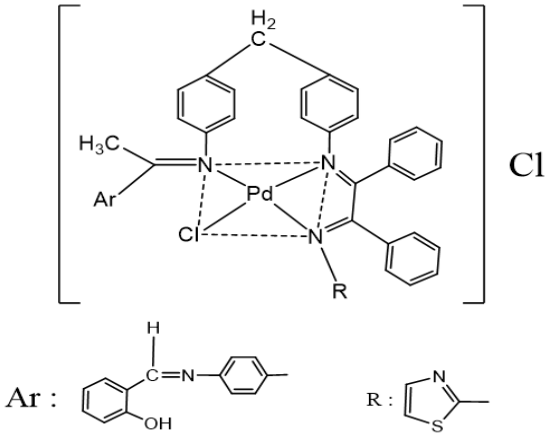


Fig. 6. Proposed square planar geometry of the prepared Pd (II) complex with dsp^2 hybridization.

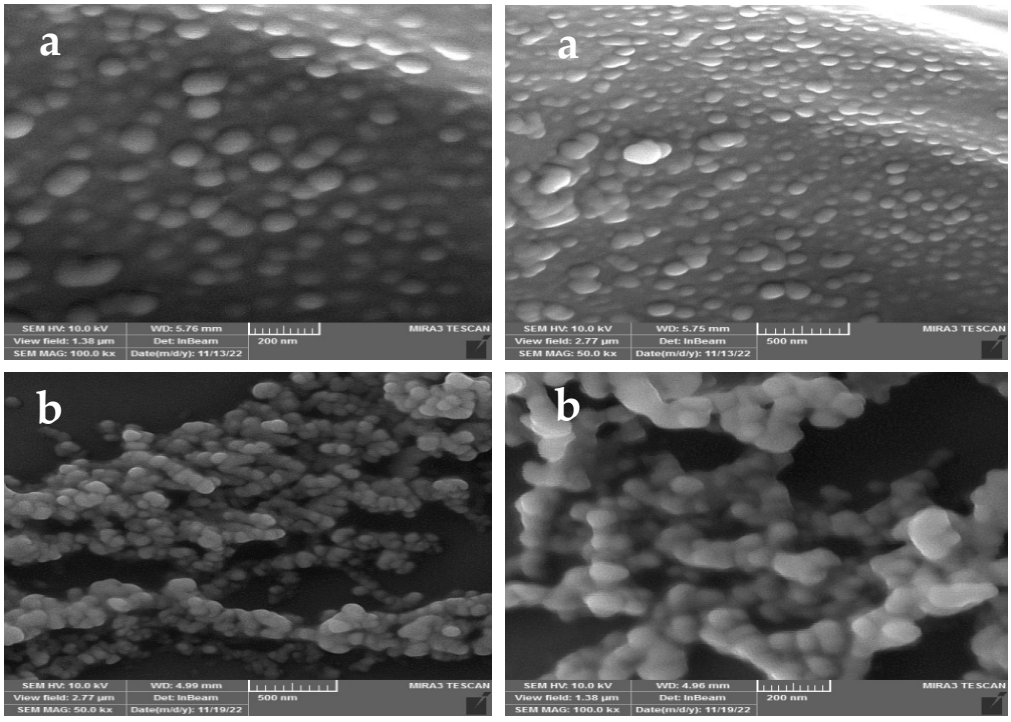
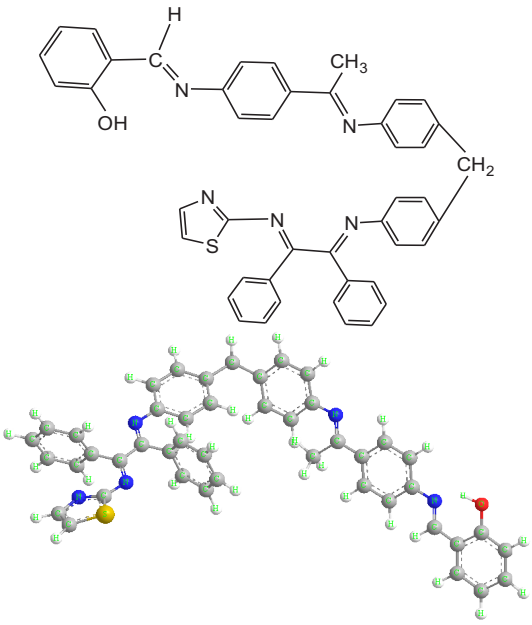


Fig. 7. The FESEM results of the newly prepared nano ligand (LH) (a) and its Pd (II) complex (b).

(a)

2D



(b)

3D

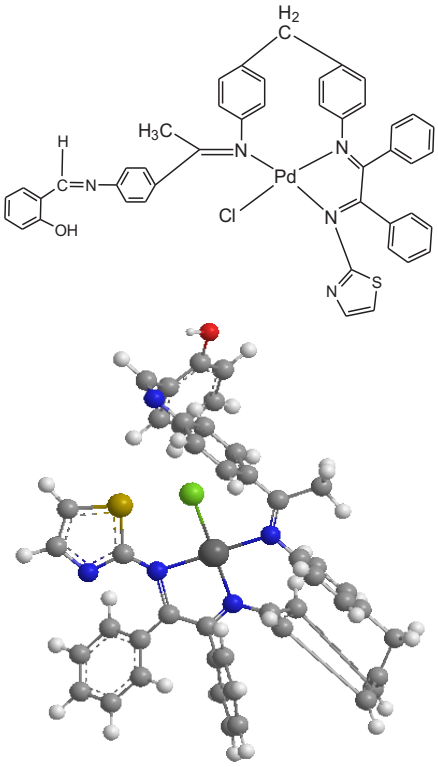


Fig. 8. Suggested structural and 3D formulae of the newly prepared ligand (LH) (a) and its Pd (II) complex (b).

2θ, d-spacing values, relative intensities, width of peaks at mid-intensity, and crystal size values for both the newly prepared nano ligand (LH) and its Pd (II) complex have been given in Tables 4 and 5.

Cytotoxicity of the prepared nano ligand (LH) and its Pd (II) complex against cancer and normal cell line

For investigating the cytotoxicity of the newly prepared nano ligand and its complex, the cell viability and cell inhibition (%) measured for both cancer (MCF-7) cells and normal (HEK-293) cells. Results of the study revealed that at a concentration of 1600 µg/ml, newly prepared nano ligand (LH) inhibited the growth of the normal (HEK-293) cells to a significant level i.e., 93.7% growth inhibition was observed. Contrary to this, nano ligand showed no substantial impact on growth inhibition of breast cancer (MCF-7) cells over a wide range of concentrations (0-1600 µg/ml) [52] as summarized in Table 6 and Fig. 10a-b.

When it comes to the cytotoxic efficiency of Pd

Table 4. Diffraction angles 2θ, d-spacing values, and relative intensities (rel. Int.%) for both the newly prepared nano ligand (LH) and its Pd (II) complex.

Compound	No.	Pos. °2Θ (Radian)	d-space in A˚	Intensity In	Rel. Int. [%]
Ligand (LH)	1-	5.99	14.7581	1760.518	100 %
	2-	6.91	12.7931	413.821	23.5%
	3-	7.83	11.2985	391.956	22.3%
	4-	8.91	10.7737	898.591	51.0%
	5-	9.77	9.9261	67.228	3.8%
[Pd(LH)Cl]Cl	1-	24.431	3.640	828.13	100 .0%
	2-	20.389	4.352	352.588	4.3%
	3-	12.157	7.274	3190	38.5%
	4-	16.36	5.413	218.106	2.6%
	5-	9.33	9.464	159.65	1.9%

Table 5. Diffraction angles, width of peaks at mid-intensity, and crystal size values for both the newly prepared ligand (LH) and its Pd (II) complex.

Compound	No.	Position °2Θ	Peak Width (FWHM)	D Crystallite size(nm)	Rel. Int. [%]	Lattice Strain
Ligand (LH)	1-	17.72	0.179	48.65	100%	0.0048
	2-	25.22	0.194	43.85	58%	0.0038
	3-	22.04	0.230	42.22	40%	0.0032
	4-	28.32	0.432	35.87	25%	0.0025
[Pd(LH)Cl]Cl	1-	24.431	0.116	73.16	100%	0.0024
	2-	17.639	0.078	89.72	5.4%	0.0022
	3-	13.543	0.125	66.84	3.2%	0.0048

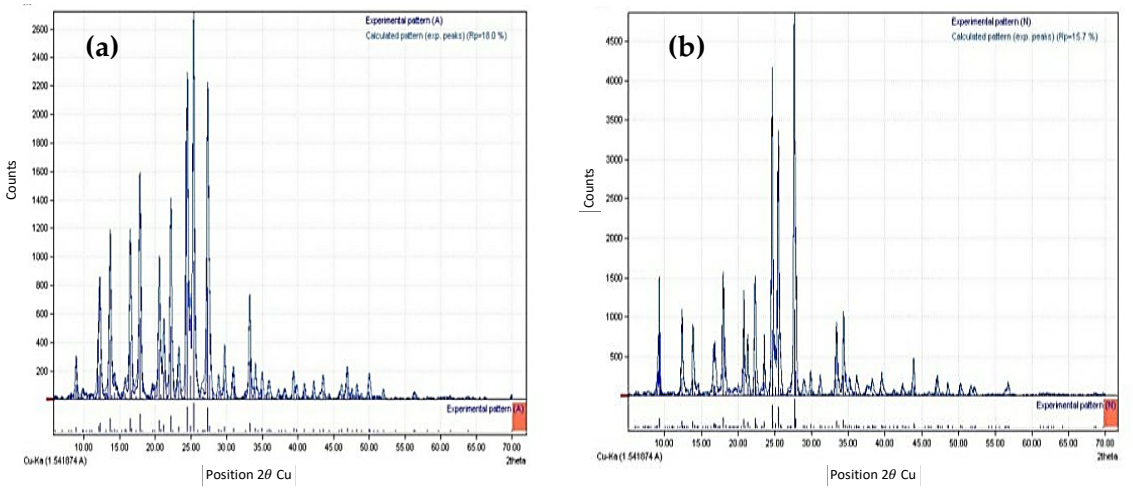


Fig. 9. The XRD analysis of the newly prepared nano ligand (LH) (a) and its Pd (II) complex (b).

(II) complex of the newly prepared nano ligand (LH), it was observed that cell inhibition efficiency (%) of the complex is higher towards the MCF-7 cells in comparison to the normal cells (HEK-293) when the concentration increases from 0 to 1600 µg/ml. The results of cytotoxicity of the Pd (II) complex of the newly prepared ligand (LH) against both cancer and normal cell lines have been

Table 6. Newly prepared nano ligand (LH) against the cells of the breast cancer cell line MCF-7 and the normal cell line HEK-293. The values are given as their average±standard deviation (S.D).

	Sample N			
Concentration	Cell viability (average ± S.D.)		Cell inhibition (%)	
µg/ml	Cancer cell MCF-7	Normal cell HEK	Cancer cell MCF-7	Normal cell HEK
0	100±0.00	100±0.00	0.00	0.00
50	81.75±2.05	86.55±1.20	8.25	14.3
100	62.20±2.82	63.75±1.34	47.8	37.2
200	43.95±5.16	63.82±10.6	26.4	43.7
400	41.55±5.72	48.67±7.24	45.6	54.0
800	33.95±3.88	35.17±0.31	63.3	65.1
1600	16.8±0.00	10.85±6.43	83.2	93.7
IC50	5.32	5.84		

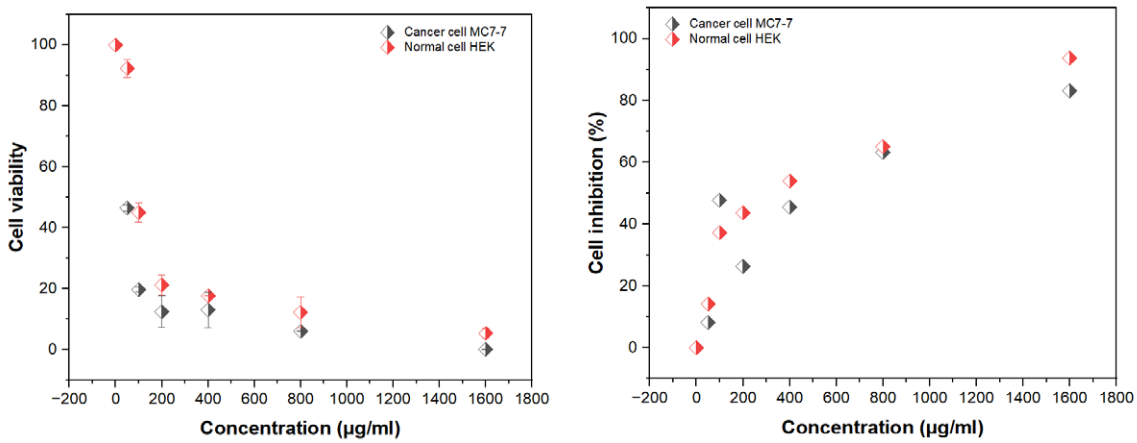


Fig. 10. Cytotoxicity of the newly prepared nano ligand (LH) against breast cancer MCF-7 cell line and normal HEK cell line in terms of cell viability (a) and % cell inhibition (b) over a range of concentration.

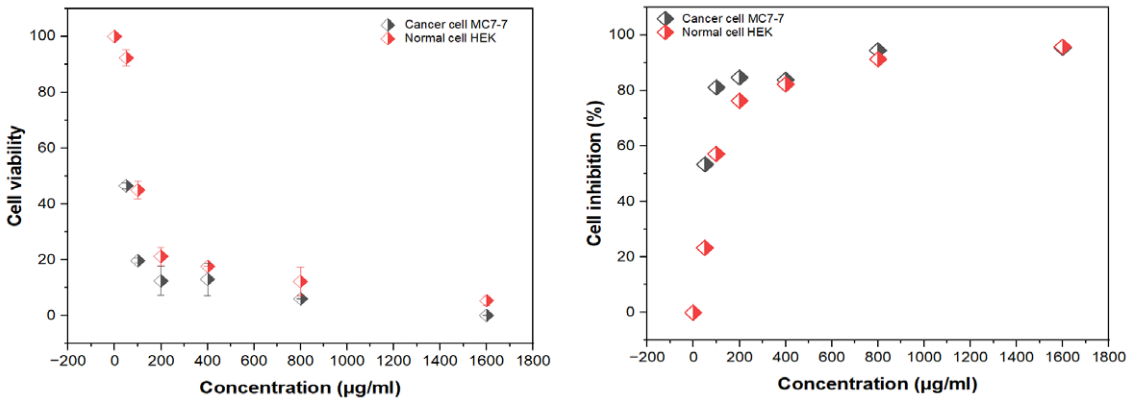


Fig. 11. Cytotoxicity of the Pd (II) complex of the prepared nano ligand (LH) against breast cancer MCF-7 cell line and normal HEK cell line in terms of cell viability (a) and % cell inhibition (b) over a range of concentration.

Table 7. Pd (II) complex of prepared nano ligand against the cells of the breast cancer cell line MCF-7 and the normal cell line HEK-293. The values are given as their average±standard deviation (S.D).

Sample 7				
Concentration	Cell viability (average ± S.D.)		Cell inhibition (%)	
µg/ml	Cancer cell MC7-7	Normal cell HEK	Cancer cell MC7-7	Normal cell HEK
0	100±0.00	100±0.00	0	0
50	46.55±1.06	92.35±2.89	53.4	23.3
100	19.80±0.84	45.00±3.11	81.2	57.2
200	12.55±5.16	21.29±3.25	84.8	76.4
400	13.05±5.86	17.72±0.01	83.8	82.3
800	6.10±0.071	12.30±5.09	94.5	91.3
1600	0.15±0.070	5.40±1.69	95.5	95.8
IC50	0.475	1.30		

summarized in Table 7 and Fig. 11a-b. Overall, the study revealed the higher cytotoxic efficiency (for the breast cancer treatment) of the Pd (II) complex of nano ligand in comparison to its free ligand (LH) for all studied concentrations [53]. This behaviour can be attributed to the presence of the anionic functionalities such as azomethine (C=N) group in both synthesized nano ligand and its complex that react with cancer cells resulting in cell apoptosis that ultimately lead to cancer treatment.

CONCLUSION

This study is devoted to the synthesis and characterization of the novel nano Schiff based ligand ad its Pd (II) complex. The cytotoxic efficiency of the synthesized complex was explored against the treatment of the breast cancer cells (MCF-7). Spectral, and analytical results for the nano ligand (LH) and palladium (II) complex revealed that the molar ratio for preparing the complex with the nano ligand (LH) is 1:1. The molar conductivity values indicate the ionic nature of the palladium (II) complex. Further, the spectral measurements suggest that the nano ligand coordinates with the metal ion through nitrogen atoms of azomethine groups, acting as a tridentate nano ligand. FESEM analysis showed that both the nano ligand and its complex have crystalline, granular structures with a nanoparticle size less than 100 nm, indicating their significance in medical and industrial applications. Crystallography measurements confirmed their crystalline nature and nano-sized features. When it comes to the mechanistic study of treating breast cancer cells, it was observed that anionic functionalities such as azomethine (C=N) group are mainly responsible for their cytotoxic activity of the synthesized nano ligand and its complex. Presence of this functional group is also confirmed from the FTIR analysis of the prepared nano ligand and its complex. Further, the MTT toxicity assays revealed that the palladium complex derived from the nano ligand (LH) selectively targets breast cancer cells (MCF-7) specifically with minor influence on the normal cell line (HEK-293).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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