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ORIGINAL ARTICLE

Pd(II) complexes bearing chromone based Schiff bases: Synthesis, characterisation and biological activity studies

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Abstract Pd(II) complexes of 3-formyl chromone Schiff bases such as 3-((2-hydroxyphenylimino)methyl)-4H-chromen-4-one (HL₁), 2-((4-oxo-4H-chromen-3-yl)methylneamino)benzoic acid (HL₂), 3-((3-hydroxypyridin-2-ylimino)methyl)-4H-chromen-4-one (HL₃), 3-((2-mercaptophenylimino)methyl)-4H-chromen-4-one (HL₄) and 3-((pyridine-2-ylimino)methyl)-4H-chromen-4-one (L₅) have been synthesised and characterised by elemental analysis, molar conductivity, IR, electronic, magnetic, TG–DTA, powder XRD and fluorescence spectral data. From the analytical, electronic and magnetic data square-planar geometry has been proposed for all the Pd(II) complexes. Powder XRD studies indicate the crystalline state of the Pd(II) complexes. The antimicrobial activity of Pd(II) complexes was performed against two Gram(–ve), two Gram(+ve) and fungal microorganisms and the results indicate that, complexes show better microbial inhibition activity than the ligands. Pd(II) complex of HL₁ displayed comparable antioxidant activity with reference to the standard drug (BHT). Agarose gel electrophoresis assay demonstrates the ability of complexes to cleave the pUC19 plasmid DNA. The cytotoxicity was tested by the MTT assay. The results indicate that the complexes exert cytotoxic effects against tested cell lines but their cytotoxicity is less than that of cisplatin.

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1. Introduction

Formyl chromone Schiff bases have been the centre of attraction for many workers in the current research due to their miscellaneous activities. Chromones are naturally occurring compounds which are able to cause cytotoxic effect in various types of cells. They are widely known to have anticancer, antioxidant, antiproliferative, antiHIV, antiinflammatory, and many other activities (Martens and Mithofer, 2005; Di Braccio et al., 2003; Middleton et al., 2000). Since the last decade, metal coordination chemistry has been one of the most efficient

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strategies in the design of drugs (Jones and Thornbak, 2007). Many literature reports revealed that chromone and its derivatives are able to form stable and coloured complexes with various metal ions (Anitha et al., 2012; Li and Yang, 2010). According to literature reports, metal complexes of the chromone moiety possess various biological activities in some cases comparable with cisplatin (which is an effective anticancer drug) (Grazul and Budzisz, 2009) and some metal complexes which are connected with reactions generating free radicals, may in some cases obtain additional antioxidant ability (Kostyuk et al., 2001; Grazul et al., 2012). These metal complexes are also used in various fields (Nijhawan and Kakkar, 1998).

A large number of platinum and palladium complexes containing amine based ligands have become the subject of intensive research, since they are structurally related to cisplatin. The success of cisplatin and other Pt(II) complexes in the treatment of ovarian, testicular, neck and head, oesophageal and non-small cell lung cancers (Todd and Lippard, 2009; Eastman, 1999; Montana and Batalla, 2009; Abu-Surrah and Kettunen, 2006; akomska, 2009) and similar properties of Pt(II) and its congener Pd(II), have led to a large effort in the search to find Pd(II) antitumour drugs that are effective against Pt(II) resistant therapies and that have fewer side effects (Garoufis et al., 2009; Abu-Surrah et al., 2008; Gao et al., 2009; Štarha et al., 2009). The main target of chemotherapy is the destruction of tumour cells without any undue influence on proper cells. Compared to palladium and platinum complexes, palladium complexes are hydrolyzed 10^5 times faster than their corresponding platinum analogues, which could lead to the hydrolysis of Pd(II) complexes before they reach their target DNA (Polyanskaya et al., 2010). Pd(II) complexes also possess antimicrobial and antioxidant activity etc. (Spera et al., 2011; Ramachandran et al., 2012). This has provoked interest in the design of Pd(II) complexes.

As part of our on-going research work on the synthesis of metal complexes with Schiff bases derived from formyl chromone and aromatic amines i.e. 3-((2-hydroxyphenylimino)methyl)-4H-chromen-4-one (HL₁), 2-((4-oxo-4H-chromen-3-yl)methyleneamino)benzoic acid (HL₂), 3-((3-hydroxypyridin-2-ylimino)methyl)-4H-chromen-4-one (HL₃), 3-((2-mercaptophenylimino)methyl)-4H-chromen-4-one (HL₄) and 3-((pyridine-2-ylimino)methyl)-4H-chromen-4-one (L₅) (Kavitha and Laxma Reddy, 2012; Kavitha et al., 2013a,b), we present here the preparation, characterisation and evolution of biological activities (antimicrobial, antioxidant, cytotoxicity and DNA cleavage) of Pd(II) complexes with the 3-formyl chromone Schiff base ligands (HL₁, HL₂, HL₃, HL₄ and L₅).

2. Experimental

2.1. Reagents and equipments

All chemicals and solvents used were of AR grade. Palladium dichloride was obtained from Jhonson Matthew Chemicals (England).

The UV–Vis spectra of the ligands and their metal complexes were recorded on an Analytikjena Specord 205 UV–Vis spectrophotometer. Molar conductance of the complexes was measured in DMF at 1×10^{-3} M using a Digisun conductivity meter. Elemental analysis (C, H, and N) was performed using Perkin Elmer CHN analyser. The chloride ion was

estimated by the Volhard's method (Vogel, 1961). The IR spectra ($4000\text{--}400\text{ cm}^{-1}$) in KBr discs were recorded on TEN-SOR 2 spectrophotometer. Thermal studies of the complexes were carried out on a Perkin Elmer diamond TGA instrument at a heating rate of $10\text{ }^\circ\text{C}$ and a nitrogen flow rate of 20 mL/min . The magnetic susceptibilities of Pd(II) complexes were measured with a Sherwood scientific balance. Fluorescence spectra were recorded using Perkin Elmer LS 55 fluorescence spectrometer. The X-ray powder diffraction analysis was carried out by using Xpert-Pro X-ray diffractometer using $\text{Cu-K}\alpha$ (1.5360 \AA) radiation.

2.2. Synthesis of ligands

Schiff base ligands HL₁, HL₂, HL₃, HL₄ and L₅ (Fig. 1) were synthesised and characterised according to the literature (Sigg et al., 1982; Khan et al., 2010; Kavitha et al., 2012, 2013b; Kavitha and Laxma Reddy, 2012).

2.3. Synthesis of Pd(II) complexes

All the palladium(II) complexes were prepared using the general procedure as given below. PdCl_2 solution in 0.1 M HCl (1 mM) was taken and treated with an equal volume of water.

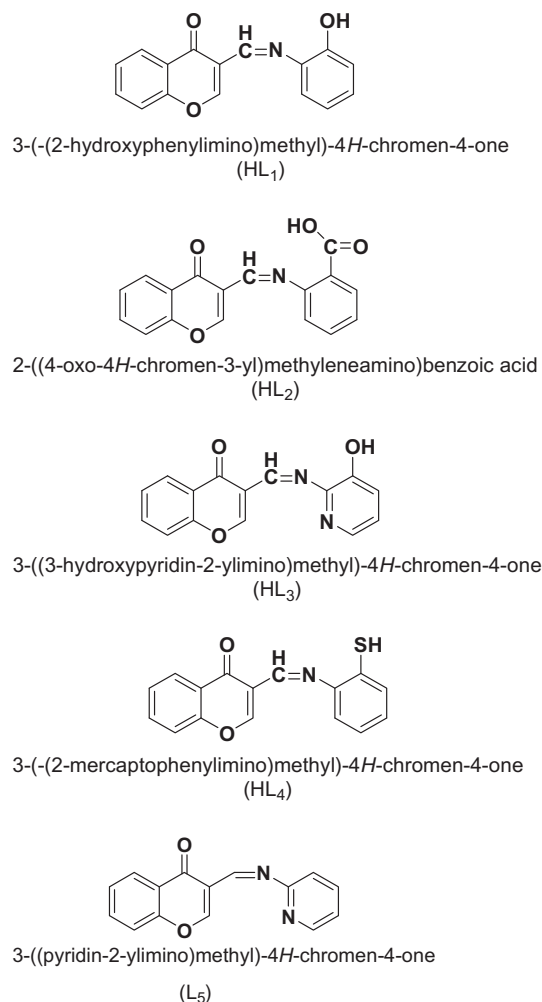


Figure 1 Structures of ligands.

The hot methanolic ligand solution (2 mM) was added drop wise to the hot metal salt solution. The reaction mixture was stirred at room temperature for 30 min. The coloured complex thus separated out was filtered, washed several times with hot water and methanol until the washings were free from excess ligand and palladium chloride and dried in vacuo.

2.4. Pharmacology studies

2.4.1. Antimicrobial activity

In vitro antimicrobial activity of the Pd(II) complexes was evaluated against *Proteus vulgaris*, *Klebsiella pneumoniae* as gram-negative, *Staphylococcus aureus*, *Bacillus subtilis* as gram-positive bacteria cultures where as *Candida albicans* fungi culture using antibiotics kanamycin and clotrimazole are the standards. The disc diffusion method was adopted for activity measurements. Standard inoculums, $1-2 \times 10^7$ cfu/mL 0.5 McFarland standards (Stemper and Matsen, 1970) were introduced onto the surface of the sterile nutrient agar plate and evenly spread by using a sterile glass spreader. Sterile antibiotic discs (6 mm in diameter, prepared using Whatmann No. 1 paper) were placed over the nutrient agar medium. Each disc was spread by 100 µg of the compounds (initially dissolved in DMSO). The plates were incubated with bacterial cultures for 24 h at 37 °C and fungal cultures at 25 °C for 48 h. The activity of the compounds was determined by measuring the diameter of inhibition zone in 'millimetres' and compared with standard antibiotics. DMSO (which has no activity) and standard antibiotics were used as negative and positive controls for antimicrobial activity studies. The activity results are calculated as a mean of triplicates.

Minimum inhibitory concentrations (MIC) of the complexes which showed antimicrobial activity were determined using the literature method (Shankar et al., 2009). All the compounds diluted within the range of 100–10 µg/mL were mixed in nutrient broth and 0.1 mL of active inoculums was added to each tube. The tubes were incubated aerobically at 37 °C for bacteria and 25 °C for fungi for 24 h. The lowest concentration of the compound that completely inhibited bacterial growth (no turbidity) in comparison to control was regarded as MIC.

2.4.2. Free radical scavenging activity

The antioxidant activity of Pd(II) complexes was evaluated in DPPH free radical scavenging method.

Test samples were applied on a TLC plate as a spot using mobile phase methanol:acetonitrile (7:1). It was allowed to develop the chromatogram for 30 min. After the completion of the chromatogram, the plate was sprayed with DPPH (0.2% w/v). The colour change (yellow spot on purple background on TLC plate) is an indication of the presence of antioxidant activity.

2.4.2.1. Quantitative analysis. 0.004% solution of the DPPH radical in methanol was prepared, and 3 mL of this solution was added to 1 mL of the compounds (stock solution is 10 mg/10 mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, the absorbance was measured at 517 nm using a spectrophotometer. DPPH solution was used as the control without the test compounds, whereas methanol was used as the blank. Lowering of the absorbance of the test compounds indicates

higher free radical scavenging activity. BHT is used as the standard. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging activity(\%)} = \left[\frac{(A_0 - A_i)}{A_0} \right] \times 100$$

where A_0 is the absorbance of the control solution, and A_i is the absorbance in the presence of sample solutions or standards for positive control. IC₅₀ values were calculated, which exhibited significant activity.

2.4.3. Cytotoxicity

2.4.3.1. Solutions. The complexes were dissolved in DMSO at a concentration of 5 mg/100 mL as stock solution, and diluted in a culture medium at concentrations of 1, 10 and 100 µg/mL as working solution.

2.4.3.2. Cell culture and drug treatment. Raw 264.7 cell line (murine macrophage cell line), MCF-7 (human breast carcinoma cell line) and COLO 205 (human colon carcinoma cell line) were obtained from NCCS Pune. Cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, amphotericin (3 µg/mL), gentamycin (400 µg/mL), streptomycin (250 µg/mL) and penicillin (250 Units/mL) in a carbon dioxide incubator at 5% CO₂. About 700 cells per well were seeded in 96 well plates using a culture medium. The cell viability was tested using trypan blue dye with the help of a haemocytometer and 95% of the viability was confirmed. After 24 h, the new medium with compounds in the concentration of 1, 10 and 100 µg/mL was added at respective wells and kept in incubation for 48 h. After incubation, MTT assay was performed. Later than, 48 h of the drug treatment, the medium was changed again for all groups and 10 µL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/mL stock solution) was added and the plates were incubated for an additional 4 h. The medium was discarded and formazan blue, which was formed in the cells, was dissolved in 50 µL of DMSO. The optical density was measured at 570 nm. The percentage toxicity was calculated by using the following formula (Sathiyaraj et al., 2012).

$$\% \text{Toxicity} = 1 - (\text{treated cells} / \text{untreated cells}) \times 100$$

IC₅₀ of compounds were calculated using Graphpad PRISM software tool.

2.4.4. DNA cleavage activity

Agarose gel electrophoresis was used to study the DNA cleavage activity of the Pd(II) complexes. pUC19 plasmid was cultured, isolated and used as DNA for the experiment. Test samples (1 mg/mL) were prepared in DMF. 25 µg of the test samples were added to the isolated plasmid and incubated for 2 h at 37 °C. After incubation, 30 µL of plasmid DNA sample mixed with bromophenol blue dye was loaded into the electrophoresis chamber wells along with control (plasmid DNA in DMF without test sample) and standard DNA markers. Finally, it was loaded onto an agarose gel and electrophoresed at a constant voltage of 50 V for 30 min. After the run, gel was removed and stained with 10.01 µg/mL ethidium bromide and image was taken in Versadoc (Bio-Rad) imaging system. The results were compared with the standard DNA marker.

The same procedure was followed in the presence of oxidising agent H_2O_2 (5 μL , 500 μM) also.

3. Results and discussion

All Pd(II) complexes are coloured, non-hygroscopic, stable in air, insoluble in water and many common organic solvents but soluble in DMF and DMSO. The analytical, physical and molar conductivity data of the metal complexes are given in Table 1. The analytical data revealed that the metal to ligand molar ratio is 1:1 for all the complexes except the complex 5. However, 1:2 M ratio was found in the case of complex 5. The molar conductance values of all Pd(II) complexes except the complex 5 in DMF at 1×10^{-3} M are found to be in the range $10\text{--}20 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ indicating their non-electrolytic nature. However, molar conductance value for the complex 5 in DMF is $128 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ which indicates its 1:2 electrolytic nature (Geary, 1971).

3.1. Electronic spectra

The absorption spectra of ligands and their Pd(II) complexes were recorded in DMF solution in the wavelength range of 200–1100 nm. The bands in the UV–visible spectra of all the complexes were assigned upon comparison with the spectra of their corresponding ligands ($\text{HL}_1\text{--}\text{L}_5$). The absorption spectrum of HL_3 and its Pd(II) complex is shown in Fig. 2. Free ligands showed the absorption bands between $25,000$ to $27,000 \text{ cm}^{-1}$ and $31,000$ to $34,000 \text{ cm}^{-1}$ and are assigned to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, respectively. These bands are shifted to the lower energy region upon complexation with the metal ion. All Pd(II) complexes exhibit one band in the visible region at around $20,000$ to $25,000 \text{ cm}^{-1}$ and are assigned to $^1A_{1g} \rightarrow ^1E_g$ transition characteristic of square-planar geometry (Al-Noaimi et al., 2012). All the palladium complexes are also found to be diamagnetic which also supports the square-planar geometry of the complexes.

3.2. IR spectra

The infrared spectra provide important information about the skeleton of the complexes. The main stretching frequencies of the Pd(II) complexes are presented in Table 2. The $\nu(\text{C}=\text{O})$ of carbonyl group of the ligands appear in the range of 1650--

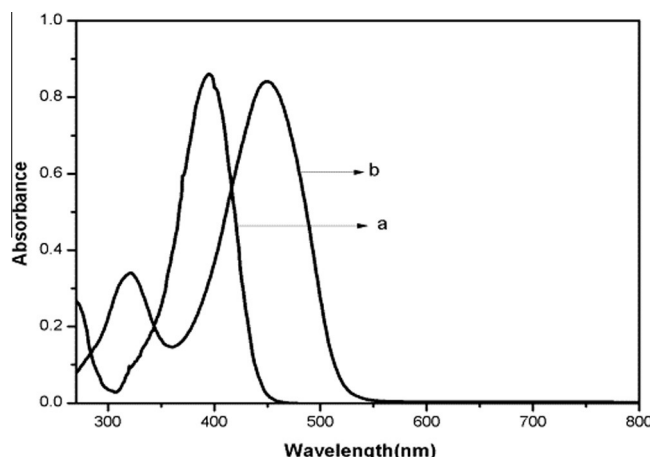


Figure 2 Electronic spectra of (a) HL_3 , and (b) Pd(II) complex.

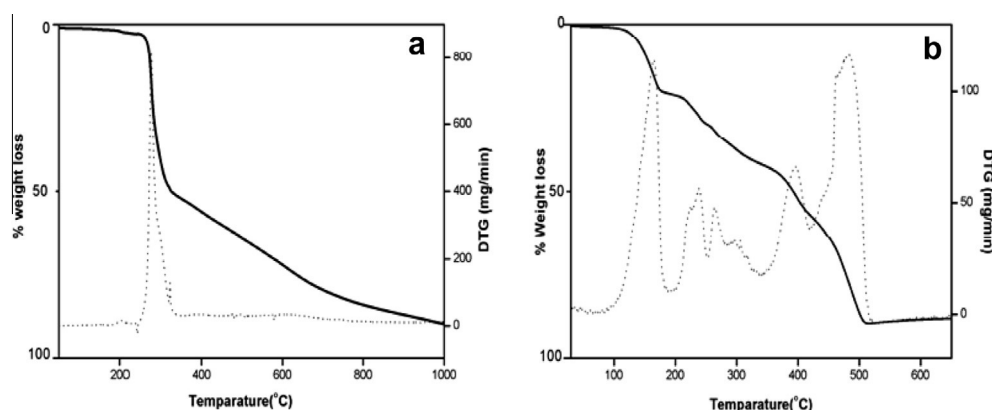
1620 cm^{-1} , while in their Pd(II) complexes the band is shifted to lower wavenumber by $6\text{--}40 \text{ cm}^{-1}$ (Wang et al., 2008). It shows that the oxygen of the carbonyl group takes part in coordination in all the complexes. The $\nu(\text{C}=\text{N})$ of azomethine group of ligands show a band in the region of $1600\text{--}1550 \text{ cm}^{-1}$ and it is shifted to $16\text{--}39 \text{ cm}^{-1}$ in Pd(II) complexes towards lower wave number region, indicating that the nitrogen of azomethine group is involved in the coordination (Li and Yang, 2010). The bands at 3241 and 3246 cm^{-1} are due to $\nu(\text{OH})$ group of HL_1 and HL_3 ligands. It disappeared in their corresponding Pd(II) complexes, indicating the participation of oxygen atom of the OH group in coordination with deprotonation. A band at 1365 cm^{-1} in HL_2 ligand is due to the $\nu(\text{C}-\text{O})$ of carboxylic group, in its complex it was shifted by 16 cm^{-1} suggesting the oxygen atom of carboxylic group participated in the coordination (Olczak-Kobza and Mrozek, 2009). The SH stretching vibration, $\nu(\text{SH})$, is not useful in ascertaining its coordination in the complexes, since it displayed very weak bands in HL_4 ligand spectra. However, the participation of the SH group in chelation is ascertained from the shift of the $\nu(\text{C}-\text{S})$ by 59 cm^{-1} to lower wavenumber region in the spectra of its corresponding Pd(II) complex (Mohamed et al., 2006). Further the confirmation of N, O and Cl in coordination is demonstrated by new bands in the range of $500\text{--}300 \text{ cm}^{-1}$ (Patel et al., 2012).

Table 1 Physical, analytical and molar conductivity data of Pd(II) complexes.

Complex no.	Molecular formula	Colour	% Yield	% Found (calcd.)					Molar conductivity ($\text{ohm}^{-1} \text{mol}^{-1} \text{cm}^2$)
				C	H	N	S	Cl	
(1)	$[\text{Pd}(\text{L}_1)\text{Cl}]$	Orange red	71	47.45 (47.30)	2.21 (2.46)	3.10 (3.44)		8.85 (8.74)	11
(2)	$[\text{Pd}(\text{L}_2)\text{Cl}]$	Yellow	65	47.55 (47.01)	2.19 (2.30)	3.18 (3.22)		8.01 (8.18)	20
(3)	$[\text{Pd}(\text{L}_3)\text{Cl}]$	Dark red	90	43.47 (44.23)	2.28 (2.21)	6.79 (6.88)		8.98 (8.72)	15
(4)	$[\text{Pd}(\text{L}_4)\text{Cl}]$	Brick red	82	45.59 (45.50)	2.09 (2.37)	3.15 (3.31)	7.68 (7.58)	8.38 (8.41)	13
(5)	$[\text{Pd}(\text{L}_5)_2]\text{Cl}_2$	Green	78	52.98 (53.14)	2.25 (2.95)	8.62 (8.26)		10.78 (10.48)	128

Table 2 Infrared spectral data and their assignments of ligands and their Pd(II) complexes.

Compound	$\nu(\text{C}=\text{O})$ (γ pyrone)	$\nu(\text{C}=\text{N})$	$\nu(\text{CO})$ (carboxylate)	$\nu(\text{C}-\text{S})$	$\nu(\text{M}-\text{N})$	$\nu(\text{M}-\text{O})$	$\nu(\text{M}-\text{Cl})$
HL ₁	1643	1605					
HL ₂	1651	1605	1365				
HL ₃	1647	1591					
HL ₄	1622	1597		790			
L ₅	1654	1596					
[Pd(L ₁)Cl] (1)	1637	1571			489	390	348
[Pd(L ₂)Cl] (2)	1611	1566	1349		470	360	346
[Pd(L ₃)Cl] (3)	1634	1575			490	375	332
[Pd(L ₄)Cl] (4)	1614	1566		731	485	399	314
[Pd(L ₅) ₂].Cl ₂ (5)	1611	1575			458	357	–

**Figure 3** TG graphs of (a) [Pd(L₁)Cl], and (b) [Pd(L₅)₂].Cl₂.

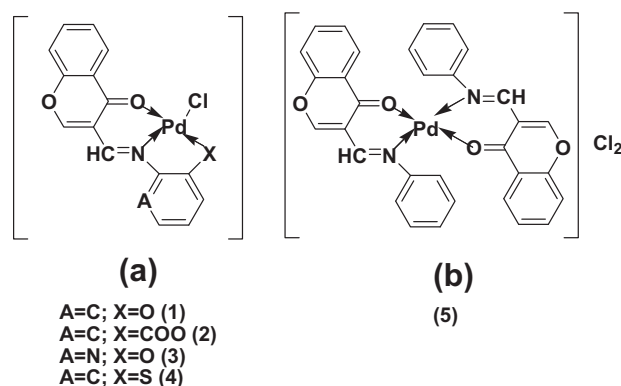
3.3. Thermogravimetric analysis

The thermal decomposition studies of the Pd(II) complexes were carried out in nitrogen atmosphere in the temperature range of 30–1000 °C. The TG graphs of complexes 1 and 5 are given in Fig. 3. In the case of complex 1 there is no weight loss up to 185 °C. However, all other complexes (2–5) undergo first step decomposition with a weight loss of 7.58–16.55% (calcd. 8.18–15.72%) in between 30 and 284 °C due to the removal of chloride ions (lattice and coordinated). All the complexes (1–5) show the second decomposition step 61.94–66.89% (calcd. 61.10–66.21%) in the temperature range of 196–710 °C due to the decomposition of organic part of the complexes. The final amount of residue of all complexes is close to calculated mass of palladium oxide.

On the basis of the above studies (elemental, conductivity, electronic and infrared data) the following structure (Fig. 4) may be proposed for all the Pd(II) complexes.

3.4. Fluorescence

The solid state emission spectra of Pd(II) complexes were studied at room temperature. The emission spectra of complexes 1–5 are illustrated in Fig. 5 and the excitation and emission data are listed in Table 3. All Pd(II) complexes exhibit weak fluorescence except complex 4 compared to their corresponding ligands when excited at the absorption wavelengths in

**Figure 4** Structures of Pd(II) complexes, (a) Complexes 1–4, and (b) Complex 5.

the range of 307–453 nm. However formyl chromone Schiff base ligands are highly emissive excitation upon different wavelengths (Kavitha et al., 2013a). It was observed that the emission spectra of metal complexes that are originated may be from the formyl chromone moiety. The emission intensity of the complexes significantly decreases compared to that of the corresponding ligands, but the emission intensity of complex 4 is much higher than those of other four complexes. This enhancement in the emission intensity may be due to low-lying

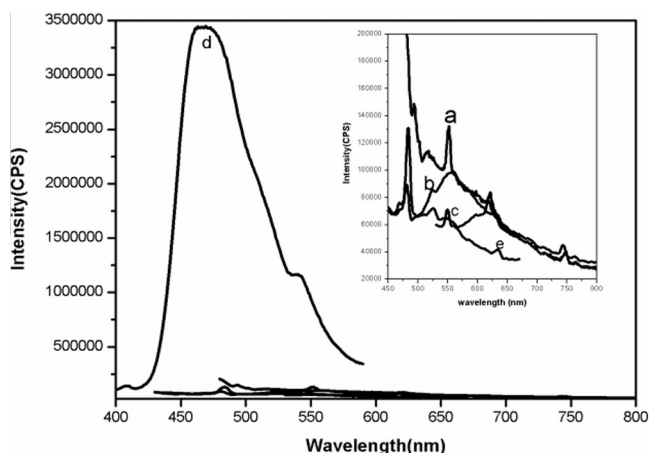


Figure 5 Fluorescence spectra of all Pd(II) complexes, (a) [Pd(L₁)Cl] (b) [Pd(L₂)Cl] (c) [Pd(L₃)Cl] (d) [Pd(L₄)Cl], and (e) [Pd(L₅)₂]Cl₂.

Table 3 Excitation and emission wavelengths of Pd(II) complexes.

Compound	Excitation wavelength (nm)	Emission wavelength (nm)
[Pd(L ₁)Cl] (1)	453	551, 621sh, 747sh
[Pd(L ₂)Cl] (2)	381	481(sh), 558
[Pd(L ₃)Cl] (3)	451	549 sh, 618, 743sh
[Pd(L ₄)Cl] (4)	307	469
[Pd(L ₅) ₂]Cl ₂ (5)	384	484, 526sh, 634sh

excited state orbital of complex 4 (Guney et al., 2010). Literature reports revealed that, blue/red shift of emission maxima was observed, when ligands are coordinated with the metal ions (Jin et al., 2012; Guney et al., 2011).

3.5. X-ray powder diffraction

Single crystals of the studied complexes could not be obtained, because the complexes are insoluble in most common organic

Table 4 PXRD spectral data of Pd(II) complexes.

Complexes	2θ	d	Relative intensity	Full width at half maximum
[Pd(L ₁)Cl] (1)	28.83	3.094	100	0.205
[Pd(L ₃)Cl] (3)	30.45	2.933	100	0.183
[Pd(L ₅) ₂]Cl ₂ (5)	46.44	1.953	100	0.438

Table 5 MIC values of antimicrobial activity of Pd(II) complexes.

Compound	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Proteus vulgaris</i>	<i>Klebsiella pneumoniae</i>	<i>Candida albicans</i>
HL ₁	–	–	–	–	–
HL ₂	80	80	80	80	80
HL ₃	–	–	–	–	–
HL ₄	–	–	–	–	–
L ₅	53	53	53	53	53
[Pd(L ₁)Cl] (1)	50	60	55	75	60
[Pd(L ₂)Cl] (2)	55	55	55	55	55
[Pd(L ₃)Cl] (3)	–	–	–	–	–
[Pd(L ₄)Cl] (4)	–	–	–	–	–
[Pd(L ₅) ₂]Cl ₂ (5)	80	80	–	–	–
Kanamycin	4	10	8	11	–
Clotrimazole	–	–	–	–	10

solvents except DMF and DMSO. The X-ray powder diffraction patterns of the Pd(II) complexes were recorded over 2θ = 10–80°. The diffractograms of the complexes are displayed in Fig. 6. The diffraction data like angle (2θ), interplanar spacing (d), and relative intensity (%) have been summarized in Table 4. From the data, all Pd(II) complexes show sharp crystalline peaks except complex 2 and 4, indicating their crystalline nature. However complex 2 and 4 show amorphous nature. The XRD patterns of all complexes are very similar and suggest that the complexes have similar structure. The average crystallite sizes of the complexes d_{XRD} were calculated using the Sherrers formula (Warren, 1990). Complexes 1, 3 and 5 have a crystallite size of 41, 46 and 20 nm respectively, suggesting that the complexes are in a nano crystalline phase.

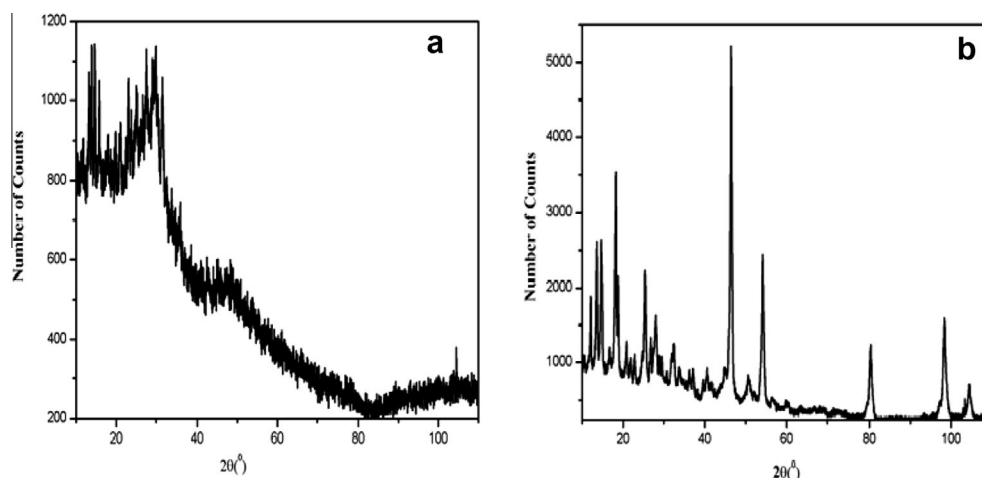


Figure 6 PXRD patterns of (a) [Pd(L₂)Cl], and (b) [Pd(L₅)₂]Cl₂.

Table 6 IC₅₀ values of antioxidant activity of Pd(II) complexes.

Compound	IC ₅₀ (μg/mL)
[Pd(L ₁)Cl] (1)	0.90
[Pd(L ₃)Cl] (3)	1.31
BHT	0.67

Table 7 IC₅₀(μg/mL) values of cytotoxicity of Pd(II) complexes.

Compound	Raw	MCF-7	COLO 205
[Pd(L ₁)Cl] (1)	44.0	62.3	63.9
[Pd(L ₂)Cl] (2)	76.0	60.1	63.1
[Pd(L ₃)Cl] (3)	51.0	49.9	56.2
[Pd(L ₄)Cl] (4)	102.4	72.2	59.9
[Pd(L ₅)Cl ₂] (5)	45.2	25.5	51.0
Cisplatin	1.2	1.7	5.6

3.6. Pharmacology

3.6.1. Antimicrobial activity

The minimum inhibitory concentration (MIC) values of Pd(II) complexes are given in Table 5. *In vitro* antimicrobial screening results (Table 5), Pd(II) complexes are potent than their corresponding ligands and compared to the antibiotics, moderately active against tested microorganisms. The activity of the ligands has enhanced on complexation, which can be explained on the basis of Tweedy's chelation theory and Overtones concept (Bahaffi et al., 2012; Mahalakshmi and Rajavel, 2010).

Among all the Pd(II) complexes, complex 2 showed better activity against all microorganisms. Complex 3 and 4 exhibit very low activity against all bacteria and fungi strains. Complex 5 is active against only *B. subtilis* and *S. aureus*.

3.6.2. Free radical scavenging activity

The stable DPPH radical scavenging is a widely used method to evaluate antioxidant activities, in a relatively short time compared with other methods. The DPPH radical contains an odd electron responsible for absorbance at 517 nm. When the reaction between antioxidant molecules and DPPH radical results in the scavenging of the radical by hydrogen or electron donation, the antioxidants cause to a decrease of the absorbance of the DPPH radical. This is visually noticeable as a dis-

coloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the activity of antioxidants (Ceyhan et al., 2011). IC₅₀ values of antioxidant activity of Pd(II) complexes were reported in Table 6. These results indicate that the complexes 1 and 3 have a noticeable activity compared to the remaining Pd(II) complexes. The complexes 2, 4 and 5 do not show antioxidant activity. Among all the complexes, complex 1 showed activity comparable to BHT (standard).

3.6.3. Cytotoxicity

The cytotoxicity of complexes 1–5 was tested on three cell lines, that is murine macrophage cell line Raw 264.7, human breast cancer cell line MCF-7 and human colon carcinoma cell line COLO 205. Cisplatin was used as a positive reference. The activity is expressed as the concentration of the complexes required to inhibit the cellular survival fraction to 50% (IC₅₀) after exposure to the compounds for 48 h. The results are presented in Table 7.

As shown in Table 7, Pd(II) complexes are found to be much less active when compared to cisplatin against three cell lines. Among all complexes pyridine ring containing complexes i.e. complexes 3 and 5 are potent.

3.6.4. Cleavage of pUC19 DNA by Pd(II) complexes

The DNA cleavage reaction of Pd(II) complexes was monitored by agarose gel electrophoresis. When circular plasmid DNA is subject to electrophoresis, relatively fast migration will be observed for the intact supercoiled form (Form I). The slower-moving migration is the open circular form (Form II) which was generated from the supercoil when scission occurred on one of its strands, and a linear form (Form III) which was generated when both strands were cleaved and migrates between Form I and II (Seng et al., 2008). To investigate the mechanism of cleavage studies of Pd(II) complexes, DNA cleavage activity was observed in the presence and absence of H₂O₂ as an oxidant. The gel electrophoretic separation of pUC19 DNA after incubation with all Pd(II) complexes is depicted in Fig. 7a and b. From Fig. 7a, no DNA-cleavage was observed for the control in which the metal complex was absent (lane control). When the pUC19 DNA was treated with the complexes (lane 1–5), no bands were observed in the lane 1, 3 and 5 probably due to the complete degradation of DNA into small pieces. It is reasonably expected due to the hydrolytic cleavage (absence of any reducing or oxidising

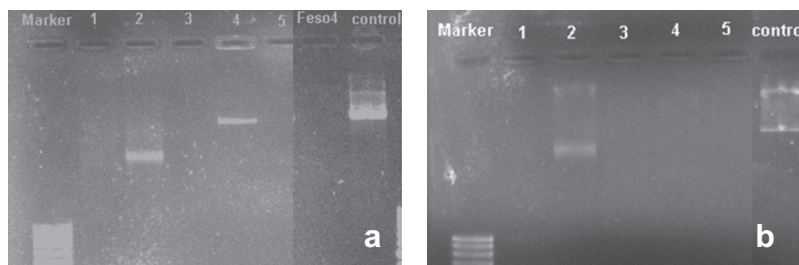


Figure 7 Gel electrophoresis graphs of Pd(II) complexes. (a) DNA cleavage studies in the absence of H₂O₂. Lane 1. DNA + Complex 1; Lane 2. DNA + Complex 2; Lane 3. DNA + Complex 3; Lane 4. DNA + Complex 4; Lane 5. DNA + Complex 5; Lane FeSO₄. DNA + FeSO₄; Lane control. DNA alone (without complexes). (b) DNA cleavage studies in the presence of H₂O₂. Lane 1. DNA + Complex 1 + H₂O₂; Lane 2. DNA + Complex 2 + H₂O₂; Lane 3. DNA + Complex 3 + H₂O₂; Lane 4. DNA + Complex 4 + H₂O₂; Lane 5. DNA + Complex 5 + H₂O₂; Lane control. DNA + H₂O₂.

agents). In lanes 2 and 4 no difference was observed compared to the control band, which indicates the inactivity of complexes 2 and 4 respectively. In the presence of H_2O_2 (Fig. 7b) complex 4 showed complete DNA cleavage and complex 2 showed partial cleavage. In this case, oxidative DNA cleavage was observed, which may be due to the formation of hydroxyl radicals. Many literature reports revealed the mechanism of the DNA cleavage of the metal complexes in the presence of oxidant H_2O_2 (Arish and Sivasankaran Nair, 2011).

4. Conclusions

A series of Pd(II) complexes with tridentate/bidentate Schiff base ligands derived from 3-formyl chromone and aromatic amines were synthesised. Complexes were characterised by physico analytical techniques. Electronic and magnetic data suggest the square-planar geometry for all Pd(II) complexes. Powder XRD data revealed the crystalline nature of the complexes. All complexes displayed solid state fluorescence at room temperature. Pd(II) complexes exhibit less to moderate antimicrobial activity. Complex 1 was considered as lead compounds worthy of further structural optimization and development as potential antioxidants. The successful DNA cleavage was observed for complexes 1, 3 and 5 without any additives. But in the case of complexes 2 and 4 effective DNA cleavage was observed in the presence of H_2O_2 . The cytotoxicity results of the complexes are not satisfactory when compared to cisplatin (anticancer drug).

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