CHAPTER VII
SYNTHETIC, DNA CLEAVAGE AND BINDING ASPECTS OF NOVEL DIAMAGNETIC PALLADIUM(II) COMPLEXES DERIVED FROM PYRIDINE SCAFFOLD
The interaction of transition metal complexes with DNA is a subject of intensive investigation with the perspective of development of newer materials for application in biotechnology, medicine and vibrant area of research\textsuperscript{1-4}. An advantage of using these complexes in such studies is that their ligands and metals can be conveniently varied to suit individual applications. These investigations have resulted in the synthesis of many new metal complexes, which bind to DNA by non-covalent interactions such as electrostatic binding, groove binding and intercalative binding\textsuperscript{5, 6}. Comparative study of DNA binding/cleavage of different metal complexes bearing same organic ligand territory is especially interesting because of their usage as active pharmaceutical ingredients in cancer therapy. Coordination compounds with labile ligands were known to inhibit the replication of cancerous cells since long back, as they can directly change the 3D-structure of DNA with the interaction of N-7 nitrogen of nucleotide. Effectiveness of the DNA cleavage/binding depends on the nucleophilic nature of the labile ligand attached to the metal centre. Hence, it is a burgeoning interest in the synthetic biomimetic anticancer agents with active metal centres which can directly interact with the DNA of infected cells and finally, these potential compounds can be used as structural probes and diagnostic tools in cancer treatment. But, many of DNA binding compounds cannot be active drug whose clinical use suffers from a few important drawbacks, such as high toxicity, lack of oral efficacy and major side effects in the long terms. Therefore attempts are required to change the existing problems.
Palladium is frequently used in the chemical industry as well as in chemical laboratories, and is particularly useful for carbon-carbon bond forming reactions. Furthermore, palladium complexes are usually stable, easy to handle, and non-hazardous. The choice of palladium as one of the metals is based on two factors: (i) it is a homologue of platinum and thus is expected to show similar activity, (ii) it is efficient catalyst precursors.

The palladium(II) complexes with similar nitrogen-donor chelating ligands have been prepared. Palladium(II) complexes are suitable model compounds for mechanistic studies on the action of platinum(II) anticancer drugs, since they exhibit a $10^4$ to $10^5$-fold higher reactivity, whereas their structural and equilibrium behaviors are rather similar\(^7\). Bis-chelated palladium(II) complexes with nitrogen-donor chelating ligands have been used as efficient catalyst precursors for the CO/Styrene copolymerization reaction\(^8\). Substitution reactions of square planar complexes in general and of Pt(II) and Pd(II) in particular have received much attention from various investigators. This interest mainly focuses on the ability to use steric and electronic effects to tune the solubility, acidity and reactivity of such complexes for various applications. Coordination compounds of Pd(II) with the tridentate ligands provide useful substrates for kinetic studies on substitution reactions of square-planar complexes. It is well known that relatively small structural modifications in a multidentate ligand can produce significant changes in the reactivity of the complexes\(^9,10\).

![Chemical structures](image-url)
Functional groups present at 2 and 6 positions of pyridine will aid the potentiality of the chelating power of the ligand and selection of the donor sites at those positions is key point in the designing of the ligand in such a way, that the complexes should retain the labile counter ions as ligands and these are responsible for the DNA cleavage/binding. 1,2,4-Triazoles are associated with diverse pharmacological activities such as analgesic, antiasthmatic, diuretic, antihypersensitive, anticholinergic, antibacterial, antifungal and anti-inflammatory activity\textsuperscript{11-14}. These biological data prompted us to synthesize new pyridine containing 1,2,4-triazole ring. The azole moiety is an important and frequent insecticidal, agrochemical structural feature of many biologically active compounds such as cytochrome P450 enzyme inhibitors\textsuperscript{15} and peptide analog inhibitors\textsuperscript{16}. Recently, much attention has been focused on 1H-1,2,4-triazole derivatives for their broad-spectrum activities, such as fungicidal, herbicidal, anticonvulsant and plant growth regulatory activities\textsuperscript{17-19}. Further, the disubstituted 1,2,4-triazole derivatives were also reported to show antifungal, insecticidal, herbicidal and anti-inflammatory properties which were similar to 1H-1,2,4-triazole derivatives\textsuperscript{20-22}.

Hence, it is an interesting target to design and synthesize the palladium(II) complexes with nitrogen-donor chelating ligands and their in vitro DNA cleavage analysis. The normal skeleton observed in the above ligands was retained in the synthesized ligand. The purpose of research work in this chapter is to synthesise the ligands having similar nitrogen donors,
synthesize, characterize palladium(II) complexes and detailed studies on the DNA interaction mechanism, acute toxicity and pharmacological activities.

**EXPERIMENTAL**

**Materials**

All reagents and solvents were purchased commercially and the chemicals used for the synthesis of ligand and complex were of AR / LR grade. PdCl₂ was obtained from s.d. fine chemicals Limited (India). Herrine sperm DNA were purchased from SRL (India). Deionized water was used for the preparation of the buffers. The concentration of DNA in nucleotide phosphate (NP) was determined by UV absorbance at 260 nm using the molar absorption coefficient as 6600 dm³ mol⁻¹ cm⁻¹. Solutions of Herrine sperm DNA in phosphate buffer gave a ratio of UV absorbance at 260 and 280 nm, A₂₆₀/A₂₈₀, of 1.8 - 1.9:1, indicating that the DNA was sufficiently free of protein²³. The solvents were purified according to the standard procedures³¹. Ligand syntheses and complexation reactions were conducted under dinitrogen unless otherwise stated. Subsequent work-up and recrystallisation procedures were performed under aerobic conditions. ¹H-NMR spectra were recorded on a Bruker 300 MHz NMR spectrometer and IR spectra (pressed KBr discs) were recorded on a Nicolet 5700 FT IR spectrometer.

**Instruments used**

The micro analyses (C, H, and N) were carried out with a Thermo Finnigan FLASH EA 1112 CHNS analyser (IISC, Bangalore). UV-vis spectra were recorded on a double beam Varian CARY 50-BIO UV–vis spectrophotometer (Mulgrave Victoria, Australia). ¹H NMR spectra were measured on a Varian-Mercury 300 MHz spectrometer with (d₆) DMSO as a
solvent at room temperature, and all chemical shifts are given relative to TMS. The infrared spectra of solid samples dispersed in KBr were recorded on a Thermo Nicolet-5700 FT-IR spectrometer (Waltham, MA, USA) equipped with a germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter. LC-ESI-MS (Wald.-bronn, Germany) spectra of ligand solution was carried out with a Hewlett-Packard GmbH, Electrochemical experiments with Pd(II) complex in DMSO solution was performed on a CH-660A (USA) electrochemical instrument in a conventional three-electrode cell assembly with a saturated Ag/AgCl reference electrode, platinum as working electrode and 0.1 mol dm$^3$ tetrabutylammonium hexafluoro phosphate ([But$_4$N]$^+$[PF$_6$]$^-$) as supporting electrolyte for all measurements.

Synthesis of 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-pyridine ($L_1$) and Synthesis of 2-[5-(3,4-dimethoxy-phenyl)-2H-[1,2,4] triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4] trizol-3-yl] -pyridine ($L_2$).

Pyridine-2, 6-dicarboxyamide was prepared from diethyl pyridine -2, 6-dicarboxylate as described previously$^{32}$. Its conversion into 2, 6-dicyanopyridine was effected using a vilsmeier complex as dehydrating agent$^{26}$. The preparation of pyridine-2, 6-dicarboxyhydrazide imide was carried out by treating the cyanide with hydrazine hydrate in the absence of solvent$^{34}$. Pyridine-2, 6-dicarboxyhydrazide imide was then converted into 2, 6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4] triazol-3-yl]pyridine($L_1$) and 2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4] triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4]trizol-3-yl]-pyridine ($L_2$) as follows: a mixture of 0.02 mole of p-anisaldehyde/
veratraldehyde and 0.01 mole of pyridine-2,6-dicarbohydrazide imide in 25 cm$^3$ of ethanol was refluxed for 6 hours. The resulting solids were filtered and recrystallised from chloroform. Ligands, 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1, 2, 4] triazol-3-yl]-pyridine and 2-[5-(3, 4-Dimethoxy-phenyl)-2H-[1,2,4] triazol -3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4] trizol -3-yl] -pyridine were confirmed by IR spectra (Fig.VII(i)(p.188) and Fig.VII(ii)(p.189)), $^1$H-NMR (Fig.VII(iii)(p.190) and Fig.VII(iv)(p.191)), $^{13}$C-NMR and CHN analysis.

The Scheme for synthesis of ligands is given as below

![Synthetic Scheme](image-url)
Fig.VII(i)

FT-IR spectrum of 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-pyridine
Fig. VII(ii)

$^1$H- NMR spectrum of 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-pyridine
Fig.VII(iii)

FT-IR spectrum of 2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4] trizol-3-yl]-pyridine
Fig.VII(iv)

$^1$H- NMR spectrum of 2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-6-
[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4] trizol-3-yl]-pyridine
L₁ Yield: (85%). Elemental analysis calculated for C₂₃H₁₉N₇O₂ (425.16): C 64.93, H 4.5, N 23.05%. Found: C 64.59, H 4.51, N 23.02 %.

¹H NMR (300 MHz, DMSO-d₆, 25 °C): δ = 6.95-8.54 (m, 11H, Ar-H), 6.43(S, 2H, -NH), 3.88(S, 6H, -OCH₃) ppm. IR (KBr): ν = 1621 cm⁻¹ (C=N of triazole ring), 1604 cm⁻¹ (C=N of triazole ring), 1564 cm⁻¹ (C=N of pyridine ring), 3393 cm⁻¹ (N-H) 1247 cm⁻¹ (O-CH₃) and M.P 211° C.

L₂ Yield: (85%). Elemental analysis calculated for C₂₅H₂₃N₇O₄ (485.18): C 61.85%, H 4.78%, N 20.2%. Found: C 61.78%, H 4.9%, N 20.08 %.

¹H NMR (300 MHz, DMSO-d₆, 25 °C): δ = 6.91-8.52 (m, 9H, Ar-H), 6.44(S, 2H, -NH), 3.95(S,6H, -OCH₃), 3.98 (S,6H, -OCH₃) ppm. IR (KBr): ν ) 1567 cm⁻¹ (C=N of pyridine ring), 1620 cm⁻¹ (C=N of 5 membered triazol ring), 3359 cm⁻¹ (N-H) 1236 cm⁻¹ (O-CH₃) and M.P 218° C.

Preparation of palladium complexes (C₁ and C₂) derived from L₁ and L₂

The palladium complexes derived from ligands L₁ and L₂ were prepared and characterized by a known procedure. PdCl₂ (0.42 g, 2.3 m mol) was added to a mixture of hydrochloric acid and water (3 cm³:10 cm³), heated under reflux to obtain a clear red solution. The solution was filtered and the warm solution was added drop wise to a stirred methanolic solution of L (0.5 g, 2.3 m mol) which immediately resulted in yellow precipitate. The solid was filtered and washed with methanol and dried. Pd(II) complexes derived from L₁ and L₂ were confirmed by IR spectra (Fig.VII(v)(p.194) and Fig.VII(vi)(p.195)), ¹H-NMR(Fig.VII(vii)(p.196) and Fig.VII(viii)(197)), ¹³C-NMR and CHN analysis.

C₁ Yield: (67%). Elemental analysis calculated for PdC₂₃H₁₉N₇O₂Cl₂ (601): C 45.83, H 3.18, N 16.27%. Found: C 46.01, H 3.11, N 16.18%.

¹H NMR (300 MHz, DMSO-d₆, 25 °C): δ = 7.03-9.18 ( m, 11H, Ar-H), 10.58(S,
1H, -NH), 10.02 (S, 1H, -NH), 3.89 (S, 6H, -OCH₃) ppm. ¹³C NMR (300 MHz, DMSO-d₆, 25 °C): 56.11, 116.48, 125.32, 8.06, 132.67, 142.51, 144.36, 156.43, 157.12, 163.16. IR (KBr): ν = 1669 cm⁻¹ (C=N of triazole ring), 1605 cm⁻¹ (C=N of triazole ring), 1570 cm⁻¹ (C=N of pyridine ring), 3393 cm⁻¹ (N-H) 1247 cm⁻¹ (O-CH₃). UV-visible (DMSO), λₘₐₓ, nm (log ε): 370(4.48). E½ (V versus Ag/AgCl in MF 25 °C, 0.1 M [(But₄ NPF₆)]: -0.201, 0.372.

C₂ Yield: (67%). Elemental analysis calculated for PdC₂₅H₂₃N₇O₄Cl₂ (661): C 45.3, H 3.5, N 14.79%. Found: C 45.8, H 3.5, N 14.80%. ¹H NMR (300 MHz, DMSO-d₆, 25 °C): δ = 7.01-9.21 (m, 9H, Ar-H), 9.91 (S, 1H, -NH), 10.50 (S, 1H, -NH), 3.82 (S, 6H, -OCH₃), 3.85 (S, 6H, -OCH₃) ppm. ¹³C NMR (300 MHz, DMSO-d₆, 25 °C): 56.33, 56.40, 115.18, 115.72, 115.39, 126.04, 131.60, 131.72, 156.01. IR (KBr): ν ) 1667.8 cm⁻¹ (C=N of 5 membered ring), 1601 cm⁻¹ (C=N of 5 membered ring), 1573 cm⁻¹ (C=N of pyridine ring), 3191 cm⁻¹ (N-H) 1269 cm⁻¹ (O-CH₃). UV-visible (DMSO), λₘₐₓ, nm (log ε): 367 (4.82), E₁/₂ (V versus Ag/AgCl in MF 25 °C, 0.1 M [(But₄ NPF₆)]: E₁/₂ - 0.244V and E₂¹/₂ 0.341V.

**LC-ESI MS spectral studies**

LC-ESI-MS analysis was carried out using a reverse phase high performance liquid chromatography (HPLC) system with a phenomenes C-18 column, HP 1100 series diode array UV/Visible detector and HP 1100 MSD series mass analyzer. 12 µL of ligand solutions were injected. The mobile phase consisted of acetonitrile (eluent A) and tri fluroacetic acid at a flow rate of 1 cm³/minute. UV detection at 367nm.
Fig. VII(v)

FT-IR spectrum of Pd(II) complex derived from 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-pyridine(C₁)
Fig. VII(vi)

$^1$H- NMR spectrum of Pd(II) complex derived from 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]- pyridine
Fig. VII(vii)

FT-IR spectrum of Pd(II) complex derived from 2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4]triazol-3-yl]-pyridine(C₂)
$^1$H-NMR spectrum of Pd(II) complex derived from 2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4] trizol-3-yl]-pyridine
DNA cleavage Analysis

a) Methodology of DNA cleavage Analysis: Nutrient broth (Peptone 10 g/l, NaCl 10 g/l and yeast extract 5 g/l) was used for the growth of the E. coli. 50 cm³ media was prepared, autoclaved for 15 minutes at 121°C, 15 lb pressure. The autoclaved media were inoculated with the seed culture and incubated at 37°C for 24 hours.

b) Isolation of DNA: DNA was isolated by using the mentioned procedure. The fresh bacterial culture centrifuged (1.5 cm³) to obtain the pellet. The pellet was dissolved in 0.5 cm³ of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 50 mM lysozyme) and 0.5 cm³ of saturated phenol added and incubated at 55°C for 10 minutes. It was centrifuged at 10,000 rpm for 10 min and to the supernatant added equal volume of chloroform: isoamyl alcohol (24:1) and 1/20th volume of 3 mol dm³ sodium acetate (pH 4.8). Again it was centrifuged at 10,000 rpm for 10 minutes and to the supernatant, added 3 volumes of chilled absolute alcohol. Finally, the precipitated DNA was separated by centrifugation. The pellet was dried and dissolved in TE buffer (10 mM tris pH 8.0, 1 mM EDTA) and stored in cold condition.

c) Sample preparation and treatment of DNA with the samples: The samples (10mg/cm³) were prepared in DMSO. The synthetic compounds (100µg) were added separately to the DNA sample of E. coli. The sample mixtures were incubated at 37°C for 2 hours.

d) Agarose gel electrophoresis: Following the treatment of DNA samples, the electrophoresis of the samples was done according to the following procedure. 200mg of agarose was weighed and dissolved in 25 cm³ of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA / 1 L) by boiling. When the gel
attains ~55°C, it poured into the gel cassette fitted with comb. Let the gel to solidify. The comb was carefully removed and the gel was placed in the electrophoresis chamber flooded with TAE buffer. 20 µl of DNA sample was loaded (mixed with bromophenol blue dye at 1:1 ratio) into the wells carefully along with standard DNA marker and the constant 50 V of electricity was passed for around 30 minutes. The gel was removed and stained carefully with ETBR solution (10 µg/cm³) for 10-15 minutes and finally the band under UV transilluminator was observed (Fig.VII(ix a)(p.201) and Fig.VII(ix b)(p.201)).

**DNA binding studies**

a) Absorption spectral studies: For electronic absorption titration, a stock 30 µM solution of the complexes were made up in a tris-HCl buffer (pH 7.2); 3000 µL of the solution was loaded into an optical glass cuvette with a path length of 1 cm, and 10 µL was removed with a micropipette and replaced with 10 µL of the complexes solution. This cuvette was then loaded into the spectrometer sample block, controlled at 25°C; 3000 µL of the buffer was loaded to an identical cuvette and placed in the reference cell. Both the cuvettes were mixed 30 times with a micropipette, and all bubbles were removed. After the cuvettes had been allowed to reach equilibrium over the course of 20 minutes, a spectrum was recorded between 700 and 200 nm. 1 to 30µL of herring sperm was added to both cuvettes and mixed thoroughly. The spectrum was recorded after checking for bubbles and showed increasing in absorptivity showing interaction between the DNA and the metal complexes (Fig.VII(x a)(p.202) and Fig.VII(xi b)(p.202)). The intrinsic binding constant $K_b$ was determined $^{30, 31}$ from the plot of $A_0/[A - A_0]$ versus $[DNA]^{-1}$ according to equation(1), where $[DNA]$ is the concentration of DNA in base pairs.
where $A_0$ and $A$ are the absorbance observed for MLCT absorption band for the free complex and absorbance observed for MLCT absorption band at given DNA concentration respectively. $[DNA]$ is the concentration of DNA in base pairs, $\varepsilon_G$ and $\varepsilon_{H-G}$ are the apparent absorption coefficients in free and DNA bounded form of complex respectively. The data were fitted to above equation to obtain graph, with a slope equal to $\varepsilon_G / [\varepsilon_{H-G} - \varepsilon_G] \times 1/K_b$ and intercept equal to $\varepsilon_G / [\varepsilon_{H-G} - \varepsilon_G]$ hence $K_b$ was obtained from the ratio of the intercept to the slope (inset Fig.VII(xa)(p.202) and inset Fig.VII(x b)(p.202))\(^{30}\).

b) Viscosity measurements: Viscosity experiments were carried out using a semimicro viscometer maintained at 23°C in a thermostatic water bath. Flow time of solutions in tris-HCl buffer (pH 7.2) was recorded in triplicate for each sample, and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio (Fig.VII(xi a)(p.203) and Fig.VII(xi b)(p.203)), where $\eta$ is the viscosity of DNA in the presence of complex and $\eta_0$ is the viscosity of DNA alone\(^{31}\).

c) Thermal denaturation study: DNA melting studies were carried out with a Varian CARY 50-BIO UV-vis. spectrophotometer (Mulgrave Victoria, Australia) equipped with a 150W Xenon lamp attached with a Peltier temperature controlling programmer ETC-717 ((0.1°C) in tris-HCl buffer (pH 7.2). The DNA melting studies were done by controlling the temperature of the sample cell with a water circulating bath. UV melting profiles were obtained by scanning A260 absorbance was monitored at a heating rate of 1 °C/min for solutions of HS-DNA (100 μM) in the absence and presence of paladium(II)
Fig. VII(ix a)

DNA cleavage of 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-pyridine(Pani, 50µg) and Pd(II) complex derived from 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-pyridine(Pdani:50µg, Pdani:100µg)

lane M  lane C  lane I  lane II  lane III

M C Pdani Pdani Pani
50 µg  100 µg

21.226 kbp
5.148
3.53
2.02

Fig. VII(ix b)

DNA cleavage of 2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4]triazol-3-yl]-pyridine (Vet, 50µg) and its Pd(II) complex (Pdvet:50µg, Pdvet:100µg)

lane M  lane C  lane I  lane II  lane III

M C Pdvet Pdvet Vet
50 µg  100 µg

21.226 kbp
5.148
3.53
2.02
**Fig. VII(x a)**

Changes in the electronic absorption spectra of Pd(II) complex derived from L₁ (10 μM) with increasing the concentrations (0-100 μM) of Herrine sperm-DNA (Tris HCl buffer pH 7.2); the inset graph shows a fit of the absorbance data used to obtain the binding constant.

![Absorbance spectra with inset graph](image1)

**Fig. VII(x b)**

Changes in the electronic absorption spectra of Pd(II) complex derived from L₂ (10 μM) with increasing the concentrations (0-100 μM) of Herrine sperm-DNA (Tris HCl buffer pH 7.2); the inset graph shows a fit of the absorbance data used to obtain the binding constant.

![Absorbance spectra with inset graph](image2)
Fig. VII(xi a)

Increasing effect of complex on the relative viscosities of Herrine sperm DNA at 25°C: (a) 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-pyridine (L₁), (b) Pd(II) complex (C₁)

Fig. VII(xi b)

Increasing effect of complexes on the relative viscosities of Herrine sperm DNA at 25°C: (a) 2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4]triazol-3-yl]-pyridine (L₂), (b) Pd(II) complex (C₂)
complexes (20 μM) from 25 to 90 °C. The data were analyzed with the use of separate thermal melting program; the temperature of the cell containing the cuvette was ramped from 50 to 90 °C. The melting temperature $T_m$ which is defined as the temperature where half of the total base pairs are unbound was determined from the midpoint of the melting curves (Fig. VII(xii a)(p.205) and Fig. VII(xii b)(p.205)).

**Antimicrobial activity**

The antimicrobial activity of ligands and its Pd(II) complexes of the G. livingstonie were tested towards 11 different microorganisms. Eight bacteria namely Bacillus subtilis, Micrococcus, Methicillin resistant Staphylococcus aureus, Staphylococcus epidermidis, Escherthia coli, Providencia alcalifaciens, Proteus mirabilis Salmonella paratyphi A. and Aspergillus niger, Penicilium notatum, Candida albicans were the three fungi used for the study. All the micro-organisms used for the analysis were obtained from S.D.M. Medical College, Dharwad, Karnataka, India. Antibacterial activity of the samples were tested by the paper disc diffusion method according to the National Committee for Clinical Laboratory Standards Guidelines$^{32}$ with some modifications, using 100 μL of suspension of the test microorganisms, containing $2.0 \times 10^6$ colony forming units (cfu/cm$^3$) for inoculating the plates$^{33}$. Test ligands and its Pd(II) complexes were prepared in DMSO (Dimethyl sulfoxide) (HiMedia Laboratories Pvt. Limited. India) and loaded (10 μL) onto sterile filter paper discs (6 mm diameter, HiMedia Laboratories Pvt. Limited. India), which finally contained 50 μg/μL of the compound per disc. Since the ligands and its Pd(II) complexes were dissolved in DMSO, it was used as control by loading 10 μL.
Fig.VII(xii a)

Relative A/Ao versus temperature for melting of Herrine sperm DNA: (a) only DNA spectra (b) DNA+2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-pyridine(L₁) (c) DNA+ Pd(II) complex (C₁)

![Graph](image1)

Fig.VII(xii b)

Relative A/Ao versus temperature for melting of Herrine sperm DNA: (a) only DNA spectra (b) DNA+2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4] trizol-3-yl]-pyridine (L₂) (c) DNA+ Pd(II) complex (C₂)

![Graph](image2)
of DMSO onto the disc. Impregnated disks were then dried for 1 hour and placed on inoculated plates. The seeded plates were incubated at 37°C for 16 hours and 25°C for 72 hours for bacteria and fungi, respectively. The diameters of the inhibition zones were measured in millimeters. Studies were performed in triplicates, and the developing inhibition zones were compared with those of reference discs. Antibiotic streptomycin was used as reference for bacteria and nystatin for fungi. The minimal inhibitory concentrations (MIC) of the compounds were determined by micro dilution assay. The compounds were two-fold serially diluted with DMSO which contains 0.125-16 μg/μL of extract. The MIC was defined, as the lowest concentration at which there was 100% inhibition of growth compared with the growth for a drug free control. In order to ensure that the solvent had no effect on microbial growth, a control test was also performed containing broth supplemented with only DMSO at the same dilution used in the assay. Each experiment for the antibacterial assay and MIC was repeated thrice.

RESULTS AND DISCUSSION

Molar conductance

The molar conductivities of the complexes in DMSO (3 x 10^{-3} mol dm^{-3}) fall in the range 0.027 x 10^{-3} and 0.023 x 10^{-3} Ω^{-1}cm^{2}mol^{-1} for C_{1} and C_{2} respectively and suggests non-electrolytic nature of the complexes.

Electronic spectra

In the present studies, both the palladium(II) complexes were found to be diamagnetic in nature, obviously the geometry is square planar. The geometries are supported by their electronic spectrum. The electronic spectral
data of ligands and their Pd(II) complexes are given in Table VII(i) (p.209). Palladium(II) in both complexes is having $d^8$ system and three spin allowed singlet–singlet $d-d$ transitions are predicted $^{34,35}$. The ground state is $^1A_{1g}$ and the three predicted transitions are $^1A_{1g}$ to $^1A_{2g}$, $^1A_{1g}$ to $^1B_{1g}$ and $^1A_{1g}$ to $^1E_g$. These transitions take place from the lower lying $d$ orbitals to the empty $d_{x^2-y^2}$ orbital. Strong charge-transfer transitions may interfere and prevent observation of all the expected bands $^{34,36-39}$. Strong bands between 350 to 400 nm are assignable to a combination of M to LCT (metal-ligand charge-transfer). Solutions of ligand in DMSO feature a strong band at ca. 367 nm (log $\varepsilon$ = 4.81) for $L_1$ and 368 nm (log $\varepsilon$ = 4.84) for $L_2$ respectively, because of the $n-\pi^*$ transition for the azomethine function with shoulders at higher and lower energy. These values show very little shift in complexes but the intensity is enhanced. An absorption band observed at ca. 280 nm, is assigned to the $\pi-\pi^*$ intraligand electron transition, $\text{N}^\equiv\text{C}^\equiv\text{N}$. Shift of this band upon complexation, reveals its involvement in the coordination.

**Infrared and $^1$H NMR spectral studies of ($L_1$), ($C_1$) and ($L_2$), ($C_2$).**

The diagnostic IR and $^1$H NMR spectral studies of the free ligands and the Pd(II)complexes are discussed below.

The pertinent infrared absorption bands of the ligands and their Pd(II) complexes are tabulated in Table VII(ii)(p.210). The spectrum of the free ligand ($L_1$) shows a band of medium intensity at 3393 cm$^{-1}$ which is assigned to $\nu$(NH) and intensity at 1247 cm$^{-1}$ which is assigned to $\nu$(OCH$_3$). The sharp band around 1564-1621 cm$^{-1}$ assigned to $\nu$(C=N) azomethine nitrogens of $L_1$. Pyridine ring $-\text{CH}$ in plane, out of plane bending appeared at 993 cm$^{-1}$ and 749 cm$^{-1}$ respectively. In the proton NMR studies of $L_1$, signal due to $-\text{OCH}_3$
appeared at 3.88 ppm and \(-\text{NH}\) appeared at 6.43 ppm (D\(_2\)O exchangeable) in the spectrum of free ligand L\(_1\). The aromatic protons signals are appeared between 6.95 - 8.54 ppm (Table VII (i) (p.209)).

The sharp band around 1564-1621 cm\(^{-1}\) assigned to \(v(\text{C=N})\) in the free ligands has been shifted in the spectra of the complex (C\(_1\)), suggesting coordination of both azomethine nitrogens to the metal ion. Appearance of amide nitrogen \(v(\text{NH})\) peak at 3393 cm\(^{-1}\) indicates, its non-involvement in the coordination. The azomethine nitrogens (\(\text{C=N}\)) of triazole ring stretching frequency 1621 cm\(^{-1}\) shifted to 1669 cm\(^{-1}\). Pyridine ring azomethine nitrogens (\(\text{C=N}\)) stretching vibrations observed at 1564 cm\(^{-1}\) and shifted to 1570 cm\(^{-1}\). Appearance of other azomethine nitrogens (\(\text{C=N}\)) stretching frequency at 1605 cm\(^{-1}\) of side ring does not take part in coordination. In the proton NMR spectrum of C\(_1\), signal due to \(-\text{OCH}_3\) appeared at of 3.89 ppm and two signals for two different NH groups appeared in the region 10.02 and 10.58 ppm (D\(_2\)O exchangeable). The aromatic protons signals are appeared between 7.03 - 9.18 ppm.

The spectrum of the free ligand (L\(_2\) shows a band of medium intensity at 3359 cm\(^{-1}\) which is assigned to \(v(\text{NH})\) and intensity at 1266 cm\(^{-1}\) which is assigned to \(v(-\text{OCH}_3)\). The sharp band around 1567-1621 cm\(^{-1}\) are assigned to \(v(\text{C=N})\) azomethine nitrogens of L\(_2\). Pyridine ring \(-\text{CH}\) in plane, out of plane bending appeared at 960 cm\(^{-1}\) and 750 cm\(^{-1}\) respectively. In the proton NMR studies of L\(_2\), signal due to two \(-\text{OCH}_3\) groups appeared at 3.95, 3.98 ppm and \(-\text{NH}\) appeared at 6.44 ppm (D\(_2\)O exchangeable) in the spectrum of free ligand L\(_2\). The aromatic protons signals are appeared between 6.91 - 8.52 ppm.
Table VII(i)

$^1$H NMR and UV absorption spectral data of ligands ($L_1$ and $L_2$) and its Pd(II) complexes ($C_1$ and $C_2$)

<table>
<thead>
<tr>
<th></th>
<th>CH(Ph)</th>
<th>–OCH$_3$</th>
<th>–NH–</th>
<th>$\lambda_{\text{max}}$ (nm) (ε(dm$^3$ mol$^{-1}$ cm$^{-1}$))</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_1$</td>
<td>6.95–8.54(m)</td>
<td>3.88(s)</td>
<td>6.43(s)</td>
<td>364 (65361)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>257(26500)</td>
</tr>
<tr>
<td>Pd(II) complex derived from $L_1$</td>
<td>7.03–9.18(m)</td>
<td>3.89(s)</td>
<td>10.02(s)</td>
<td>378 (32422)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.58(s) 281(31305)</td>
</tr>
<tr>
<td>$L_2$</td>
<td>6.91–8.52(m)</td>
<td>3.95(s)</td>
<td>3.98(s)</td>
<td>370 (68430)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>268 (14948)</td>
</tr>
<tr>
<td>Pd(II) complex derived from $L_2$</td>
<td>7.01–9.21(m)</td>
<td>3.82(s)</td>
<td>9.91(s)</td>
<td>372 (30769)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.50(s) 372(30769)</td>
</tr>
<tr>
<td></td>
<td>-C = N</td>
<td>-NH</td>
<td>-OCH₃</td>
<td>N → Pd</td>
</tr>
<tr>
<td>----------------</td>
<td>----------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>L₁</strong></td>
<td>1621 cm⁻¹</td>
<td>3393 cm⁻¹</td>
<td>1247 cm⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1604 cm⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1564 cm⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pd(II)</strong></td>
<td>1669 cm⁻¹</td>
<td>3390 cm⁻¹</td>
<td>1245 cm⁻¹</td>
<td>474 cm⁻¹</td>
</tr>
<tr>
<td>complex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>derived from</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L₁</td>
<td>1605 cm⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1570 cm⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>L₂</strong></td>
<td>1621 cm⁻¹</td>
<td>3359 cm⁻¹</td>
<td>1266 cm⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1567 cm⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pd(II)</strong></td>
<td>1668 cm⁻¹</td>
<td>3359 cm⁻¹</td>
<td>1268 cm⁻¹</td>
<td>473 cm⁻¹</td>
</tr>
<tr>
<td>complex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>derived from</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L₂</td>
<td>1601 cm⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1573 cm⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The sharp band at 1567, 1621 cm\(^{-1}\) are assigned to v(C=N) in the free ligand have been shifted in the spectra of the complexes, suggesting coordination of both azomethine nitrogens to the metal ion. Appearance of amide nitrogen v(NH) peak at 3359 cm\(^{-1}\) indicates, its non-involvement in the coordination. The azomethine nitrogens (C=N) of triazole ring stretching frequency 1621 cm\(^{-1}\) shifted to 1667 cm\(^{-1}\). Pyridine ring azomethine nitrogens (C=N) stretching vibrations observed at 1567 cm\(^{-1}\) and shifted to 1573 cm\(^{-1}\). Appearance of other azomethine nitrogens (C=N) stretching frequency at 1601 cm\(^{-1}\) of side ring does not take part in coordination. In the proton NMR spectra of C\(_2\), signal due to two –OCH\(_3\) appeared at of 3.82, 3.85 ppm and two signals for two different NH groups appeared in the region 9.91 and 10.50 ppm (D\(_2\)O exchangeable). The aromatic protons signals are appeared between 7.01-9.21 ppm.

**LC-ESI MS spectral studies of ligands**

LC-ESI-MS analysis of the ligands showed the molecular ion of m/z 430 (Yield 99%) corresponds to 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-pyidine(L\(_1\)) (Fig.VII(xiii a)(p.212)) and the molecular ion of m/z 485 (Yield 99%) corresponds to 2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4]trizol-3-yl]-pyridine (L\(_2\)) (Fig.VII(xiii b)(p.212)).
Fig. VII(xiii a)

LC-ESI-MS spectrum of 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-pyridine

Fig. VII(xiii b)

LC-ESI-MS spectrum of 2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4]trizol-3-yl]-pyridine
Cyclic voltammetric studies

The ligands and complexes (0.003 mol dm\(^3\)) were scanned in the potential range -1.0V to 1.0V. In this potential range, studied ligand was observed to be electrochemically inactive. Analysis of the voltammograms reveals that only the Pd(II) complexes (Fig.VII(xiv a)(p.214)and Fig.VII(xiv b)(p.214)) shows response at the scan rate of 0.1V/s found to be electrochemically active. An anodic peaks observed in the voltammogram at \(E_{pa1} = 0.0072V\) & \(E_{pa2} = 0.65V\) for C\(_1\) and \(E_{pa1} = -0.0925V\) & \(E_{pa2} = 0.6120V\) for C\(_2\), showed the oxidation of \(\text{Pd(II)} \rightarrow \text{Pd(III)} & \text{Pd(III)} \rightarrow \text{Pd(IV)}\). During the reverse scan two cathodic peaks are observed at \(E_{pc1} = -1.2196V\) & \(E_{pc2} = -0.626V\) for C\(_1\) and \(E_{pc1} = -1.191 V\) & \(E_{pc2} = -0.5857 V\) for C\(_2\) corresponding to \(\text{Pd(IV)} \rightarrow \text{Pd(III)} & \text{Pd(III)} \rightarrow \text{Pd(II)}\) reductions. The couple \(\text{Pd(II)}/\text{Pd(III)}\) shows \(\Delta E_p = E_{pa} - E_{pc} = 1.23 \text{ mV}\) and \(1.1V\) and \(\text{Pd(III)}/\text{Pd(IV)}\) shows \(\Delta E_p = E_{pa} - E_{pc} = 1.28 \text{ mV}\) and \(1.2V\) indicating the quasi reversible redox process.

The voltammogram concludes the two electron process and the detailed studies necessary to understand the nature the chemical reactions following electron transfers which is helpful in the enzymatic catalysis are beyond the scope of the present investigation. Further scan rate was increased from 0.01 to 0.2 and the effect of which on peak current was observed. It was observed that as scan rate was increased peak current also got increased. From the plot of peak current versus square root of scan rate (Fig.VII(xv a)(p.215)and Fig.VII(xv b)(p.215)) concludes that redox process of palladium is diffusion controlled electrode process\(^40\).

213
Fig. VII(xiv a)
Cyclic voltammograms of Pd(II) complex derived from 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]- pyridine (3 mM) with different scan rate. (a) 0.01; (b) 0.02; (c) 0.03; (d) 0.05; (e) 0.08; (f) 0.1 and (g) 0.2

Fig. VII(xiv b)
Cyclic voltammograms of Pd(II) complex derived from 2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4] trizol-3-yl]-pyridine (3 mM) with different scan rate (a) 0.01; (b) 0.02; (c) 0.03; (d) 0.05; (e) 0.08; (f) 0.1 and (g) 0.2
Fig. VII(xv a)

Plot of peak current versus square root of scan rate of Pd(II) complex derived from 2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]- pyridine (3 mM)

Fig. VII(xv b)

Plot of peak current versus square root of scan rate of Pd(II) complex derived from 2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4] trizol-3-yl]-pyridine (3 mM)
DNA Cleavage

Gel electrophoresis is an extensively used technique for the analysis of nucleic acids; in this method segregation of the molecules will be on the basis of their relative rate of movement through a gel under the influence of an electric field. DNA is negatively charged and when it is placed in an electric field, it migrates towards the anode; the extent of migration of DNA is decided by the strength of electric field, buffer, and density of agarose gel and size of the DNA. Generally it is seen that mobility of DNA is inversely proportional to its size. Gel electrophoresis pictures are shown in Fig.VII(ix a)(p.201) and Fig.VII(ix b)(p.201). The photograph shows the bands with different bandwidth and brightness compared to the control. The difference observed in the intensity and the band width is the criterion for the evaluation of binding ability of ligands and its Pd(II) complexes with DNA of E. coli. Lane-C representing control experiment, where unaided DNA is used, does not show any significant cleavage of DNA even after a longer exposure time. Lane-I, Lane-II corresponding to Pd(II) complexes (50µg and 100µg) and Lane-III corresponding to ligands (50µg) showed the streak in all the treated samples indicating the activity unspecific cleavage of DNA by the samples.

DNA binding studies

Absorption spectroscopy is one of the most useful techniques to study the binding of any drug to DNA. The binding behavior of ligand and its Pd(II) complexes to DNA helix have been followed through absorption spectral titrations. The absorption spectra of the complexes, palladium complex C₁ and C₂, in the absence and in the presence of Herrine sperm DNA, are shown in
Fig.VII(x a)(p.202) and Fig.VII(xi b)(p.202). With increasing concentration of Herrine sperm DNA, the absorption bands of the ligand and its complex were affected, resulting in the tendency of hyperchromism and a slight blue shift. The palladium complex C₁ and C₂ can bind to the DNA in different binding modes on the basis of their structure and charge and type of ligands. Since DNA possesses several hydrogen bonding sites in the minor as well as major grooves, and the palladium complex C contain –NH groups, there could be hydrogen bonding between the complexes and the base pairs in DNA⁵²⁻⁵⁵. However, the hyperchromism effects observed in the present study in respect of both the ligand and complex suggest that there is a strong hydrophobic association between the palladium complex and the hydrophobic interior of DNA. In order to compare the binding strengths of the complexes, the intrinsic binding constant, Kₘ, was determined using the equation (1). We have calculated the intrinsic binding constant value for palladium complex C. The intrinsic binding constants for the L₁, L₂ and their palladium complexes C₁, C₂ are shown in Table VII(iii)(p.219). The table shows that the binding constants of complexes are higher than ligands. Due to the higher hydrophobicity, the complexes binds with DNA more strongly than ligands.

The thermodynamic parameters, enthalpy change (ΔH⁰) and entropy change (ΔS⁰) of Pd (II) complexes derived from L₁ and L₂ interaction are important for confirming binding mode. For this purpose, the temperature dependence of binding constant was studied. Binding studies were carried out at 286 and 306K at which DNA does not undergo any structural degradation. The molecular forces contributing to DNA interactions with small molecular
substrates may include van der Waals interactions, hydrogen bonds, electrostatic and hydrophobic interactions and so on\textsuperscript{49}. The thermodynamic parameters were evaluated using the equations:

\[ \Delta G^0 = \Delta H^0 - T\Delta S^0 \]  \hspace{1cm} (5)

The plot of \( \log K_b \) versus \( 1/T \) enabled the determination of the values of \( \Delta H^0 \) and \( \Delta S^0 \) (Fig.VII(xvi a)(p.220) and Fig.VII(xvi a)(p.220)). Ross and Subramanian\textsuperscript{57} have characterized the sign and magnitude of the thermodynamic parameters associated with various individual kinds of interaction. For typical hydrophobic interactions, both \( \Delta H^0 \) and \( \Delta S^0 \) are positive, while these are negative for van der Waals forces and hydrogen bond formation in low dielectric media\textsuperscript{50, 51}. Moreover, the specific electrostatic interaction between ionic species in an aqueous solution is characterized by positive \( \Delta S^0 \) value and negative \( \Delta H^0 \) value (small). From Table VII(iii)(p.219), it can be seen that the negative sign for free energy (\( \Delta G \)) means that the interaction process is spontaneous. For the Pd complexes, the main source of \( \Delta G^0 \) value was derived from a large contribution of \( \Delta S^0 \) term with a little contribution from factor \( \Delta H^0 \). So, the main interaction between Pd complexes and DNA was believed to be hydrophobic forces. The positive enthalpy (\( \Delta H^0 \)) and entropy (\( \Delta S^0 \)) values of the interaction of DNA-Pd complexes derived from \( L_1 \) and \( L_2 \) and DNA, indicate that the binding is mainly entropy-driven and the enthalpy is unfavorable for it, the hydrophobic forces played major role in the reaction\textsuperscript{50}.
Table VII(iii)

Thermodynamic parameters of the interaction of ligands \((L_1 \text{ and } L_2)\) and its Pd(II) complexes with DNA system

<table>
<thead>
<tr>
<th>System</th>
<th>Temp. (K)</th>
<th>Intrensic Binding constant ((K_b \times 10^{-4}) \text{ L mol}^{-1})</th>
<th>(\Delta H^0 \text{ (k J mol}^{-1}))</th>
<th>(\Delta S^0 \text{ (J K}^{-1} \text{ mol}^{-1}))</th>
<th>(\Delta G^0 \text{ (k J mol}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,6-Bis-[5-(4-methoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-pyridine((L_1))-DNA Pd(II) complex derived from (L_1)</td>
<td>296</td>
<td>0.21 ± 0.01</td>
<td>29.9 ±3</td>
<td>164.7 ±1.0</td>
<td>-19.7 ±3.0</td>
</tr>
<tr>
<td></td>
<td>301</td>
<td>0.26 ± 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>306</td>
<td>0.32 ± 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pd(II) complex derived from (L_2)</td>
<td>296</td>
<td>0.46 ± 0.01</td>
<td>70.44 ±3</td>
<td>308±3.0</td>
</tr>
<tr>
<td></td>
<td>301</td>
<td>0.74 ± 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>306</td>
<td>1.17 ± 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-[5-(3,4-Dimethoxy-phenyl)-2H-[1,2,4]triazol-3-yl]-6-[5-(3,4-dimethoxy-phenyl)-4H-[1,2,4]triazol-3-yl]-pyridine ((L_2))-DNA Pd(II) complex derived from (L_2)</td>
<td>296</td>
<td>0.18 ± 0.001</td>
<td>41.7 ±3</td>
<td>203.0±1</td>
<td>-18.5 ±3</td>
</tr>
<tr>
<td></td>
<td>301</td>
<td>0.25 ± 0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>306</td>
<td>0.32 ± 0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pd(II) complex derived from (L_2)</td>
<td>296</td>
<td>0.48 ± 0.001</td>
<td>22.5 ±3</td>
<td>147.0±1</td>
</tr>
<tr>
<td></td>
<td>301</td>
<td>0.58 ± 0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>306</td>
<td>0.65 ± 0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig.VII(xvi a)

van’t Hoff plot for the binding of Pd(II) complex (C₁) with Herrine sperm DNA

Fig.VII(xvi b)

van’t Hoff plot for the binding of Pd(II) complex (C₂) with Herrine sperm DNA
To further clarify the binding modes of the complexes with DNA, viscosity measurements were carried out. Spectroscopic experiments provide necessary data, but not sufficient clues to support a binding mode. Viscosity measurements that are sensitive to length change are regarded as the least ambiguous and the most critical tests of the binding model in solution in the absence of crystallographic structural data\textsuperscript{52}. The viscosity of DNA increases as the increase in the ratio of complexes to DNA indicates the intercalative mode of binding\textsuperscript{52}. The representative graph of the (L\textsubscript{1}), (L\textsubscript{2}) and their Pd(II) complexeses (C\textsubscript{1}), (C\textsubscript{2}) are shown in Fig.VII(xi a)(p.203) and Fig.VII(xi b)(p.203).

Thermal behaviors of DNA in the presence of complexes can give insight in to their conformational changes when temperature is raised, and provide the information about the interaction strength of complexes with DNA\textsuperscript{53}. The T\textsubscript{m} of Herrine sperm DNA in absence of the complexes is found to be 60 \textdegree{}C\textsuperscript{54} (Fig.VII(xii a)(p.205)and Fig.VII(xii b)(p.205)). On the bases of viscometric measurements, (L\textsubscript{1}), (L\textsubscript{2}) and (C\textsubscript{1}), (C\textsubscript{2}) have chosen for the thermal denaturation experiment, due to their stronger binding capability with the Herrine sperm DNA. However, with addition of (L\textsubscript{1}) and (C\textsubscript{1}) the T\textsubscript{m} of the DNA increases to 63.0 and 64.0 \pm 1\textdegree{}C respectively. While, with addition of (L\textsubscript{2}) and (C\textsubscript{2}), the T\textsubscript{m} of the DNA increases to 62.0 and 65.0 \pm 1\textdegree{}C respectively. These are characteristic of an intercalative binding behavior of the complexes\textsuperscript{55}.

**Acute toxicity of palladium complexes derived from L\textsubscript{1} and L\textsubscript{2} against Wister albino rats**

The study was carried out according to OECD guidelines 423(OECD, 1992). Nine female Wister albino rats weighing (150-200gm) were taken and fasted over night prior to the acute experimental procedure. Next day, body
weight was taken and synthesized compounds were orally fed to the animals at the dose of 3000mg/kg in 0.3% w/v of carboxy methyl cellulose sodium. Then the animals were observed for mortality at, 0, $\frac{1}{2}$, 1, 2, 4, 6, 8, 12 and 24 hours. Food was given to these animals after 4 hours of dosing and the body weight was checked at 6 hours after dosing, mobility like convulsions, tremours, grip strength lethargy, ptosis and pupil dilation were observed. The animals were observed twice a day for one week and body weight was noted. One tenth of median lethal dose ($LD_{50}$) was taken as an effective dose ($ED_{50}$). The testing of the acute toxicity of palladium complexes derived from $L_1$ and $L_2$ against Wister albino rats suggests that palladium complexes ($C_1$ and $C_2$) have shown $ED_{50}$ 300mg/Kg and 270mg/Kg body weight, respectively. Effect of ligands against Wister albino rats were not significant.

**Antimicrobial activity**

The microbial results of ligand (L) and its pd(II) complexes are systemized in Table VII(iv)(p.223), Fig. VII(xvii a)(p.224) and Fig. VII(xvii b)(p.224). Pd(II) complexes have shown moderate activity against microbial like Bacillus subtilis, Micrococcus, Methicillin resistant Staphylococcus aureus, Escherthia coli, Proteus mirabilis and Salmonella paratyphi A, Candida albicans and prominent activity against Providencia alcalifaciens. Ligands have shown moderate activity against bacteria namely Bacillus subtilis, Proteus mirabilis, Escherthia coli, Salmonella paratyphi A and fungi Candida albicans. The prepared ligand was found to be moderately active against microbes and show enhancement in its activity upon complexation. This higher toxicity is may be due to the effect of metal ions in the normal cell process.
Table VII(iv)

Antimicrobial activity of ligand and its Pd(II) complex for microorganisms with Disc diffusion method (mm) and Minimum inhibitory concentration (µg/µl)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Ligand(L₁) mm MIC</th>
<th>Pd(II) complex(C₁) Mm MIC</th>
<th>Ligand(L₂) mm MIC</th>
<th>Pd(II) complex(C₂) mm MIC</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>9 ≤2.0</td>
<td>11 1.0</td>
<td>10 1.0</td>
<td>8 ≤2.0</td>
<td>25 ≤0.010</td>
</tr>
<tr>
<td>Micrococcus</td>
<td>- ≤2.0</td>
<td>9 2.0</td>
<td>9 ≤2.0</td>
<td>- ≤2.0</td>
<td>35 ≤0.005</td>
</tr>
<tr>
<td>M.R.S.A</td>
<td>- ≤4.0</td>
<td>8 2.0</td>
<td>- 2.0</td>
<td>- 2.0</td>
<td>29 ≤0.005</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 ≤0.005</td>
</tr>
<tr>
<td>E.coli</td>
<td>13 ≤1.0</td>
<td>15 ≤1.0</td>
<td>11 ≤1.0</td>
<td>- ≤0.020</td>
<td>16 ≤0.020</td>
</tr>
<tr>
<td>Pr. alcalifaciens</td>
<td>≤0.125</td>
<td>≤0.125</td>
<td></td>
<td></td>
<td>11 ≤0.020</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>8 ≤2.0</td>
<td>11 ≤1.0</td>
<td>10 ≤1.0</td>
<td>9 ≤1.0</td>
<td>25 ≤0.010</td>
</tr>
<tr>
<td>S. paratyphi A</td>
<td>≤2.0</td>
<td>≤1.0</td>
<td>≤1.0</td>
<td>≤2.0</td>
<td>18 ≤0.020</td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>223</td>
</tr>
<tr>
<td>A. niger</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13 ≤0.040</td>
</tr>
<tr>
<td>P. notatum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.9 ≤0.160</td>
</tr>
<tr>
<td>C. albicans</td>
<td>8 ≤4.0</td>
<td>11 ≤1.0</td>
<td>10 ≤1.0</td>
<td>8 ≤2.0</td>
<td>12 ≤0.160</td>
</tr>
</tbody>
</table>
Fig.VII(xvii a)

Antibacteriogram of ligand (1), Pd(II) complex (2) and streptomycin(3).

Fig.VII(xvii b)

Antibacteriogram of ligand (1), Pd(II) complex (2) and streptomycin(3).
FINDINGS OF THE CHAPTER VII

The new ligands and their complexes \([\text{PdCl}_2(L)]\) have been characterized by elemental analyses (CHN analysis), electro chemical and spectroscopic studies (IR, \(^1\text{H}-\text{NMR}, \text{UV- Vis. and LC-ESI MS}\)). The complexes were prepared for detailed studies on the DNA interaction mechanism, pharmacological activities and cytotoxic activity. Strong bands in the range of 350-400 nm are assignable to a combination of M to LCT (metal-ligand charge-transfer) and d–d bands, which support the idea of a square-planar environment for the metal ions. The testing of the acute toxicity of palladium complexes derived from \(L_1\) and \(L_2\) against Wister albino rats suggests that palladium complexes \((C_1\) and \(C_2)\) have shown \(\text{ED}_{50}\) 300mg/Kg and 270mg/Kg body weight, respectively. \(\text{Pd(II)}\) complexes bind to Herrine sperm DNA through hydrophobic forces. Due to the higher hydrophobicity, the complex binds with DNA more strongly than ligand. So, the main interaction between Pd complex and DNA was believed to be hydrophobic forces. The positive enthalpy \((\Delta H^0)\) and entropy \((\Delta S^0)\) values of the interaction of Pd complexes with DNA, indicate that the binding is mainly entropy-driven and the enthalpy is unfavorable for it. The ligand and its \(\text{Pd(II)}\) complexes have been found cleavage/binding and antimicrobial agents. It would contribute to the synthesis of biomimics and help our understanding of the functioning of metalloenzymes related to electron transport as well as its pharmacological action.
REFERENCES

1. B. M. Zeglis, V. C. Pierre, and J. K. Barton,

2. C. Metcalfe, and J. A. Thomas,

3. K. E. Erkkila, D. T. Odom and J. K. Barton,

4. D. S. Sigman, A. Mazumder and D. M. Perrin,
   *Chem. Rev.*, 93, 2295 (1993)

5. J. A. Cowan,

6. S. Mathur and S. Tabassum,

7. T. Rau and R. V. Eldik,
   "*Metal Ions in Biological Systems*", A. Sigel and H. Sigel, (Ed.), vol.

8. B. Milani, A. Anzilutti, L. Vicentini, A. S. Santi, E. Zangrando, S.
   Geremia and G. Mestroni,
   *Organometallics*, 16, 5064 (1997)

9. M. R. Plutino, L. M. Scolaro, R. Romeo and A. Grassi,
   *Inorg. Chem.*, 39, 2712 (2000);
   D. Jaganyi, A. Hofmann and R. V. Eldik,
*Dalton Trans.*, 2984 (2006);
A. Shoukry, T. Rau, M. Shoukry and R. V. Eldik,

11. N. G. Birenda, C. S. K. Jiban and N. B. Jogendra,

12. P. J. Kothari, M. A. Mehlhoff, S. P. Singh, S. S. Parmar and V. I. Stenberg
*J. Heterocycl. Chem.*, 17, 1369 (1980)

13. A. K. Sengupta and H. K. Misra,

14. S. C. Sarmah and S. C. Bahel,

15. H. J. Vanden Bossche,

16. T. D. Meek,
*J. Enzyme Inhib.*, 6, 65 (1992)

17. K. Toyabe, M. Nezu and H. Shimazu,
*Chem. Abstr.*, 121, 9409q (1989)

18. S. J. Shaber, K. E. Flyn and T. T. Fujimoto,
*Chem. Abstr.*, 119, 72612z (1993)
19. S. Stankoversusky, E. Jedloversuska, K. Spirkova and Collect
   Czech

20. M. B. Talawar, U. V. Laddi, Y. S. Somannavar, R. S. Benner and S.
    C. Bennur,

21. Z. Y. Zhang and H. Yan,
    Acta Chimica Sinica., 45, 403 (1987)


23. J. Marmur,

24. A. I. Vogel,
    "A Text Book of Practical Organic Chemistry" 5th edn., ELBS,

25. H. Meyer,
    Montash. Chem., 24, 207 (1903)

    Russell,

27. F. H. Case,
    J. Heterocycl. Chem., 8, 1043 (1971)

28. Z. D. Bugarcic, G. Liehr and R. V. Eldik,
29. **J. Sambrook, E. F. Fritsch and T. Maniatis,**


30. **A. Wolfe, G. H. Shimer and T. Meehan,**

*Biochemistry*, 26, 6392 (1987)

31. **G. Cohen, and H. Eisenberg,**

*Biopolymers*, 8, 45 (1969)

32. **P. A. Wayne,**

"In Performance standards for anti-microbial susceptibility testing: 11th informational supplement", Document M100-S11, National Committee for Clinical Laboratory Standard, (2001)

33. **H. William,**


34. **A. B. P. Lever,**


35. **S. P. Perlepes, P. Jacobs, H. O. Desseyn and J. M. Tsangaris,**


38. D. X. West, M. S. Lockwood, A. Liberta, X. Chen and R. D. Willet, 

39. D. Kovala-Demertzi, A. Michaelides and A. Aubry,
   D. Kovala-Demertzi, A. Domopoulou, D. Nicholls, A. Michaelides
   and A. Aubry,

40. A. J. Bard and L.R. Faulkner,
   "Electrochemical Methods Fundamentals and applications", Wiley,
   New York, (1980)

41. T. M. Kelly, A. B. Tossi, D. J. McConnell and T. C. Strekas,

42. J. K. Barton, A. T. Danishefsky and J. M. Goldberg,

43. S. A. Tysoe, R. J. Morgan, A. D. Baker and T. C. Strekas,

44. R. F. Pasternack, E. J. Gibbs and J. J. Villafranca,
   Biochemistry, 22, 2406 (1983)

45. J. Liu, T. Zhang, T. Lu, L. Qu, H. Zhou, Q. Zhang and L. Ji,

46. C. Liu, J. Y. Zhou, Q. X. Li, L. J. Wang, Z. R. Liao and H. B. Xu,

47. S. Zhang, Y. Zhu, C. Tu, H. Wei, Z. Yang, L. Lin, J. Ding, J. Zhang
   and Z. Guo,
48. M. T. Carter, M. Rodriguez and A. J. Bard, 

49. K. H. Ulrich, 

50. P. D. Ross and S. Subramanian, 
   *Biochemistry*, 20, 3096 (1981)

51. A. Mallick, B. Haldar and N. Chattopadhyay, 

52. S. Satyanarayana, J. C. Dabrowiak and J. B. Chaires, 
   *Biochemistry*, 31, 9319 (1992)

53. J. M. Kelly, A. B. Tossi, D. J. McConnell and C. Oh. Uigin, 
   *Nucleic Acids Research*, 13, no. 17, 6017 (1985)

54. Tselepi-Kalouli and N. Katsaros, 

55. R. B. Lopez, B. L. Loeb, T. Boussie and T. J. Meyer, 