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Structurally different mono, bi and trinuclear Pd(II) complexes and their DNA/Protein interaction, DNA cleavage, anti-oxidant, anti-microbial and cytotoxicity studies

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Abstract

A series of new structurally different Pd(II) complexes were obtained from the reactions between $K_2[PdCl_4]$, 3-methoxysalicylaldehyde-4(*N*)-substituted thiosemicarbazone [H_2L^1-H_2L^4] and bis(diphenylphosphino)ethane [dppe]. All the complexes were characterized by various spectral techniques (IR, electronic, ^{1}H-NMR and mass spectroscopy). The crystal structures of the complexes **1**, **3**, **4** and **3a** & **4a** have been determined by X-ray crystallographic technique. The interaction of new complexes with calf thymus DNA (CT-DNA) and bovine serum albumin (BSA) have been evaluated by various spectroscopic methods, which showed the interaction potential of the complexes with CT-DNA and BSA. Further, they cleaved supercoiled DNA pBR322. Anti-oxidant profile of the complexes found better than the standards against 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) and superoxide anion radicals. Antibacterial activity of the complexes against five pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* found better than their parent ligands. The complexes exhibited potential cytotoxicity against human breast cancer cells (MCF-7) in the following order **4>3>1>*cisplatin*>2**.

Keywords: Palladium(II) thiosemicarbazone; spectroscopy; X-ray crystallography; DNA binding and DNA cleavage; BSA protein binding; anti-oxidant; anti-microbial; cytotoxicity.

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1 Introduction

Metal based compounds offer substantial potential in medicinal chemistry where the meticulous choice of metal may afford compounds possessing coordination, geometrical and catalytic properties. The most well-known metal-based anticancer drug *cisplatin* arose from serendipity.[1,2]Globally, the demand for platinum-based drugs has grown steadily, although several side effects ranging from nephrotoxicity to drugs resistance of tumour cells.[3-5] Side effects associated with *cisplatin* administration, along with limited applicability arising from specificity shown towards cancer cell lines. One of the alternatives that have shown considerable promise has been the development of other transition metal based drugs.[6-8] The combination of transition metals with biologically active molecules has been exploited showing promising activity due to their unique ability to bind with biological targets.[9,10]Palladium(II) complexes are good alternative candidates for metal-organic drugs due to their thermodynamic and structural similarities to platinum(II) complexes.[11,12]Thiosemicarbazones and their metal complexes have more consideration in the area of medicinal chemistry, due to their pharmacological properties.[13,14]The present level of interest in metal complexes of thiosemicarbazone stems from the fact that the biological activities are frequently ascribed to the chelation of thiosemicarbazones to a metal ion.[15-17]Metal complexes of salicylaldehydethiosemicarbazones have been considered for their antitumor activity.[18,19] Das and Livingstone suggested that sulphur containing ligands chelated to palladium(II) are better antitumor agents than those of other metals, as the palladium(II) chelates possess the proper liability to transport the metal to DNA, its primary target.[20]A number of palladium(II) thiosemicarbazone complexes have been synthesised and examined for their potential as antitumor agents.[21-23]Bisphosphines are important ligand backbones exhibiting differential bite angle upon metal coordination, diphenylphosphinoethane (dppe) provides both mono and bidentate coordination mode.[24] These ligands were recently employed in the development of antitumor cyclopalladated complexes.[25]The cytotoxicity of 1,2-bis(diphenylphosphino)ethane copper(I) complexes was discovered and the mechanism of activity was also proposed.[26] The fact that DNA and protein biomolecules are electron-rich and metal ions electron-deficient compounds to have strong interactions between metal ions and biomolecules. Therefore, the study of the binding stuffs of metal complexes with DNA and protein is of great importancein the designing the new drugs and their application.[27] Metal based antioxidants have received recent responsiveness for their capacity to protect organisms and cell damage induced by oxidative stress.[28]In our current article, we attempted to synthesis palladium(II) complexes containing

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3-methoxysalicylaldehyde-4(*N*) substituted thiosemicarbazone [H_2L^1-H_2L^4] and diphenylphosphinoethane [dppe] in dichloromethane-methanol medium. Surprisingly, the reactions afforded there structurally different products from four of the reactions. The products were separated and characterised by various spectral, analytical methods. Further, the structures of them were proved by X-ray crystallographic technique. The new complexes were subjected to study their potential DNA/protein binding, DNA cleavage, antioxidant, antimicrobial and cytotoxicity.

2 Experimental

1,2-bis(diphenylphosphino)ethane was purchased from Sigma Aldrich Ltd. The ligands [H_2L][1-4], palladium precursor $K_2[PdCl_4]$and palladium(II) complexes were synthesized according to the standard literature procedures.[17a,29,30] All the reagents used were analargrade, were purified and dried according to the standard procedure.[31] Protein free calf thymus DNA (CT-DNA), Ethidium bromide (EB), Bovine serum albumin (BSA) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT)were purchased from Hi Media. Double distilled water was used to prepare Tris–HCl buffer and phosphate buffer. Infrared spectra were measured as KBr pellets on a JASCO FT-IR 4100 instrument between 400–4000 cm^{-1}at the Department of Chemistry, Bharathiar University, Coimbatore, India. Melting points were measured in a Lab India apparatus. The electronic spectra of the complexes were recorded in dichloromethane using a JASCO V-630 Spectrophotometer in the 800-200 nm range. Emission spectra were recorded by using JASCO FP-6600 Spectrofluorimeter. ^{1}H-NMR spectra were recorded in DMSO at room temperature with a Bruker 400 MHz instrument, chemical shift relative to tetramethylsilane. The mass spectrum of the complex **2** was recorded in DMSO using Waters Q-Tof Micro instrument. Single crystal data collection and correction for the new Pd(II) complexes **1**, **3** and **4** were done at 293 K with CCD kappa diffractometer using graphite mono chromated Mo Kα (λ = 1.54184 Å) radiation.[32] The structural solution were done by using SHEL-XTL-97[33]and refined by full matrix least square on F2 using SHEL-XTL-97.[34]

2.1 Preparation of [(Pd_2(Msal-tsc)$_2$)(μ-dppe)] (1)

0.086 g of 3-methoxysalicylaldehyde 4(*N*)-thiosemicarbazone [H_2-Msal-tsc] (0.3063 mmol) was dissolved in dichloromethane (30 cm^3) and added to $K_2[PdCl_4]$ (0.100 g, 0.3063 mmol) in hot methanol (30 cm^3). The mixture was refluxed for 10 min. To this 0.061 g of 1,2-bis(diphenylphosphino)ethane (0.1531 mmol) was added. After 5 hours refluxing, the

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reaction mixture was allowed to stand for 3 days at room temperature. A reddish brown solid formed was subjected to thin layer chromatography and a red band was eluted with benzene :methanol (95:5). The reddish brown solid obtained was recrystallized from toluene/ methanol to yield brown crystals of (**1**).

Yield: 59 %, M.p. 187 ºC. FT-IR (cm^{-1}) in KBr: 1623 ($\nu_{C=N}$), 1301 (ν_{C-O}), 733 (ν_{C-S}), 1455, 1024, 696 cm^{-1} (for PPh_3); UV-Vis (CH_2Cl_2), λ_{max} (nm) (dm^3 mol^{-1} cm^{-1}): 244 (46,918) (intra-ligand transition); 304 (20,722), 339 (16,289), 398 (7,614) (LMCT); ^{1}H-NMR (DMSO-d_6, ppm, *J*= Hz): δ 8.219-8.254 (t, (*J*= 8), CH=N), δ 6.567-6.604 (d, (*J*= 14.8), C4-H), δ 6.502-6-541 (t, (*J*= 7.8), C5-H), δ 6.824-6.840 (d, (*J*= 6.4), C6-H), δ 7.033-7.051 (d, (*J*= 7.2), -NH_2), δ 3.75 (s, OCH_3), δ 2.48 (m, (-CH_2-)$_2$), δ 7.220-7.610 (m, dppe protons).

2.1.1Preparation of [(Pd_2(Msal-mtsc)$_2$)(μ-dppe)] (2)

Complex **2** was prepared by the procedure as described for (**1**), with 3-methoxysalicylaldehyde 4(*N*)-methylthiosemicarbazone [H_2-Msal-mtsc] (0.085 g, 0.3063 mmol), $K_2[PdCl_4]$ (0.100 g, 0.3063 mmol) and 1,2-bis(diphenylphosphino)ethane (0.061 g, 0.1531 mmol). A reddish brown solid formed was subjected to thin layer chromatography and a red band was eluted with benzene : methanol (95:5). The reddish brown solid obtained was recrystallized from chloroform / methanol to yield brown crystals of (**2**).

Yield: 60 %, M.p. 196 ºC. FT-IR (cm^{-1}) in KBr: 1592 ($\nu_{C=N}$), 1306 (ν_{C-O}), 723 (ν_{C-S}), 1452, 1027, 671 cm^{-1} (for PPh_3); UV-Vis (CH_2Cl_2), λ_{max} (nm) (dm^3 mol^{-1} cm^{-1}) : 241 (57,488) (intra-ligand transition); 313 (21,051), 347 (15,607), 393 (7,780) (LMCT); ^{1}H NMR (DMSO-d_6, ppm, *J*= Hz): δ 8.295-8.316 (t, *J*= (4.4), CH=N), δ 6.688- 6.705 (d, (*J*=6.8), C4-H), δ 6.483- 6.522 (t, (*J*=7.8), C5-H), δ 6.962- 6.982 (d, (*J*=8), C6-H), δ 6.878-6.890 (q, –$NHCH_3$), δ 3.39 (s, –OCH_3), δ 2.670-2.682 (d, (*J*=4.8), –CH_3), δ 2.775 (s, (–CH_2–)$_2$), δ 7.325- 7.593 (m, dppe protons).

2.1.2 Preparation of complex (3)

Complex **3** was prepared by the procedure as described for (**1**), with 3-methoxysalicylaldehyde 4(*N*)-ethylthiosemicarbazone [H_2-Msal-etsc] (0.090 g, 0.3063 mmol), $K_2[PdCl_4]$ (0.100 g, 0.3063 mmol) and 1,2-bis(diphenylphosphino)ethane (0.061 g, 0.1531 mmol). The resulting reddish orange solution was concentrated. It was subjected to thin layer chromatography where two spots were identified and isolated by silica gel column chromatography using benzene–methanol solvent mixture. The light yellow band was eluted

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with 98:2 benzene–methanolwhich afforded yellow crystals of **3a**. Followed by **3a**, complex **3** was isolated from an orange band by using 90:10 benzene: methanol as eluent and was crystallized by using chloroform-ethylacetate mixture to afforded yellowish orange crystals.

[$PdCl_2$(dppe)] (**3a**). Yield: 21 %,M.p. >250 ºC.Anal.Calcd for $C_{26}H_{24}Cl_2P_2Pd$: C, 54.24; H, 4.20. Found: C, 54.17; H, 4.12 %. FT-IR (cm^{-1}) in KBr:1456, 1092, 694 cm^{-1} (for PPh_3); UV-Vis (DMSO), λ_{max} (nm) (dm^3 mol^{-1} cm^{-1}): 272 (22,251) (intra-ligand transition); 326 (12,020) (LMCT);^{1}H-NMR (DMSO-d_6, ppm): δ 2.34 (s, $(–CH_2–)_2$), δ 7.140-7.288 (m, dppe protons).

[Pd_3(μ-S-Msal-etsc)$_3$] (**3**).Yield: 38 %, M.p. 192 ºC. FT-IR (cm^{-1}) in KBr: 1596 ($\nu_{C=N}$), 1307 (ν_{C-O}), 727 (ν_{C-S}); UV-Vis (CH_2Cl_2), λ_{max} (nm) (dm^3 mol^{-1} cm^{-1}): 252 (21,868) (intra-ligand transition); 319 (13,446) (LMCT); 404 (3,889) (MLCT);^{1}H-NMR (DMSO-d_6, ppm, *J*= Hz): δ 8.177 (s, CH=N), δ 6.543-6.582 (t, (*J*= 7.8), C4-H), δ 6.912-6.934 (q, C5-H), δ 7.091-7.112 (t, (*J*= 4.2), C6-H), δ 7.189-7.214 (t, (*J*=5), $-NHC_2H_5$), δ 3.750 (s, OCH_3), δ 2.486-2.495 (t, (*J*= 3.6), CH_2), δ 1.077-1.095 (t, (*J*= 7.2), CH_3).

2.1.3 Preparation of complex (4)

Complex **4** was prepared by the procedure as described for (**1**), with 3-methoxysalicylaldehyde 4(*N*)-phenylthiosemicarbazone [H_2-Msal-ptsc] (0.104 g, 0.3063 mmol), $K_2[PdCl_4]$ (0.100 g, 0.3063 mmol) and 1,2-bis(diphenylphosphino)ethane (0.061 g, 0.1531 mmol). The resulting red coloured solution was concentrated. It was subjected to thin layer chromatography where two spots were identified and isolated by silica gel column chromatography using benzene–methanol solvent mixture. The first yellow band was isolated using a 98:2 benzene–methanol solvent as the eluent which afforded yellow crystals **4a**. Followed by **4a**, complex 4 was isolated from a red band by using benzene : methanol (95:5) as eluent and the obtained orange red solid was recrystallized from chloroform-methanol mixture to yield red crystals of complex **4**.

[$PdCl_2$(dppe)] (**4a**). Yield: 25 %,M.p. >250 ºC.Anal.Calcd for $C_{26}H_{24}Cl_2P_2Pd$: C, 54.24; H, 4.20. Found: C, 54.17; H, 4.12 %. FT-IR (cm^{-1}) in KBr:1456, 1092, 694 cm^{-1} (for PPh_3); UV-Vis (DMSO), λ_{max} (nm) (dm^3 mol^{-1} cm^{-1}): 272 (22,251) (intra-ligand transition); 326 (12,020) (LMCT);^{1}H-NMR (DMSO-d_6, ppm): δ 2.34 (s, $(–CH_2–)_2$), δ 7.140-7.288 (m, dppe protons).

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[Pd(Msal-ptsc)(Msal-ptaz)] (**4**). Yield: 63 %, M.p. 202 ºC. FT-IR (cm^{-1}) in KBr: 3384 (ν_{OH}), 3062 (ν_{N-H}), 1596 ($\nu_{C=N}$), 1316 (ν_{C-O}), 816 ($\nu_{C=S}$), 730 (ν_{C-S}); UV-Vis (CH_2Cl_2), λ_{max} (nm)(dm^3 mol^{-1} cm^{-1}): 243 (36,935), 265 (30,997) (intra-ligand transition); 351(9,373) (LMCT); 405 (3,964) (MLCT); ^{1}H-NMR (DMSO-d_6, ppm, *J*= Hz): δ 11.45 (s, -OH), δ 6.714-6.811 (m, C4-H & C5-H), δ 7.018-7.032 (t, *(J*= 5.6), C6-H), δ 8.533 (s, 1H, CH=N), δ 3.968 (s, 3H, OCH_3), δ 9.439 (s, N(2)HC=S), δ 7.670-7.689 (d, (*J*=7.6), NHPh), δ 7.092-7.463 (m, phenyl protons).

2.2 DNA binding studies

DNA binding studies have been carried out according to the method described in the earlier reports.[17]Various concentrations of CT-DNA (0.5-5 μM) in Tris-HCl buffer (pH 7.2) were treated with complexes (**1-4**) (10 μM) in 1 % aqueous DMSO. The detailed procedures for DNA binding experiment was provided in the Supporting Information.

2.2.1 Competitive binding with ethidium bromide

Ethidiumbromide studies have been carried out according to the earlier reported methods.[16,17]The detailed procedures for DNA binding experiments were provided in the Supporting Information.

2.2.2 DNA cleavage studies

The cleavage of DNA was monitored by using agarose gel electrophoresis[35] and the detailed producer was given in supporting information.

2.3 Bovine serum albumin binding studies

Protein binding studies were done according to the earlier reported methods[16,17]and were given in the Supporting Information.

2.4. Evaluation of antioxidant activity

The potential antioxidant activity of new palladium(II) complexes was evaluated by DPPH and superoxide anion radical-scavenging assaysby using standard literature methods.[36,37] Further reductive ability of the complexes were assayed by Oyaizu method.[38] Further, estimation of total antioxidant capacity of the complexes has been done by phosphomolydenum method.[39]The experimental procedures for above said methods were given in the Supporting Information.

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2.5 Antibacterial activity studies

The palladium(II) complexes (**1-4**) have been screened for their antibacterial activities against various pathogenic bacteria *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Bacillussubtilis*, *Pseudomonas aeruginosa* and *Klebsiellapneumoniae* by disc diffusion method[40] and were given in supporting information.

2.6 Cytotoxicity studies

2.6.1 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[41]

Cytotoxic activity of new palladium(II) complexes have been evaluated and the IC_{50} values were obtained from nonlinear regression using GraphPad Prism 5.[42] The experimental procedure was given in supporting information.

3 Results and discussion

3.1. Synthesis of new palladium(II) complexes

The reaction of two equivalents 3-methoxysalicylaldehyde-4(*N*)-substituted thiosemicarbazone $(H_2L)^{1-4}$ with two equivalents $K_2[PdCl_4]$ and one equivalent 1,2-bis (diphenylphosphino)ethane [dppe] in 1:1 methanol-dichloromethane resulted in the formation of new complexes (**Scheme 1**). The structure of the complexes (**1**, **3** and **4**) was confirmed by X-ray crystallographic studies. The complex $[PdCl_2(dppe)]$obtained along with complex **3** and **4** was isolated by column chromatography with 98:2 benzene: methanol as eluent. The identity of the complex has been confirmed by elemental analysis, IR, UV-Vis and NMR spectroscopic studies and left without further characterisation. Further, the complexes are soluble in common organic solvents such as dichloromethane, chloroform, ethanol, methanol, dimethylformamide and dimethylsulfoxide.

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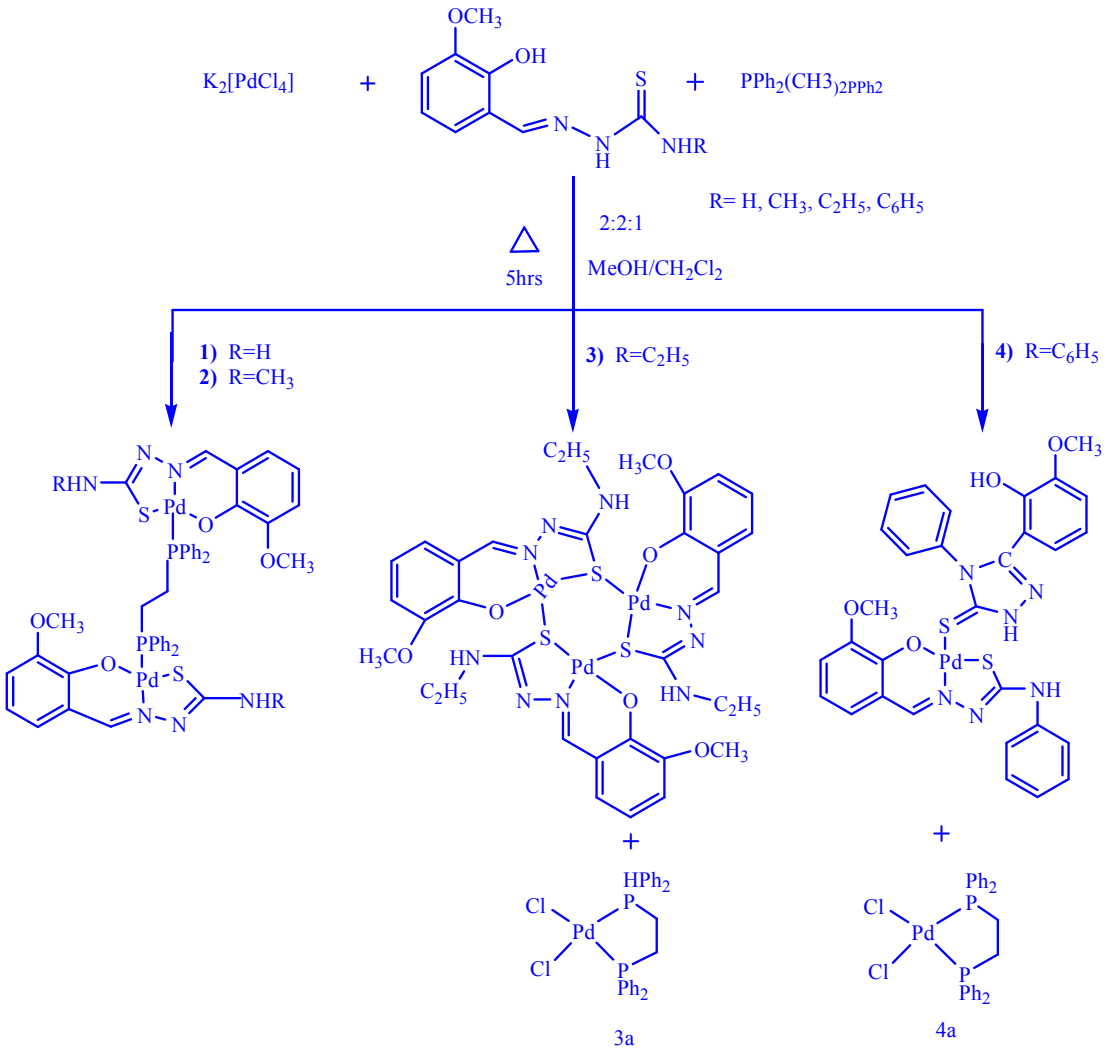

Scheme1. Synthesis of new palladium(II) complexes

3.2 Spectroscopic studies

The IR spectra of the ligands $(H_2L)^{1-4}$ and the corresponding complexes (**1-4**) provided substantial information about the metal ligand bonding (Fig. S1-S5). Strong stretching frequency observed at 1593-1539 cm^{-1} in the ligands corresponding to $\nu_{(C=N)}$ was shifted to 1626-1597 cm^{-1} in all complexes (**1-4**) indicating the coordination of azomethine nitrogen to metal atom.[43] A band in the region 3310-3457 cm^{-1} due to the presence of –OH group in the free ligands $(H_2L)^{1-3}$ was completely disappeared in the IR spectra of the new complexes (**1-3**) indicating the coordination of phenolic oxygen to palladium after deprotonation. This was further supported by increase in the phenolic C-O stretching frequency from 1260-1275 to 1307-1317 cm^{-1}.[16b] However, in the complex **4**, a broad peak corresponding to $\nu_{(OH)}$at 3382 cm^{-1}, suggested that non participation of phenolic OH in bonding. A sharp band observed at

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772-788 cm^{-1}ascribed to $\nu_{(C=S)}$ in the ligands (H_2L^{1-3}) was completely disappeared in the spectra of new complexes (**1-3**) and the appearance of a new band around 726-727 cm^{-1} due to $\nu_{(C-S)}$ indicating the coordination of the sulphur atom after enolisation followed by deprotonation.[44,17b] Whereas in complex **4**, there were two signal at 827 $cm^{-1}$and 728 cm^{-1}, corresponding to $\nu_{(C=S)}$ and $\nu_{(C-S)}$ respectively and a peak at 3026 cm^{-1}corresponding to NH stretching added more possibility to predict the presence of thione sulphur. In addition, the absorption bands in the region 1434 and 1456 cm^{-1}, 1094 and 1097 cm^{-1}, 696 and 689 cm^{-1} confirms the presence of (diphenylphosphino)ethane in the complexes **1** and **2** respectively.[17b] However, such signal were absent in the complexes **3** and **4** suggested the non-participation of (diphenylphosphino)ethane in coordination with the metal ions. The electronic spectra of the complexes (**1-4**) have been recorded in chloroform and they displayed bands in the region around 241-400 nm (Fig. S6). The bands that appeared at region 241-252 nm have been assigned to intra ligand transition and the bands around 304-400 nm have been assigned to ligand to metal charge transfer (LMCT) transition.[45]

In the ^{1}H-NMR spectra of ligands (H_2L^{1-3}), a singlet appeared around δ 9.13-9.14ppm has been assigned to N(2)HCS proton.[46] However, in the spectra of new complexes (**1-3)**, there was no resonance attributable to N(2)HCS, indicating the coordination of sulphur in the anionic form after deprotonation at N(2) (Fig. S7-S11). A sharp singlet appeared at δ 11.34-11.40 ppm corresponding to the phenolic –OH group in the free ligands (H_2L^{1-3})was completely disappeared in complexes **1-3**confirming the involvement of phenolic oxygen in coordination.[43]In complexes **1**and **2**, a triplet appeared at δ 8.21 and δ 8.29 ppm corresponding to the azomethine group which may be due to the nuclear quadrupolar effect of the nitrogen atom[44] and in complex **3**, a singlet appeared at δ 8.17 for azomethine (CH=N) proton.[16b] Moreover in complexes **1** and **2**, a multiplet was observed at δ 2.48 andδ 2.77 ppm respectively due to the presence of ethylene protons of the 1,2-bis(diphenylphosphino)ethane.[24]A sharp singlet corresponding to the phenolic –OH proton appeared at δ 11.76 ppm in the free ligand H_2L^4 and the complex **4**, the appearance of a singlet at δ 11.45 ppm indicating the non-participation of the phenolic oxygen in coordination.[44] The spectrum of H_2L^4 showed a sharp singlet atδ 10.00 ppm analogous to N(2)HCS proton. However, this signal completely disappeared and a new singlet appeared at δ 9.43 ppm in complex **4** indicating the coordination of the thione form of the sulphur to the palladium ion.[16a]In complex **4**, a singlet observed at δ8.53 ppm was assigned to azomethine(CH=N) proton.[17b]

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3.3 X-ray crystallography

In order to confirm the exact structure, X-ray crystallographic studies were done for the new complexes (**1**, **3**,**4**and **3a & 4a**). The ORTEP diagrams of the complexes are given in Fig. 1-4. The crystallographic data, selected bond distances and bond angles are listed in Table S1 and S2a-S2c.Single crystals of $[Pd_2(Msal\text{-}tsc)_2(\mu\text{-}dppe)]$ (**1**) complex crystallised in the triclinic space group P-1(Fig. 1). In the dimeric complex (**1**), each palladium atom is coordinated with a binegative tridentate ligand through phenolic oxygen, N1 hydrazinic nitrogen and thiolate sulphur atom by forming one six member and another five member ring with a bite angle of 84.47(7)°[S1-Pd1-N1]. The remaining coordination site is occupied by the phosphorous atom of 1,2-bis(diphenylphosphino)ethane. Inspection of the angles formed between the metal and the coordinated atoms showed that the metal complex is slightly distorted from square-planar geometry. The angles [N1-Pd1-S1] 84.47(7)°and [O1-Pd1-P1] 84.12(6)° formed between the thiosemicarbazone ligand and the metal are less than 90°. The angles [O1-Pd1-N1] 93.85(9)°and [S1-Pd1-P1] 97.58(3)° are therefore greater than 90°. Collectively the bite angles observed in the molecular structure compare favourably with those of analogous palladium(II) complexes.[47]The Pd1-O1 bond distance 2.008(2)Å, Pd-N1 bond distance 2.023(2)Å and Pd1-S1 bond distance 2.233(8)Å were found as similar to the reported values.[17b,47,48] The strong *trans* influence of the phosphorous atom is reflected in the Pd-N1 bond distance which is slightly longer than the expected length calculated from the covalent radii of palladium and nitrogen (2.01 Å).[49] The *trans* angles [P1-Pd1-N1] and [O1-Pd1-S1] were found as 177.52(1)° and 178.29(6)° indicating the considerable deviation from the ideal symmetry and significant distortion around square planar palladium ion.[47,48] Two of these symmetrical units are bridged through dppe ligand in the complex **1**. Complex **1**exhibited an intermolecular hydrogen bonding between hydrogen atom H(3A) of amino group N(3)-H(3A) with hydrazinic nitrogen atom N(2) of a second molecule. In addition, the hydrogen atom H(3A) of amino group N(3)-H(3A) in the second molecule is involved in intermolecular hydrogen bonding with hydrazinic nitrogen atom N(2) of the first one. This intermolecular hydrogen bonding leads to an unremitting 1D chain formation (Fig. S13 and Table S3).

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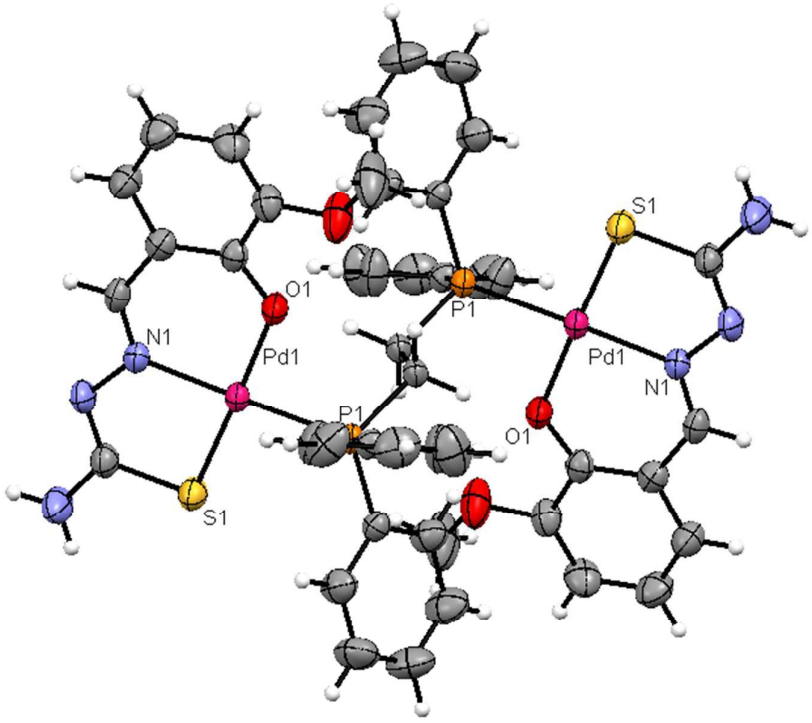

Fig. 1. ORTEP diagram of [(Pd_2(Msal-tsc)$_2$)(μ-dppe)] (**1**)

The palladium complex **3** was crystallized by chloroform-ethylacetate mixture. From the unit cell dimensions, it is clear that the complex**3** is crystallised in triclinic system with P-1 space group (Fig. 2). The complex **3** consists of six-member ring of alternating Pd(II) and sulphur atoms in a chair-like configuration. The remaining two sites of each square planar Pd(II) centres are occupied by the hydrazinic nitrogen and the phenolic oxygen. The whole structure formed like a bowl type with (3Pd-3S) three palladium(II)-sulphur ring as the base. This type of complex has been reported previously for palladium complexes.[49] The distance between the palladium centres [Pd1-Pd2] 3.376 Å; [Pd2-Pd3] 3.887 Å and [Pd3-Pd1] 4.106 Å indicate that there is no direct bond between the palladium atoms.[50]The three bond angles involving bridging sulphur atoms are as follows: [Pd1-S1-Pd2] 95.56(3)°; [Pd2-S2-Pd3] 117.53(4)° and [Pd3-S3-Pd1] 127.34(4)°. The Pd-O bond distances 2.003(3) to 2.014(3) Å and Pd-N bond distances 1.988(3) to 1.994(3)Å and Pd-S bond distances 2.244(10) to 2.336(10)Å are similar to those found in other palladium complexes.[50] An intramolecular N(9)-H(9)-O(1) hydrogen bonding between the terminal nitrogen N(9) and phenolic oxygen O(1) of the ligand and Complex **3** also exhibited an intermolecular N(3)-H(3)-O(7) hydrogen bonding between the terminal nitrogen N(3) and oxygen atom of the ethyl acetate moiety which is present in the crystal lattice (Fig. S14 and Table S3).

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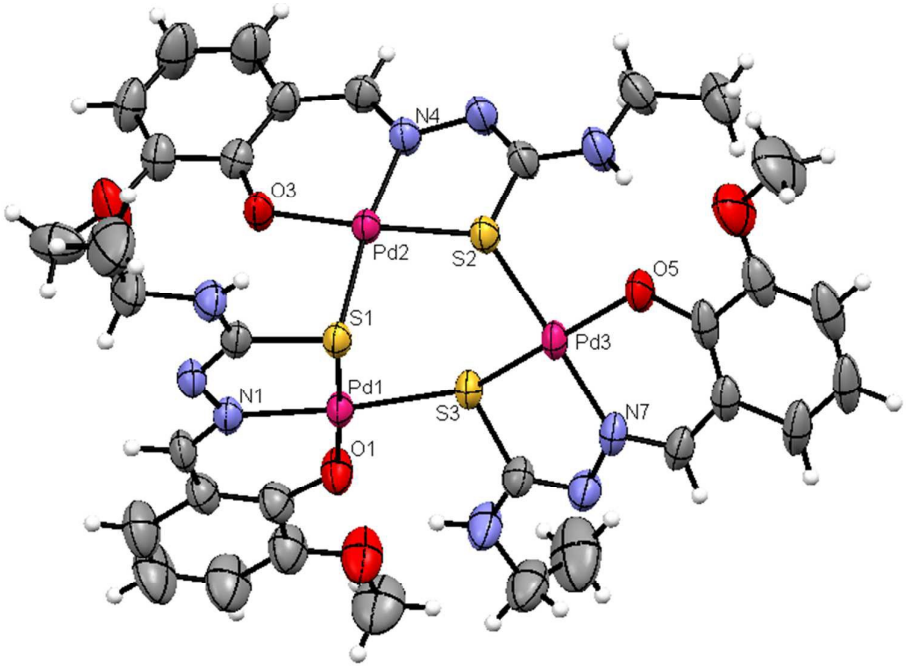

Fig. 2. ORTEP diagram of $[Pd_3(\mu\text{-S-Msal-etsc})_3]$ (**3**)

Though the spectroscopy characterisations of the complex **4** gave some idea about the composition of the complex, where they failed to indicate the definite binding mode of the thiosemicarbazone. Hence, the identity of the complex **4** was determined by X-ray crystallography. The unit cell dimensions clearly showed that the crystal of complex **4** is monoclinic belonging to P_{21}/c space group (Fig. 3). The structure of the complex **4**consistsof ONS chelated thiosemicarbazone ligand and the fourth coordinate site is occupied by sulphur atom of 5-(2-hydroxy-3-methoxy-phenyl)-4-phenyl-2,4-dihydro[1,2,4]triazole-3-thione which is formed by the cyclisation of the H_2L^4 ligand. The [Pd1-O1] bond distance 2.0337(16) Å, [Pd1-N1] bond distance 1.9911(18) Å and [Pd1-S1] bond distance 2.2327(7) Å and [Pd1–S2] bond distances of 2.3167(6) Å were found as similar to the reported values.[49]Theelongation of Pd(1)-S(2) bond may be due to the strong *trans* influence of N(1) nitrogen on thione sulphur-palladium in Pd(1)-S(2). This is further evident from the shorter bond distance of (1.698(2) Å) C16-S2 bond of coordinated ligand.[51]The *trans* angles [N1-Pd1-S2] and [O1-Pd1-S1] were found as 170.54(6)° and 176.81(5)° respectively indicating the significant distortion around square planar palladium ion.[47]

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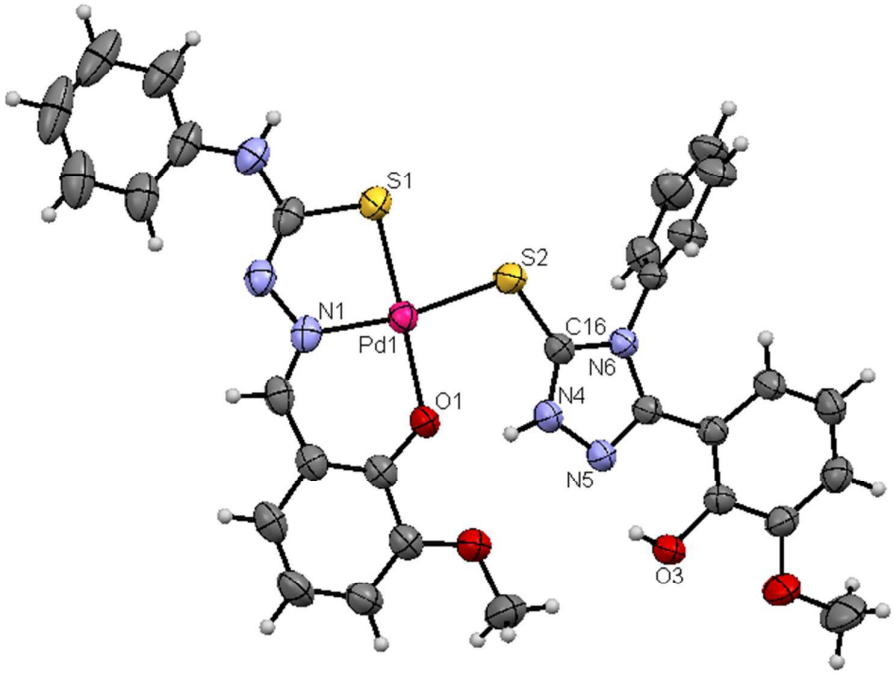

Fig.3. ORTEP diagram of [Pd(Msal-ptsc)(Msal-ptaz)] (**4**)

Single crystals of [$PdCl_2$(dppe)] (**3a** and **4a**) complex crystallised in the monoclinic space group P1 21/C1(Fig. 4). In this complex, palladium atom is coordinated with two chloride atoms and third and fourth site was occupied by phosphours atom of 1,2-bis(diphenylphosphino)ethane. The [Pd1-C30] bond distance 2.3552(11) Å, [Pd1-C30] bond distance2.3618(12) Å and [Pd1-P8] bond distance 2.2355(10) Å and [Pd1–P11] bond distances of 2.2377(11) Å were found as similar to the reported values.[38]The *trans* angles [P8-Pd1-Cl30] and [P11-Pd1-Cl31] were found as 175.43(4)° and 172.35(4)° indicating the considerable deviation from the ideal symmetry and significant distortion around square planar palladium ion.[52]

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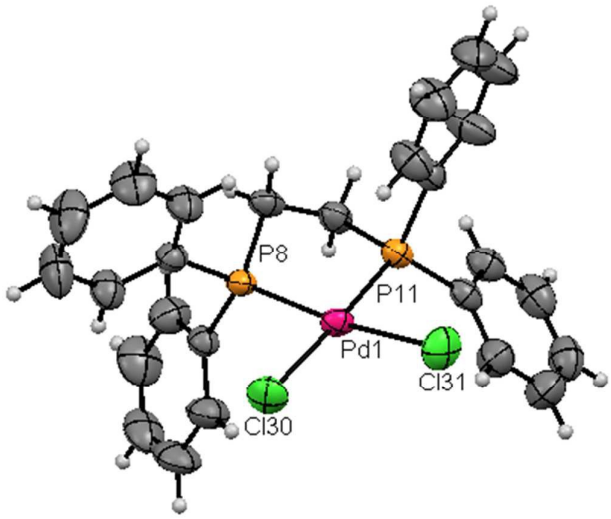

Fig.4. ORTEP diagram of [$PdCl_2$(dppe)] (**3a**&**4a**)

Repeated attempts to get good crystals of complex **2** suitable for the X-ray single crystallographic studies were unsuccessful. Hence, the stoichiometry of complex **2** was further confirmed by Electro spray Ionization Mass Spectroscopy (ESI-MS), the m/z value is 1085.0372 (Fig. S12).

3.4 DNA binding studies

3.4.1 Electronic absorption titration

The investigation of the binding of metal complexes to DNA is of major importance in the development of drugs. The transition metal complexes interact with DNA via both covalent and/or non-covalent interactions. The electronic absorption spectra of complexes**1-4** exhibited two to three absorption bands at 256-268 nm and 305-408 nm, which were assigned to intra ligand transition (IL) and ligand to metal charge transfer transition (LMCT). The absorption spectra of the complexes (**1-4**) at constant concentration (10 µM) in the absence and the presence of different concentrations of nucleotide CT-DNA (0.5-5 µM) are shown in Fig. 5. Upon increasing the concentration of CT-DNA, hyperchromism was observed in the complexes **1** and **2** with a small blue shift of 2 nm and 5 nm in the wavelength. However, in the complexes **3** and **4**, hyperchromism without shift in the wavelength was observed in the intra ligand bands. The LMCT bands exhibited modest hyperchromism with and without blue shifts from 1-7 nm for complexes (**1-4)** was observed, which indicated that the new

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palladium(II) complexes (**1-4**) bind to CT-DNA.[53] However, the exact mode of binding can be established only after calculating the intrinsic binding constant K_b values (Table 1) and confirmation studies by either EB-displacement studies or thermal denaturation or viscosity measurement or recording circular dichroism. Even though, hyperchromic effect was observed, the very high binding constant values suggested that the binding may be an intercalative.[46] This has been further verified by doing EB-displacement studies. From the intrinsic binding constant (K_b) values (Table 1), it is inferred that all the complexes (**1-4**) bind with CT-DNA efficiently (Fig. 6). Among the four complexes, complex **4** binds more strongly with CT-DNA as compared to trinuclear complex (**3**) and binuclear complexes (**1** and**2**), which may be due to the presence of 5-(2-hydroxy-3-methoxy-phenyl)-4-phenyl-2,4-dihydro[1,2,4]triazole-3-thione.Trinuclear complex **3** exhibited better binding than the binuclear complexes **1** and **2**, owing to the presence of palladium-sulphur bridge and the order of binding affinity is **2**<**1**<**3**<**4**. The binding constants of the complexes are sufficiently high (10^6 M^{-1}). This is may be due to the strong supramolecular interaction between cationic palladium complexes and negatively charged phosphodiester backbone of DNA.[54]

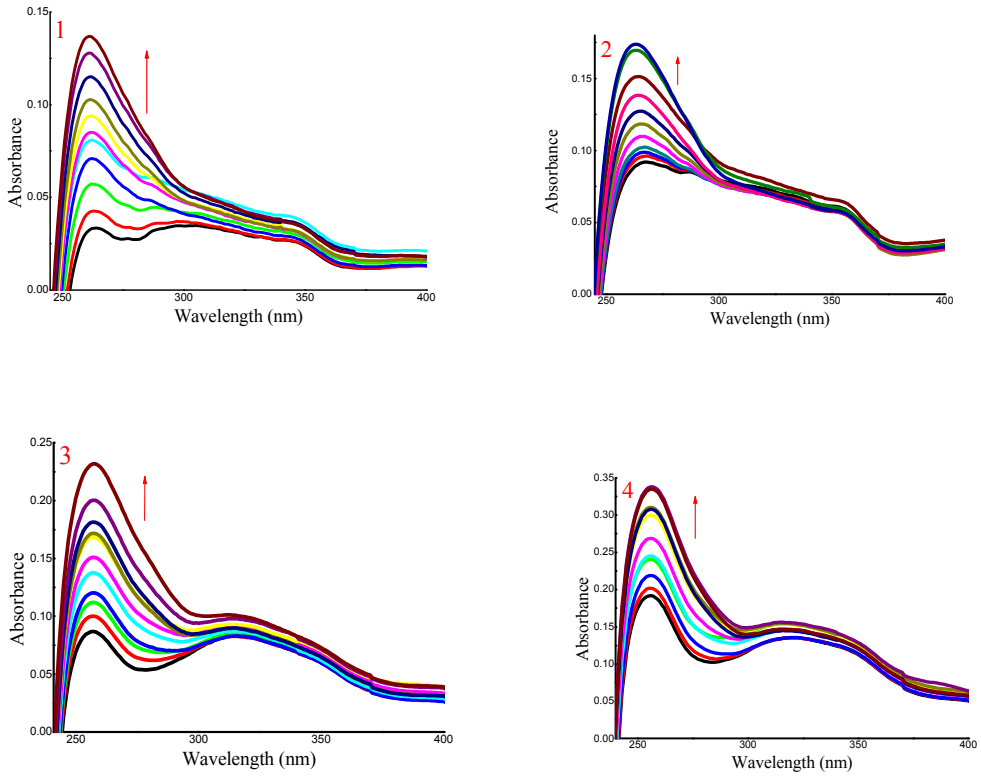

Fig.5. Absorption titration of fixed concentration (10 μM) of complexes **1-4** with increasing concentrations (0.5-5 μM) of CT-DNA (TrisHCl, pH 7.2).

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Table 1 Binding constant for interaction of complexes with CT-DNA

System	K_b ($\times 10^6$ M^{-1})
CT-DNA + **1**	2.024±0.17
CT-DNA + **2**	1.921±0.12
CT-DNA + **3**	3.612±0.15
CT-DNA + **4**	3.975±0.18

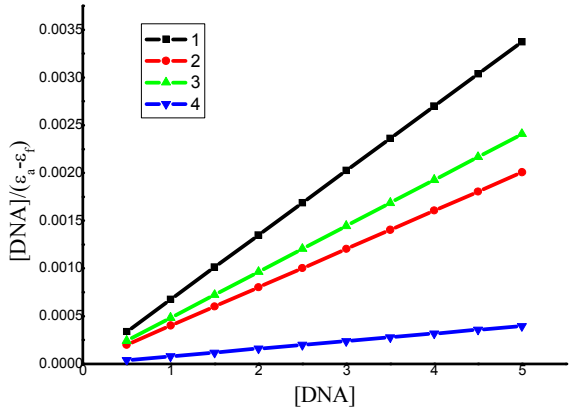

Fig.6. Plot of [DNA] versus [DNA] / (ε_a- ε_f)

3.4.2 Competitive studies with ethidium bromide

From the electronic absorption titration studies, it is inferred that the complexes can bind with the DNA. Whereas, the observed hyperchromic effect with slight red shift showing the intrinsic binding constant values in the magnitude of 10^6, may not be proposed for electrostatic interaction. Hence, in order to confirm the binding interaction of the complexes to CT-DNA, competitive studies of the palladium(II) complexes with ethidium bromide were undertaken. EB displacement technique provides indirect evidence for the DNA binding mode. The fluorescence intensity of EB-DNA was gradually decreased with increasing concentrations of the complexes (**1-4**) indicating that the complexes bound to DNA by competing with EB(Fig. 7). From the quenching plots and quenching constant values, it is clear that EB was replaced by complexes **1-4** from the EB-DNA system (Fig. 8, Table 2). Such a characteristic change is an indication that all the complexes interacted with DNA through intercalation mode.[16a,55] The calculated value of the quenching constant (K_q) and binding constant (K_{app}) are listed in Table 2. The quenching constants and binding constants

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of the palladium(II) complexes suggest that the interactions of allof the complexes with DNA should be intercalation.[55]

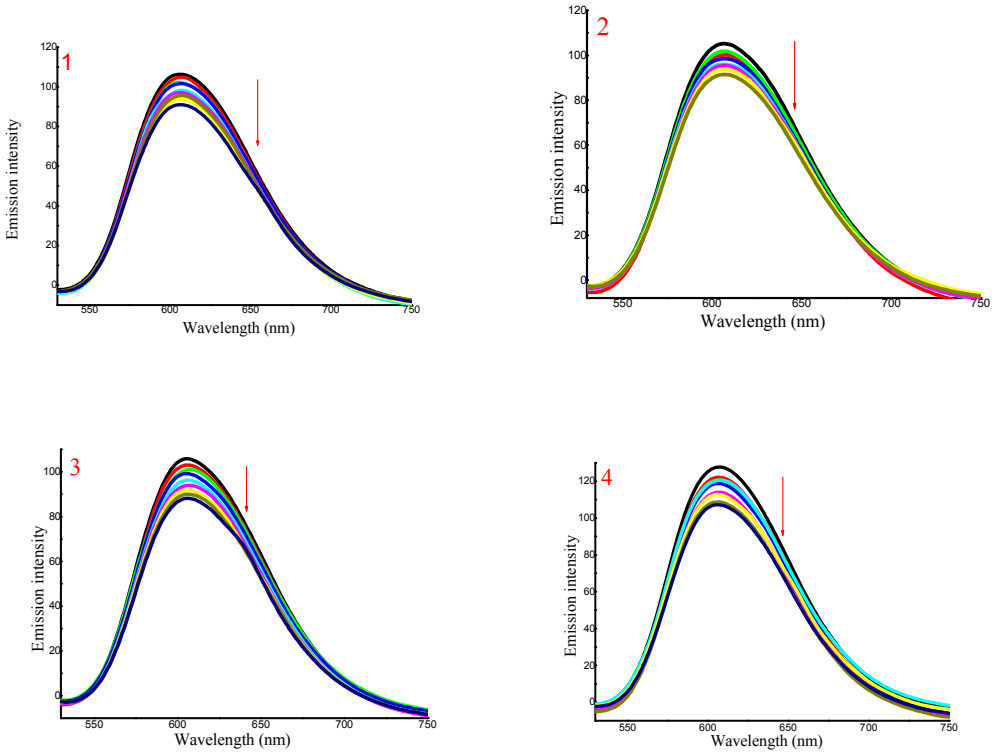

Fig. 7. Fluorescence quenching curves of ethidium bromide bound to DNA by complexes **1-4**. [DNA] = 10 μM, [EB] = 10 μM and [compound] = (0-80) μM.

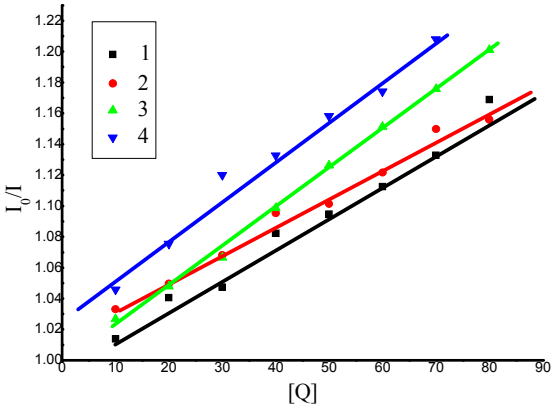

Fig. 8. Plot of [Q] versus I_0/I

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Table 2 Quenching constant and Binding constant for interaction of complexes with DNA

System	K_q ($\times 10^3 M^{-1}$)	K_{app} ($\times 10^6 M^{-1}$)
1	1.89±0.10	1.90
2	1.83±0.09	1.99
3	2.55±0.13	1.94
4	3.15±0.16	1.63

3.4.3 DNA cleavage studies

To assess the DNA cleavage ability of complexes **1-4**, supercoiled (SC) pBR322 DNA was incubated with 50 μM concentration of the complexes in a 5 mMTris-HCl/50 mMNaCl buffer at pH 7.2 for 2 h without the addition of a reductant (Fig. 9). Upon gel electrophoresis of the reaction mixture, DNA cleavage was observed during which the SC DNA was converted into nicked circular (NC) DNA and linear DNA. The complexes did not require any addition of external agents to cleave the DNA. Moreover, the metal precursors $K_2[PdCl_4]$, free ligands (H_2L^{1-4}) and 1,2-bis(diphenylphosphino)ethane (dppe) did not show any cleavage activity. From this result, it is clear that the palladium(II) complexes (**1-4**) has the potential to cleave the supercoil DNA and the new complexes (**1-4**) alone are responsible for the cleavage of DNA.[56] Among the four complexes, binuclear complexes **1** and **2** exhibited better DNA cleavage as compare to the other trinuclear **3** and cyclised mononuclear **4** complexes. The highest activity of the binuclear complexes **1** and **2** may be due to the presence of two planar metal centres which are separated by bridged bis(diphenylphosphino)ethane can undergo greater interaction as compare with other complexes. Complex **4** showed good DNA cleavage as compared to trinuclear complex **3**, which may be due to the presence of 5-(2-hydroxy-3-methoxy-phenyl)-4-phenyl-2,4-dihydro[1,2,4]triazole-3-thione. Oxidative cleavage is usually mediated by the presence of additives and photo induced DNA cleaving agents i.e. an external agent like light or H_2O_2 is required to initiate cleavage. Photo cleavage of nucleic acids allows the use of light to trigger nuclease activity. However, the general mechanism of hydrolytic cleavage is the hydrolysis reaction is facilitated by the presence of metal ions, acting as Lewis Acids. These Lewis acids can activate the phosphate group towards nucleophilic attack, activate water or hydroxide as nucleophile or increase the leaving group ability of the departing alcohol. The general accepted mechanism of the DNA hydrolysis reaction is a nucleophilic attack at the DNA

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phosphate backbone, to form a five coordinate intermediate, which can be stabilized by the catalyst. Thus hydrolytic cleavage mechanism was found in our complexes.[57]

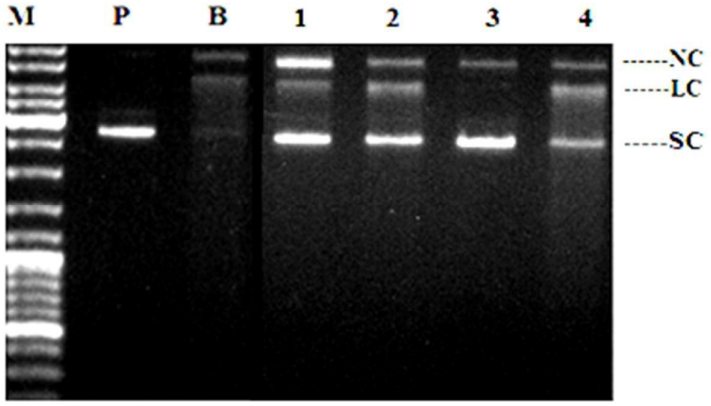

Fig. 9. Gel electrophoresis diagram showing the cleavage of supercoiled pBR322 DNA by complexes **1-4** in 5% DMSO and 95% 5 mMTris−HCl/50 mMNaCl buffer at pH 7.2 and 37 °C with an incubation time of 2 h. Lane B: Buffer+plasmid; Lane P: Plasmid alone ; Lane M: DNA ladder; Lane 1: Complex **1** (50 μM); Lane 2: Complex **2** (50 μM); Lane 3: Complex **3** (50 μM); Lane 4: Complex **4** (50 μM). Forms SC, NC, and LC are supercoiled, nicked, circular and linear circular DNA, respectively.

3.5 Protein binding studies

The crucial step in accessing a drug's bioavailability is assigned to its interaction to plasma protein. In screening potential therapeutic agents, plasma protein binding is required.[58]Bovine serum albumin (BSA) is the most extensively studied serum albumin, which is able to bind a variety of substrates including metal cations, hormones, and most therapeutic drugs. BSA possesses three fluorophores(tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe)).

3.5.1 UV absorption spectra of BSA

UV absorption spectrometric titration is useful techniqueto distinguish between static and dynamic quenching based on the absorption spectra of BSA in the presence of complexes.[59]The UV absorption spectra of BSA in the absence and presence of four complexes (**1-4**) (Fig. 10) revealed that the absorption intensity of BSA was enhanced as concentration of the compounds increased, and there was a blue shift for all the compounds. The changes in the absorption spectra for BSA + complexes indicate that the test compounds interact with BSA and altering the secondary state of protein. Based on the enhancement of the absorption intensity of BSA with blue shift suggesting the static type of quenching of BSA by the complexes.[16a,46]

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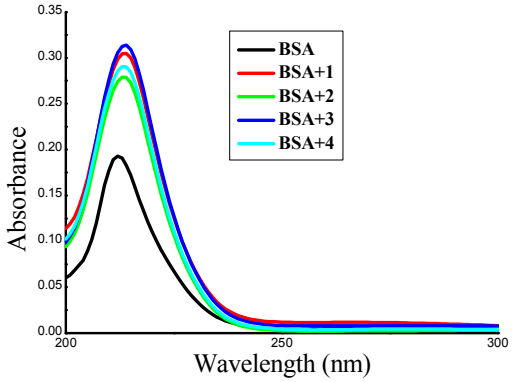

Fig.10.UV absorption spectra of BSA (10 μM) in the presence of complexes (10 μM).

3.5.2 Fluorescence quenching studies of BSA

The intrinsic fluorescence of BSA will provide considerable information on their structure and is often utilized in the study of protein folding and association reaction. The interactions of BSA with the complexes (**1-4**) were studied by emission titration at room temperature. A solution of BSA (10 μM) was titrated with various concentrations of complexes (0-100 μM). The effects of the complexes on the fluorescence emission spectra of BSA are shown in Fig. 11. The addition of the complexes (**1-4**) to a solution of BSA resulted in a significant decrease in the fluorescence intensity of BSA at 348 nm, up to 57.86, 57.06, 70.65 and 72.86 % from the initial fluorescence intensity of BSA accompanied by a blue shift of 1-3 nm for all the complexes (**1-4**). This is mainly due to the active site in protein is buried in a hydrophobic environment.[60]The results suggested a definite interaction of all the complexes with BSA.[46]The quenching constants (K_{sv}) were calculated from slope of the plot I_0/I versus [Q], the concentration of complexes (**1-4**) resulted in a linear plot (Fig. 12) and this linear fit plot represents a single quenching mechanism for all the complexes (**1-4**). The value of binding constant (K) can be determined from the slope of the plot log [F_0 – F/F] versus log[Q] (Fig. 13). The calculated value of the quenching constant (K_{sv}), binding constant (K_b) and the number of binding sites (n) are provided in Table 3. Complex **3** has a high degree of binding than other complexes.

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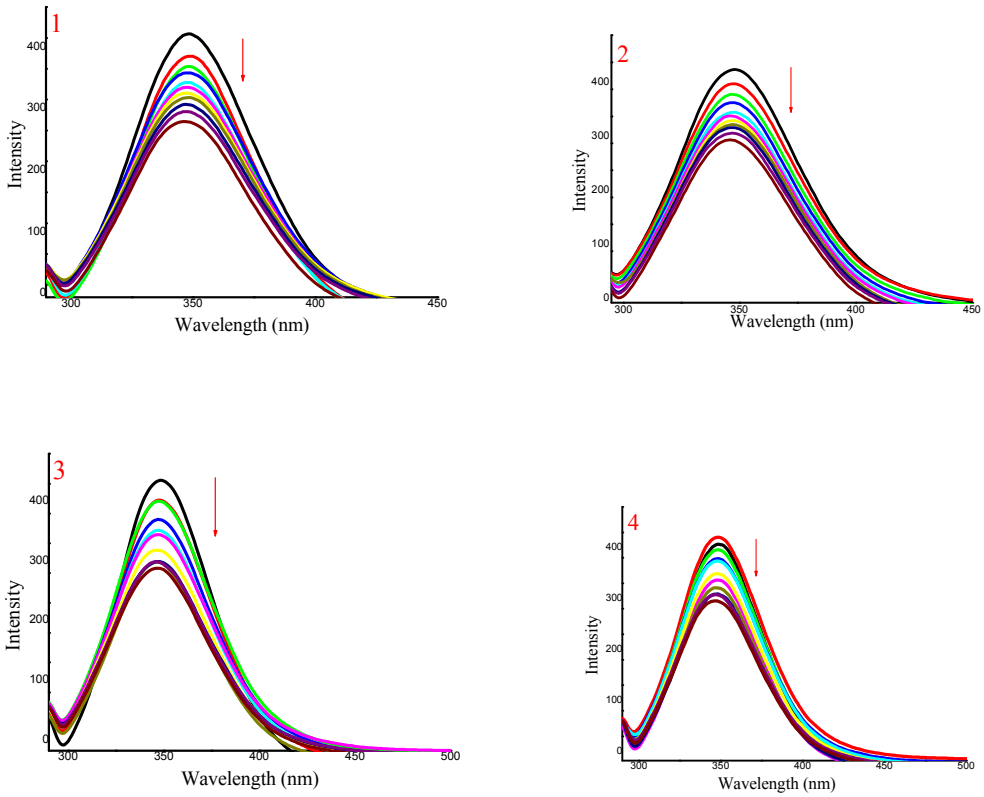

Fig. 11. Fluorescence quenching of BSA (1×10^{-5} M; λ_{exi} = 280; λ_{emi} = 346 nm) in the absence and presence of various concentration of complexes (0-100 μM).

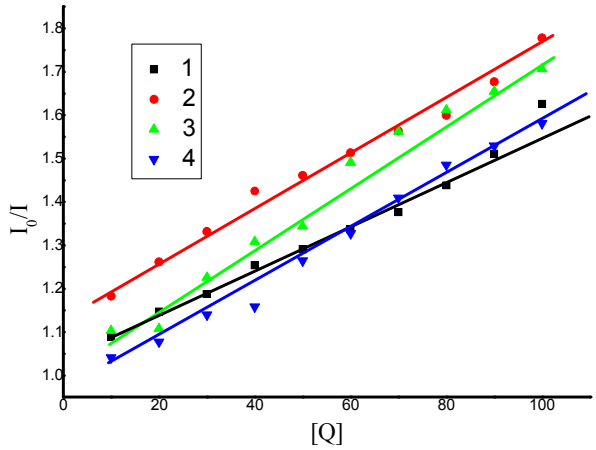

Fig. 12. Stern-VolmerPlot of BSA

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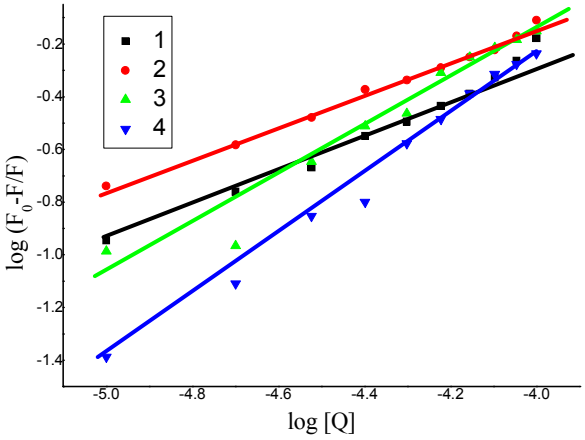

Fig. 13. Plot of log [Q] versus log (F_0-F/F)

Table 3 Quenching constant (K_{sv}) binding constant (K_b) and number of binding sites (n) for interaction of complexes (**1-4**) with BSA.

System	K_{sv}($\times 10^3 M^{-1}$)	K_b($\times M^{-1}$)	n
BSA + **1**	5.48±0.20	$4.92\times 10^2 \pm 0.21$	0.703
BSA + **2**	6.11±0.13	$1.98\times 10^2 \pm 0.12$	0.601
BSA + **3**	7.33±0.24	$4.69\times 10^3 \pm 0.29$	0.952
BSA + **4**	6.40±0.39	$3.98\times 10^4 \pm 0.32$	1.207

3.5.3 Synchronous fluorescence spectroscopic studies of BSA

The synchronous fluorescence method is usually applied to find out the conformation changes in the active site of the protein, that is around tryptophan and tyrosine.[61] Hence, the synchronous fluorescence spectra of BSA were measured before and after the addition of the complexes to get valuable information on the structural changes and molecular micro-environment. Synchronous fluorescence spectra displays tyrosine residue of BSA at wavelength interval Δλ of 15 nm whereas tryptophan residues of BSA at Δλ of 60 nm. As the concentration of complexes (0-100 μM) added to BSA (10 μM) is increased, a decrease in the fluorescence intensity was observed for all the complexes in both tryptophan and tyrosine but the tryptophan spectra were accompanied by a blue shift (Fig. S15). It reveals that the binding around tryptophan residues was strengthened. The results clearly indicate that all the complexes bind to the active site of protein and brought conformational change in the

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secondary structure of protein, which makes them potential molecules for biological applications.

3.6 Antioxidant activity

Free radicals play an important role in the inflammatory process. Number of thiosemicarbazone derivatives are reported to act either as inhibitors of free radical production or as radical scavengers.[62-65,16a]Thus, the compounds possessing antioxidant properties might play a vital role against inflammation and lead to potentially effective drugs. Since the experiments conducted so far revealed that the Pd(II) complexes have good DNA and protein binding ability, it is considered worthwhile to investigate their antioxidant activity.

3.6.1 DPPH$^{\cdot}$ scavenging assay

Antioxidants with DPPH radical scavenging activity are receiving much attention since they can explore interesting anticancer, anti-ageing and anti-inflammatory activities.[16a]The free radical scavenging activity of the compounds increased with an increase in the concentration of the compounds[17b,64]and it is measured in terms of IC_{50} values (Table 4 and Fig. S16). The results of the DPPH test showed that the new complexes exhibited a remarkable radical scavenging activity than the well-established standard (STD) vitamin C[17a] and also found to be significantly higher than the parent ligands and metal precursors.[16,17] Complexes**4**and **3** being good DPPH scavenger by comparing with the other complexes (Fig. 14).The observed antioxidant activity of the palladium complex may be due to the neutralization of free-radical character of DPPH by transfer of either an electron or a hydrogen atom.[65]All the complexes exhibited significant antioxidant activity than the reported complexes.[16,17b,67]

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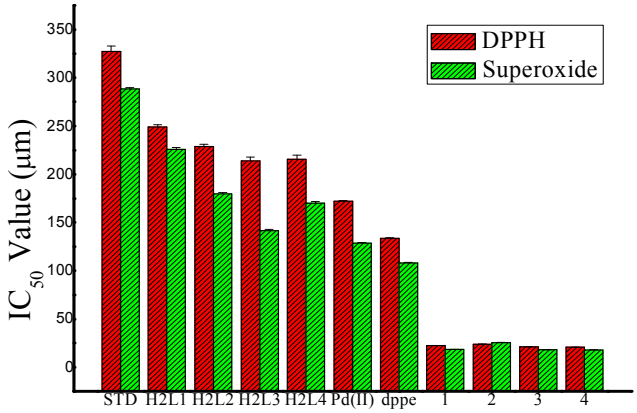

Fig. 14. Antioxidant activity (IC_{50} values) of ligands, bis(diphenylphosphino)ethane, $K_2[PdCl_4]$ and new Pd(II) complexes (**1**-**4**).Error barsrepresent the standard deviation of the mean (n=3).STD (Standard) = Vitamin C ($DPPH^{\cdot}$) and Butylatedhydroxytoluene ($O_2^{-\cdot}$)

3.6.2 $O_2^{-\cdot}$scavenging assay

Reactive oxygen species (ROS) are generated during cellular metabolic process and assist in different biochemical reactions. The superoxide anion $O_2^{-\cdot}$may lead to heavy effects including tissue injury and inflammation.[68]Superoxide ($O_2^{-\cdot}$) is highly reactive among the reactive oxygen species (ROS). We have tried to examine the antioxidant potential of $K_2[PdCl_4]$, ligands, 1,2-bis(diphenylphosphino)ethane and new Pd(II) complexes and their corresponding IC_{50} values were shown in Table 4. From the values, it can be concluded that the Pd(II) complexes exhibited better scavenging activity than thefree ligands, metal precursorsand the standard (STD) Butylatedhydroxytoluene (BHT)(Fig. S17 & 14).[67] Among the four complexes, complex **4** showed the better scavenging ability in bleach out the free radicals than other complexes. The order of the activity follows in the order **4**>**3**>**1**>**2.** The lower IC_{50} values observed in antioxidant assays have demonstrated that these complexes have strong potential to be applied as scavengers for eliminating radicals.

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Table 4 The radical scavenging activity of the compounds and the results are expressed as the mean ± SD (n=3).

Compounds	IC_{50} values (μM)	
	$DPPH^{\cdot}$	$O_2^{-\cdot}$
Standard (STD)	327.16±5.68	288.55±1.14
H_2L^1	248.97±2.44	225.86±2.00
H_2L^2	228.83±2.29	179.58±1.41
H_2L^3	213.84±3.76	141.72±1.45
H_2L^4	215.65±4.12	170.29±1.24
$K_2[PdCl_4]$	172.01±0.69	128.60±0.53
dppe	133.61±0.51	108.29±0.29
1	22.47±0.09	18.51±0.07
2	23.97±0.12	25.54±0.15
3	21.09±0.11	18.32±0.09
4	20.84±0.05	18.13±0.10

*(Standard) STD= Vitamin C ($DPPH^{\cdot}$) and Butylatedhydroxytoluene ($O_2^{-\cdot}$)

3.6.3 Reducing ability

Depending on the reducing power of each compound, the test solution changes its colour from yellow to various shades of blue and green. The presence of reducers (i.e., antioxidants) reduces the Fe^{3+}/ferricyanide complex to the ferrous form, the Perl's Prussion blue after the addition of trichloroacetic acid and ferric chloride that can be monitored at 700 nm. The reducing power of the standard BHT at various concentrations was higher than that of test compounds. Increase in concentration of the compounds increases the reducing ability and the new palladium(II) complexes (**1-4**) which have strong reducing ability than the free ligands, dppe and $K_2[PdCl_4]$. The complex **4** has higher reducing ability when compared to the other complexes (Fig. 15).

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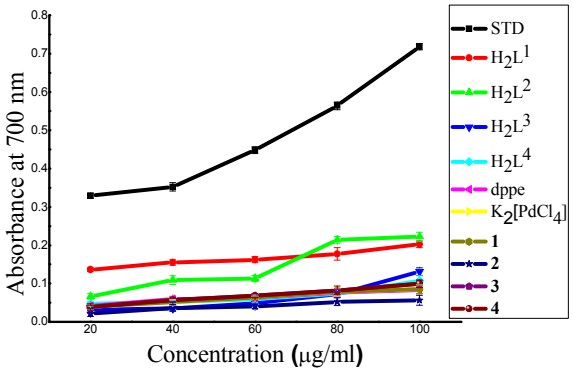

Fig. 15. Reductive ability of ligands, bis(diphenylphosphino)ethane, $K_2[PdCl_4]$ and new Pd(II) complexes (**1-4**). Error barsrepresent the standard deviation of the mean (n=3).

3.6.4 Total antioxidant activity assay

The total antioxidant activity of Pd(II)precursors, free ligands and their corresponding palladium(II) complexes (**1-4**) was assessed by phosphomolybdenum method. This method is quantitative, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The total antioxidant activities of the complexes were higher than the free ligands and the values are listed in Table 5. Among the four complexes, complex **4** showed the good activity.

From the above results, it can be concluded that the scavenging effects of$K_2[PdCl_4]$, 1,2-bis(diphenylphosphino)ethane and free ligands are comparatively less than their corresponding Pd(II) complexes, which is mainly due to the chelation of the organic ligand with the Pd(II) ion.

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Table 5 Estimation of Total antioxidant capacity of new Pd(II) complexes and the results are expressed as the mean ± SD (n=3).

Compounds	µg Ascorbic acid equivalents/ml
H_2L^1	9.84±0.55
H_2L^2	10.95±0.56
H_2L^3	9.52±0.61
H_2L^4	12.26±0.45
$K_2[PdCl_4]$	9.52±0.48
dppe	7.87±0.53
1	39.64±0.44
2	31.37±0.31
3	47.61±0.37
4	52.82±0.33

3.7 Antibacterial activity studies

Metal ions are adsorbed on the cell walls of the microorganisms, disturbing the respiration process of the cells and thus blocking the synthesis of protein required for their growth. Hence, metal ions are vital for the growth-inhibitory effects.[69] Antibacterial studies of synthesised compounds have been carried out with some bacterial microorganisms namely *Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi*and *Escherichia coli*. From the result, it is noted that the $K_2[PdCl_4]$, ligands and dppe did not show any significant activity at 1 mg/ml concentration. From the bactericidal activity, it is clear that the complexes (**1-4**) were more effective towards gram positive bacterial strains than gram negative bacterial strains (Fig. 16), due to the different structure of the cell walls.[70]All the complexes were found to be good activity on all the pathogens except *E. Coli*, in particularly, complex **4** exhibited better activity on *B. subtilis, S.aureus*and *S. typhi*. The activities of the complexes may be explained by chelation theory.[71] The variation of activity exhibited by different complexes against various organisms is dependent on either the differences in ribosome in microbial cells or the impermeability of the cells of the microbes.[72]

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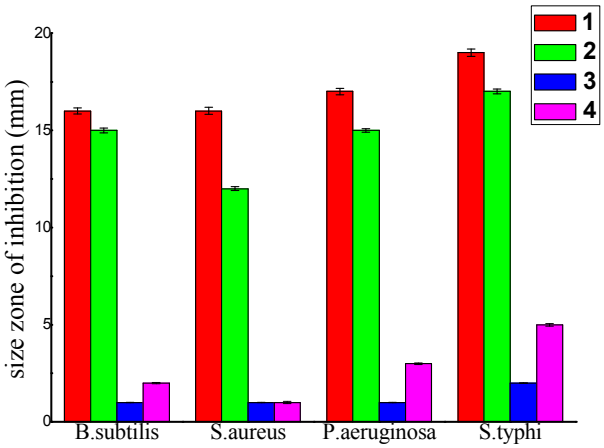

Fig.16. Anti-bacterial screening of new Pd(II) complexes (**1-4**). Error bars represent the standard deviation of the mean (n=3).

3.8 *In vitro* cytotoxicity studies

3.8.1 Cytotoxic activity against human tumour cell lines

The positive results obtained from the DNA binding and DNA cleavage, protein binding, antibacterial and antioxidant studies encouraged us to test the cytotoxicity of the complexes against MCF-7 human breast cancer cell line and HeLa human cervical cancer cell line using the colorimetric MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Cells were incubated with each compound for a period of 24 h and *cisplatin* was used for comparison. There were no substantial effect was observed in cell proliferation by the $K_2[PdCl_4]$, ligands and dppe when compared with control cells (Table 6). All the complexes exhibited better activity compared to ligands, metal precursors and bis(diphenylphosphinoethane) (dppe). Moreover, on comparing the IC_{50} of these complexes with that of *cisplatin* for the MCF-7 cell line, complexes **1**, **3** and **4** showed better activities with exception of complex **2**(Table 6).[23]Whereas in HeLa cell line, all the complexes showed very low cellular growth inhibition at the maximum concentration of 100 μM and therefore had no evaluable cytotoxicity (IC_{50}> 100 μM).HeLa is a cervical cancer cell line. Whereas, MCF-7 is a breast adenocarcinoma cell line. These two cell lines are originated from distinct organs. In generally, cytotoxic molecules internalise into cells through various mechanisms such as endocytosis, diffusion and cell surface receptors mediated. If molecules internalise into cells through particular receptors and those molecules can only enter into the cells. The

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cytotoxic ability of the compounds has been investigated by using the percentage of cell viability versus concentration of compounds (Fig. 17& S18). The observed cytotoxic effects by the new palladium(II) complexes towards the MCF-7 cell line was better than those of previously reported palladium complexes.[23]The overall results observed in the cytotoxicity of the complexes are very similar to that of the DNA/protein binding and antioxidant activity. In general, the Pd(II) complexes exhibited anticancer activity in the order of **4**>**3**>**1**>**2**.Complex **4** exhibited better activity than other complexes and this may be due to the presence of 5-(2-hydroxy-3-methyl-phenyl)-4-phenyl-2,4-dihydro[1,2,4]triazole-3-thione. Complex **3** showed moderate activity than the bisphosphine bridged binuclear complexes (**1** and **2**).Further cytotoxicity of complexes was tested against non-cancerous normal human keratinocyteline (HaCaT) and the complexes showed less toxic towards the normal cells which is evident from its higher IC_{50} values (IC_{50}> 100 μM). These results suggest that the complexes possess anticancer activities in human breast cancer cells with less toxic against normal cells.

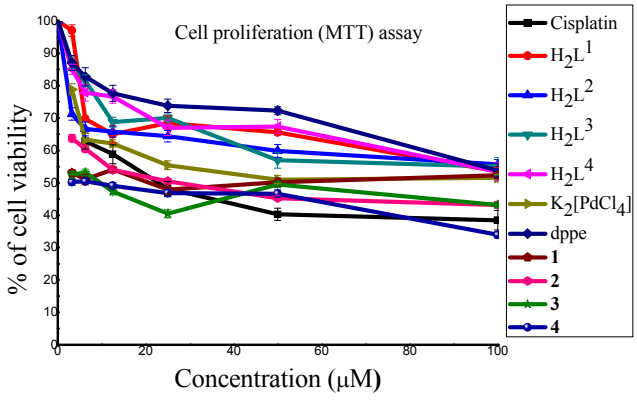

Fig. 17. MCF-7 cells were treated with different concentrations of complexes (**1-4)**, ligands, bis(diphenylphosphino)ethane, $K_2[PdCl_4]$ and *cisplatin* (3.125, 6.25, 12.5, 25, 50, 100) for 24 h. cell viability was assessed by cell proliferation (MTT) assay. Error bars represent the standard deviation of the mean (n=3).

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Table 6.*In vitro* cytotoxicity activity of the complexes (**1-4)**, ligands, bis(diphenylphosphino)ethane, $K_2[PdCl_4]$ and *cisplatin* against MCF-7 cell line expressed as the IC_{50} values (μM). The results are expressed as the mean ± SD (n=3).

Compounds	IC_{50} values (μM)
	MCF-7
Cisplatin	23.7±0.07
H_2L^1	>100
H_2L^2	>100
H_2L^3	>100
H_2L^4	>100
$K_2[PdCl_4]$	>100
dppe	>100
1	20.38±0.11
2	27.11±0.13
3	9.74±0.09
4	8.44±0.05

4 Conclusion

Newly synthesised palladium(II) complexes were characterised by analytical and spectral techniques. Further, their exact structure except complex **2** was confirmed by X-ray crystallographic studies. Complex **1** is a bincuelar complex containing bridged diphenylphosphino ethane with ONS chelation of thiosemicarbazone ligand. Complex **3**, was identified as a trinuclear complex with the unusual cyclised Pd-S bonds. Complex **4**, a mononuclear complex with ONS chelation of the ligand and sulphur bonded 5-(2-hydroxy-3-methoxy-phenyl)-4-phenyl-2,4-dihydro[1,2,4]triazole-3-thione which is formed through the cyclization of H_2L^4.The complexes bound intercalatively with CT-DNA and interaction with albumin followed static quenching mechanism. Antioxidant activity of the complexes found as better than the free ligands and standard. All the complexes showed effective antibacterial activity against five pathogenic bacteria. *In vitro* cytotoxicity of the compounds was evaluated against the HeLa (human cervical cancer) and MCF-7 (human breast cancer) cell lines by comparing with *cisplatin*. The palladium(II) complexes did not show any activity in HeLa cells up to 100 μM concentration. However, in MCF-7cell line, they showed higher cytotoxicity as compared to the ligands and the standard.

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Supplementary Information

CCDC1538585, 1538586 and 1538587contain the supplementary crystallographic data for complexes $[Pd_2(Msal\text{-}tsc)_2(\mu\text{-}dppm)]$ (**1**), [Pd(Msal-ptsc)(Msal-ptaz)] (**4**) and $[(Pd_3(\mu\text{-}S\text{-}Msal\text{-}etsc)_3)]$ (**3**) respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data centre via www.ccdc.cam.ac.uk.

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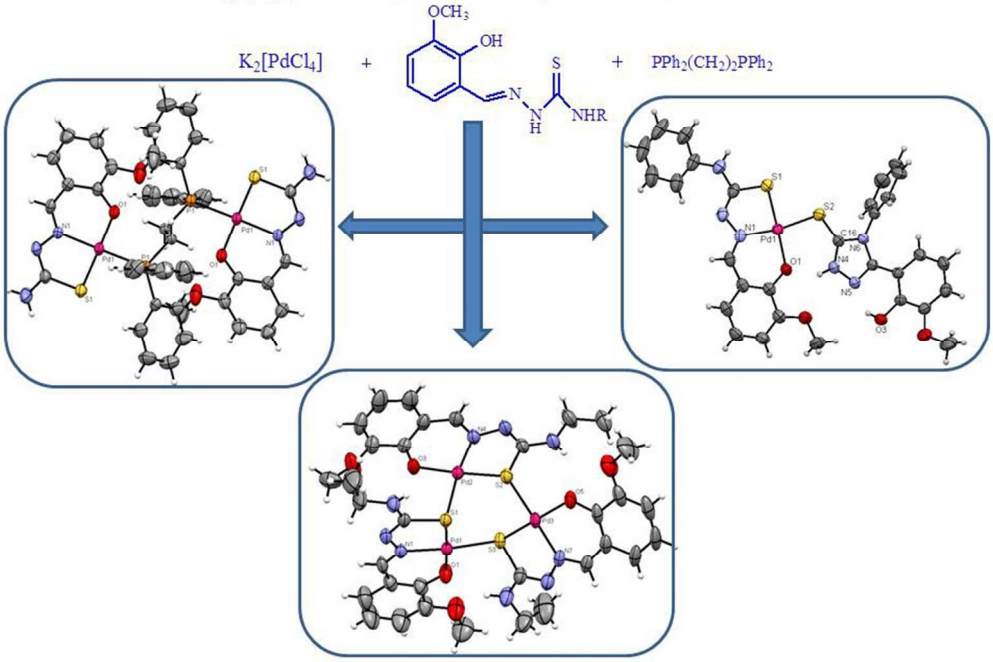
Structurally different mono, bi and trinuclear Pd(II) complexes and their DNA/Protein interaction, DNA cleavage, anti-oxidant, anti-microbial and cytotoxicity studies

A. Shanmugapriya, Ruchi Jain, D. Sabarinathan, G. Kalaiarasi, F. Dallemer and R. Prabhakaran



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