A new series of Pd (II) complexes of methyl substituted benzylamine ligands (BLs) has been synthesized and characterized via spectroscopic techniques such as UV/Vis, FTIR, LCMS, $^1$H, and $^{13}$C NMR. The UV/Vis study in DMSO, DMSO + water, and DMSO + PBS buffer (pH = 7.2) confirmed their molecular sustainability in liquids. Their \textit{in vitro} anticancer activity against breast cancer cell lines such as MCF-7 and MDA-MB-231 makes them interesting for \textit{in vivo} analysis. Their stronger DNA binding activity (DBA) compared with free ligand suggested them as a good DNA binder. DBA was further confirmed by physicochemical studies such as surface tension and viscosity of complex + DNA which inferred the disruption of DNA and intercalation of complexes, respectively. Their % binding activity, % disruption of DNA base pairs (DNABP), and % intercalating strength are reported in this paper for the first time for better understanding of DNA binding mechanism. Along with this, their scavenging activity (SA) determined through DPPH free radical and the results indicate good antioxidant behaviour of complexes.

1. Introduction

From the last few decades, the transition metal complexes with different amine ligands have drawn a significant interest in exploring anticancer activities, specifically related to solid tumor chemotherapies [1–4]. The breast cancer, a solid tumor, is one of the major issues in health care in terms of morbidity, mortality, and therapy costs [5]. To overcome these problems many more drugs have been introduced into the market but their response to therapy is still very poor. However, such drugs have not been found to be much effective for treatment of solid tumors due to their pertinent side effects such as nephrotoxicity, drug resistance, and renal and cervical problems [6, 7]. Therefore, the foremost target of most research groups is to find a convenient anticancer drug that can be used efficiently for the treatment of solid tumors. Recently, many Pd (II) complexes with promising anticancer activity against tumor cell lines have been synthesized and reported elsewhere [8]. In such studies, a good correlation was observed between the cytostatic activity and lipophilicity of the Pd complexes [6–8]. In fact, the Pd complexes, as a nonplatinum complex, have recently been reviewed to have a significant antitumor activity and lower side effects compared to cisplatin [5]. As an essential feature of metal-containing anticancer agents, Pd complexes are expected to have less kidney toxicity than cisplatin [7, 8]. Further many new Pd complexes with amine ligands having promising anticancer activities with lower side effect have recently been reported [5–12]. Bearing in mind that Pd (II) complexes are about 105 times more reactive than their Pt (II) analogues, the lower antitumor activity of Pd compounds has been attributed to very rapid hydrolysis of the leaving groups that dissociate readily in solution, leading to reactive species far from their pharmacological targets [9, 10].

Keeping above inconveniences, a new series of Pd (II) complexes has been synthesized with methyl substituted BLs and analyzed their \textit{in vitro} antitumor activity on breast cancer cell lines MCF-7 and MDA-MB-231 which expressed effective anticancer potential. Since DNA is a primary molecular target of anticancer drugs and ascertains an extent of
2. Experimental Section

2.1. Materials and Methods. Palladium dichloride (PdCl₂), benzylamine ligands (BLs), CT-DNA, Tris buffer, DMSO, and ethanol (>99.5%) were procured from Sigma-Aldrich and used as received. Elemental analysis was made with a Euro vector CHN analyzer and UV/Vis spectra were recorded with a Spectro 2060 plus spectrophotometer over 200–600 nm by using 1 cm path length cuvette. FTIR (Perkin Elmer) spectra were taken with KBr palate where polystyrene thin film was used as a calibration standard. 1H and 13C NMR spectra were recorded in DMSO-d6 (NMR, 99.99%) with a Bruker-Biospin Avance-III 500 MHz FT-NMR spectrometer. Mass spectra were obtained with PE SCIEX API 165 with +ve ESI mode. 1H NMR (500 MHz; DMSO-

2.2. General Consideration for Synthesis. Initially, PdCl₂ and BLs were separately dissolved in freshly prepared aqueous ethanol (absolute ethanol and Milli-Q water in 1:1.5) in 1:2 molar ratio, using 1 MLH magnetic stirrer. The BLs solutions were added dropwise in metal compound solution with continuous stirring at room temperature. After 16 h, the mixture turned from light brown to greenish for Pd complexes and precipitates were formed. The ppt were filtered off and washed several times with chilled water/ethanol in 1:1 ratio and kept overnight in vacuum oven at room temperature for absolute dryness.

2.3. Characterization Data

2.3.1. Complex 1: C₁₆H₂₀N₂Pd [Pd₂MBA]. Yield: 0.1810 g, 74.76%. Elemental analysis, found: C, 55.74; H, 5.26; N, 8.13%. Calcd for C₁₆H₂₀N₂Pd: C, 55.82; H, 5.44; N, 8.22%. IR (KBr): v_max/cm⁻¹ = 3300 and 3214 (NH₂), 1493 and 1449.7 (Ph, C=C), 740.7 (mono substituted Ph), 1051.9 (C–N), 479.6 (Pd–N). 1H NMR (125 MHz; DMSO-d6; Me₄Si) δ 2.09 (2H, s, PhCH₂N₂H₂), 3.93 (2H, s, PhCH₂N₂H₂), 2.50 (3H, s, PhCH₃), 7.37–7.43 (1H, d, PhH, J = 13.9 Hz), 7.45–7.47 (1H, d, PhH, J = 7.3 Hz) and 7.35–7.38 (1H, m, PhH).

2.3.2. Complex 2: C₁₆H₂₀N₂Pd [Pd₃MBA]. Yield: 0.1785 g, 74.20%. Elemental analysis, found: C, 55.74; H, 5.26; N, 8.13%. Calcd for C₁₆H₂₀N₂Pd: C, 55.79; H, 5.33; N, 8.19%. IR (KBr): v_max/cm⁻¹ = 3257 and 3210 (NH), 1493 and 1453 (Ph, C=C), 744.64 (mono substituted Ph), 1099 (C–N), 437.42 (Pd–N). 1H NMR (500 MHz; DMSO-d6; Me₄Si) δ 0.2086 (2H, s, PhCH₂N₂H₂), 3.932 (2H, s, PhCH₂N₂H₂), 2.404 (3H, s, PhCH₃), 7.21–7.23 (1H, d, PhH, J = 7.2 Hz), 7.08–7.10 (1H, d, PhH, J = 7.2) and 7.12–7.20 (1H, s, PhH).

2.3.3. Complex 3: C₁₆H₂₀N₂Pd [Pd₄MBA]. Yield: 0.1785 g, 74.20%. Elemental analysis, found: C, 55.74; H, 5.26; N, 8.13%. Calcd for C₁₆H₂₀N₂Pd: C, 55.88; H, 5.29; N, 8.21%. IR (KBr): v_max/cm⁻¹ = 3257 and 3210 (NH), 1493 and 1453 (Ph, C=C), 756.46 (mono substituted Ph), 1000.7 and 1036.1 (C–N), 492.6 (Pd–N). 1H NMR (500 MHz; DMSO-d6; Me₄Si) δ 2.09 (2H, s, PhCH₂N₂H₂), 3.93 (2H, s, PhCH₂N₂H₂), 2.31 (3H, s, PhCH₃), 7.33–7.35 (1H, d, PhH, J = 6.0 Hz), 718–720 (1H, d, PhH, J = 10.72 Hz) and 7.25 (1H, s, PhH).

2.3.4. Complex 4: C₁₆H₂₀N₂Pd [Pd₅MBA]. Yield: 0.1785 g, 74.20%. Elemental analysis, found: C, 55.74; H, 5.26; N, 8.13%. Calcd for C₁₆H₂₀N₂Pd: C, 55.88; H, 5.29; N, 8.21%. IR (KBr): v_max/cm⁻¹ = 3257 and 3210 (NH), 1493 and 1453 (Ph, C=C), 756.46 (mono substituted Ph), 1000.7 and 1036.1 (C–N), 492.6 (Pd–N). 1H NMR (500 MHz; DMSO-d6; Me₄Si) δ 2.09 (2H, s, PhCH₂N₂H₂), 3.93 (2H, s, PhCH₂N₂H₂), 2.31 (3H, s, PhCH₃), 7.33–7.35 (1H, d, PhH, J = 6.0 Hz), 718–720 (1H, d, PhH, J = 10.72 Hz) and 7.25 (1H, s, PhH).

2.3.5. Complex 5: C₁₆H₂₀N₂Pd [Pd₆MBA]. Yield: 0.1785 g, 74.20%. Elemental analysis, found: C, 55.74; H, 5.26; N, 8.13%. Calcd for C₁₆H₂₀N₂Pd: C, 55.88; H, 5.29; N, 8.21%. IR (KBr): v_max/cm⁻¹ = 3257 and 3210 (NH), 1493 and 1453 (Ph, C=C), 756.46 (mono substituted Ph), 1000.7 and 1036.1 (C–N), 492.6 (Pd–N). 1H NMR (500 MHz; DMSO-d6; Me₄Si) δ 2.09 (2H, s, PhCH₂N₂H₂), 3.93 (2H, s, PhCH₂N₂H₂), 2.31 (3H, s, PhCH₃), 7.33–7.35 (1H, d, PhH, J = 6.0 Hz), 718–720 (1H, d, PhH, J = 10.72 Hz) and 7.25 (1H, s, PhH).

3. Biological Evaluation

3.1. In Vitro Anticancer Activity. Cell viability was estimated colorimetrically using 2-(3-diethylamino-6-diethylaminonaphtalene-9-yI)-5-sulfobenzenesulfonate, SRB (sulfurhodamine B) as standard assay with high reproducibility [17].

3.1.1. Cell Lines and Culture Conditions. Human breast cancer cell lines MCF-7 and MDA-MB-231 were obtained from NCI, USA, and grown in minimal essential medium (MEM). Eagles media were supplemented with 10% heat inactivated fetal bovine serum (FBS, Sigma-Aldrich), 2 mM L-glutamine, and 1 mM sodium pyruvate (Hyclone) in humidified CO₂ incubator.
3.1.2. Assay of Cytotoxicity in Cancer Cell Lines. Cytotoxicity of Pd (II) complexes was determined by SRB assay where E5000 cells were seeded into each well of a 96-well clear flat bottom polystyrene tissue culture plate and incubated for 2 h in MEM. An additional 190 mL cell suspension was added in each well containing 10 mL test sample in 10% DMSO with 10 mL Adriamycin (doxorubicin) as a positive drug control. Each experiment was carried out in 3 replicate wells. After an incubation of 48 h, 100 mL of 0.057% SRB solution (w/v) was added in each well. Then 200 mL of 10 mM Tris base solution (pH = 10.5) was added into each well and shaken smoothly. The cell viability was assayed by absorption at 510 nm with a microplate reader. The experiments were repeated thrice with 3 replicates each time and 99% reproducibility was obtained.

3.2. DNA Binding. CT-DNA (analytical grade, Sigma-Aldrich) was used as received. Tris-HCl buffer (10 mM, pH = 7.2) was prepared in Milli-Q water for each experiment well and used for absorption titration, viscosity, surface tension, conductivity, and zeta potential measurements.

3.2.1. Absorption Spectroscopy. DNA concentration was determined using an absorption spectrophotometer that gave 6600 M⁻¹cm⁻¹, a molar absorption coefficient at 260 nm [18, 19]. The CT-DNA in buffer gave a ratio of UV absorbance at 260 and 280 nm which is found to be 1.8 to 1.9 and indicates a presence of protein free DNA [20–22]. The absorbance at 260 and 280 nm [18, 19] gave 6600 M⁻¹cm⁻¹. The CT-DNA absorbance was 6600 M⁻¹cm⁻¹ where the absorbance of DPPH· was measured at 517 nm with UV/Vis spectrophotometer. Later, the radical scavenging activity was measured and a decrease in DPPH· absorbance indicated a radical scavenging activity, calculated with the following equation [23]:

\[
\text{Scavenging activity} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100. \quad (2)
\]

The A_0 is absorbance of DPPH· with a test compound and A_1 absorbance of DPPH· without a test compound. Absorbance data are presented as means ± SD of three determinations.

4. Results and Discussion

4.1. Synthesis and Characterizations. Initially, the PdCl₂ and BL ligands were mixed in 1:2 molar ratio for the synthesis of Pd(BLs₂)₂ complexes (Figure 1). The reaction was conducted in aqueous ethanol solution for 14 to 16 h as per reaction scheme given below.

**Synthesis of Pd (II) Complexes.** Consider

\[
PdCl_2 + 2BLs \xrightarrow{\text{Aqueous C}_2\text{H}_5\text{OH}} \text{Pd (BLs)}_2 \quad (3)
\]

The 3300 to 3119 cm⁻¹ stretching frequencies inferred presence of –NH₂ of benzylamine in the Pd(BLs)₂ and similarly from 1497 to 1453 cm⁻¹ predicted C=C in phenyl ring. The 495.92 to 438.78 cm⁻¹ and 380–348 cm⁻¹ indicates Pd–N coordinate and Pd–Cl bands, respectively [26, 27]. In ¹H NMR, the 2H of –NH₂ and PhCH₂– appeared at δ 3.93 to 2.09 with singlet for Pd(BLs)₂. The PhCH₃ proton of benzylamine appeared at δ 2.50 to 2.31 with singlet for all Pd(BLs)₂ [28].
Two doublets at $\delta$ 7.37–7.42 and 7.49–7.47 having $J = 13.9$ and 7.3 MHz, respectively, in Pd2MBA for H of C4 and C6 appeared. Similarly, a singlet for 1H of C3 and C4 at 7.20 value and doublets for 1H of C5 and C6 at $\delta$ 7.08–7.10 and 7.21–7.23 ($J = 7.2$ MHz), respectively, were found for Pd3MBA. A doublet at $\delta$ 7.33–7.35 and 7.18–7.20 with $J = 6.0$ and 10.72 MHz, respectively, was confirmed for 1H of C3 and C4 positions while a singlet was confirmed for 1H of C6 at $\delta$ 7.25 in Pd4MBA. In $^{13}$C NMR, the benzyl carbon (PhCH$_2$–) appears at $\delta$ 47.66 for all the Pd(BLs)$_2$ [28]. The aromatic ortho, meta, and para –CH$_3$ attached carbon appeared at $\delta$ 145.72, 129.036, and 138.23, respectively, for the Pd(BLs)$_2$. The carbon of –CH$_3$ at ortho, meta, and para appeared within $\delta$ 20.98 to 18.72 ppm. The +ve ESI mass spectra of Pd(BLs)$_2$ have found [M + 1] confirming their molecular mass. In UV study, the absorption spectrum consists of a band at 400 nm and may be assigned as $1A_{1g} \rightarrow 1A_{2g}$ ($d_{xy} \rightarrow d_{x^2-y^2}$) transition occurring with Pd complexes (see ESI, Figures 1–3, in Supplementary Material available online at http://dx.doi.org/10.1155/2016/9245619) [29]. To investigate their molecular stability in solution, the UV/Vis spectral behaviour was investigated in DMSO, DMSO + water, and DMSO + phosphate buffer for Pd(BLs)$_2$. (ESI, Figures 1–3). The overall patterns of spectra for complexes solution were found to be similar to the different mediums to ensure their stability. Thus our synthesized Pd (II) complexes having UV/Vis absorption from 265 to 270 nm and $^1$H NMR coupling constant between 5 and 10 MHz have confirmed their trans geometry (Figure 2).

4.2. Anticancer Activity. Moving towards the higher anticancer potential of Pd (II) complexes, many of the in vitro studies on different cell lines have been reported, suggesting their cytotoxicity up to some extent [30–33]. In such studies authors have synthesized some Pd complexes with different amine ligands and report their cytotoxicity when tested in vitro [30–33]. It seems that these amine ligands and their derivatives can further improve the cytotoxicities when they reacted with different metals. Therefore, in our study we tried to focus on the factors, namely, nature of ligands and methyl group substitution on ligand, which can modulate the cytotoxicity of such complexes. In this context, we have demonstrated an in vitro anticancer study of some novel Pd complexes using BLs as ligands for better anticancer activity. The complexes were tested in vitro against MCF-7 and MDA-MB-231 human breast tumor cell lines by colorimetric microculture 2-(3-diethylamino-6-diethylazaniumylidene-xanthen-9-yl)-5-sulfobenzenesulfonate (SRB) assay and compared with Adriamycin (ADR) and cisplatin [6, 34]. The MCF-7 and MDA-MB-231 cell lines anticancer activities for 10, 20, 40, and 80$\mu$M Pd(BLs)$_2$ have been illustrated in Figures 3 and 4. The analyzed GI$_{50}$, TGI, and LC$_{20}$ in $\mu$M have inferred 50% growth inhibition, resultant total growth inhibition, and a net loss of 50% cells after treatment, respectively (Table 1).

The GI$_{50}$ value less than 10$\mu$M depicts the anticancer activity with respect to ADR and cisplatin, where the closer GI$_{50}$ values of Pd2MBA, Pd3MBA, and Pd4MBA from standards make them interesting for testing against other cancerous cell lines.

4.3. DNA Binding Activity

4.3.1. Spectrophotometric Method. The DBA explains the anticancer nature of the complex or the drug and can be analyzed by the absorption spectral study. In light of this,
the Pd (II) complexes were mixed with CT-DNA at certain concentration and shown the changes in absorbance. Generally, the hypochromism effect in UV study reveals all synthesized complexes intercalative binding strength attributed to an interaction with DNA bases [35, 36]. Similarly, a hyperchromic effect ascribes an external contact or a partial uncoiling of DNA structure; exposing more bases of DNA may be due to electrostatic binding [37, 38]. Such observations have been noticed in the present study where a significant hypochromic effect in absorption titrations of DNA with Pd (II) exposed an intercalation with the base pairs of DNA [37, 38] (ESI, Figures 4–6).

The decrease in UV absorption predicted the strong interactions of Pd2MBA, Pd3MBA, and Pd4MBA, whose intercalating strengths depend on size and electron densities of interacting aromatic rings with an amine group [35–38]. Their DBA has been found stronger in comparison to a free ligand like benzylamine (ESI, Figure 7).

Comparative % Binding Affinity of Complexes. For a better understanding of their binding potential, a % binding affinity (BA) with DNA has been calculated through well known spectrophotometric method at a characteristic wavelength of 260 nm. The % BA was calculated as per the following equation (Figure 5):

\[
\text{% BA of Complexes} = \frac{\text{Absorbance of complex at 260 nm} - \text{Absorbance of DNA at 260 nm}}{\text{Absorbance of DNA at 260 nm}} \times 100. 
\]

Figure 4 shows the BA of each complex where negative sign explains their strong binding with DNA and hypochromism of the complexes.

The all Pd (II) complexes show approximately the same binding activity at each concentration indicating the same binding potential of each complexes. The same binding potential can further be explained by the same geometry of the all complexes. Therefore, it can be understood that the Pd complexes with the same geometry may equally participate when they interacted with DNA.

4.3.2. Physicochemical Method. Along with spectrophotometric study, the physicochemical study, such as viscosity, surface tension, conductivity, and zeta potentials, is also an effective method for better understanding of DBA. Thus, we have mathematically established a comparison of their physicochemical data that presumes their binding affinity with DNA at structural level.

(1) Study of Structural Disruption of CT-DNA. Initially, the complex entertains the DNA and attacks on its base pairs; therefore it weakens the intermolecular forces of bases which results in the disruption of base pairs. Since, it is an intermolecular force disruption and such disruption can be measured by the analysis of surface tension, therefore, we have analysed and compared the surface tensions of DNA with and without complexes. Generally, the decrease in surface tension infers the force disruption and we have also obtained such results where we compared the surface tension of pure DNA and DNA + complexes. For instance, the surface tension values of the DNA solution with increasing amounts of Pd (II) complexes (1/R = 0.2, 0.6, 1.0, 1.4, 1.8) have decreased as compared to pure DNA (Figure 6).

A decrease in surface tension of Pd (II) complexes + DNA inferred the weakening of intermolecular interaction of DNA bases and has lost interaction with complexes.

Complex Contributions on Disruption of DNA Base Pairs. Surface tension is an important analysing aspect for the disruption of DNA base pairs; therefore, for the advanced study we have also measured and compared the % disruption of DNA base pairs. The % complex contribution was calculated on the disruption of DNA (Figure 7):

\[
\text{% disruption of DNA base pairs} = \left(\frac{\gamma_{\text{DNA with complexes}} - \gamma_{\text{pure DNA}}}{\gamma_{\text{pure DNA}}} \right) \times 100. 
\]

Figure 7 shows the disruption of DNABP with respect to complexes and with negative values indicating the weakening
interaction of DNA bases towards disruption. This negative values support the binding affinity (BA) of complexes where the lower –ve values of Pd complexes indicate the disruption of DNABP.

(2) Study on Intercalation Phenomenon of Complexes. For the classical intercalative mode, the viscosity of the DNA solution is increased due to an increase in overall DNA length while it is decreased for a partial nonclassical intercalation process causing a bend or kink in the DNA helix that reduces its effective length concomitantly [39, 40]. For instance, a decrease in relative viscosity with cisplatin is explained due to covalent binding and shortening in an axial length of double helix DNA. On the other hand, classical organic intercalator such as ethidium bromide increased the relative viscosity with increasing of an axial length of the DNA [41–43].

To observe covalent binding or classical organic interaction, their relative specific viscosities \( \eta/\eta^0 \) \( 1/3 \) (\( \eta^0 \) and \( \eta \) being specific viscosity contributions of DNA with and without complexes, resp.) were plotted against \( 1/R \) (\( R = [\text{DNA}]/[\text{complex}] = 0.2, 0.6, 1.0, 1.4, 1.8 \)) (Figure 6).

On increasing concentration of Pd(Bl)s2, an increase in viscosity of DNA was observed, proving the Pd(Bl)s2 as DNA intercalators (Figure 6). For ethidium bromide, the relative viscosity of DNA is increased with a slope from 0 to 0.9448 L/mM [44], whereas with 0.001 the relative viscosity of DNA has increased for Pd2MBA, Pd3MBA, and Pd4MBA. The viscosity of free ligand (2-methylbenzylamine) with DNA was measured and found lower than the complexes + DNA, which inferred that interaction of DNA with free ligand is weaker as compared to complex. Therefore, a higher increase in viscosity of Pd complex + DNA as compared to free ligands clearly explained the stronger intercalation between complexes and DNA [42]. The constant value of the slope and increasing values of viscosity infer almost similar intercalating nature for all the complexes.
Complex Contributions towards Intercalation with DNA Base Pairs. Apart from the above discussion, we have also established the % binding affinity with respect to viscosity ($\eta$) using two different methods. In both methods the % complex on the intercalation with DNA was calculated:

$$\% \text{Intercalating strength of complexes} = \frac{\eta \text{ of DNA with complexes} - \eta \text{ of pure DNA}}{\eta \text{ of pure DNA}} \times 100.$$ (6)

Figure 8 clearly explains the individual BA of complexes with DNA base pairs (DNABP). The Pd2MBA, Pd3MBA, and Pd4MBA expressed lower BA when they interacted with DNA.

(3) Conductivity and Zeta Potential. The DNA and complexes are charge molecules; therefore their conductivity and zeta potential have also been considered as interaction parameters as well as key factors for an analyzing conformational behaviour of an isolated DNA chain with the complexes. A DNA solution of 50 $\mu$M had 3426 $\mu$S conductivity while, with increasing amounts of complexes, the decrease in zeta potential as well as conductivities has been found (Figure 9 and Table 2). DNA molecules are negatively charged due to phosphate groups, but due to interaction with complexes their negative charge density is decreased due to Columbic interaction of positively charged complexes and the disassociation of counter ions in DNA.

The decrease in conductivity and zeta potential confirmed that the DNA could structurally be modified due to such interactions.

4.4. Drug Efficacy Studies or DFI with DNA Binding. For the better understanding of Pd(BLs)$_2$ + DNA interaction and their disruption during an intercalation process, DFI has found reliable data in support of anticancer activity [42]. Therefore, our study infers that the Pd(BLs)$_2$ + DNA interactions at different concentration have shown their higher viscosities and lower surface tension with respect to DNA solution (Figure 6). Thus, a disruption of DNABP decreases surface tension by developing an interaction between complexes and DNA.

4.5. Scavenging Activities. The scavenging activities have been investigated to support the anticancer potential of such complexes [43, 44]. Recently it is reported that, by using hydroxyl radicals, an inorganic radical (most dangerous reactive oxygen species) with its precursor such as H$_2$O$_2$ proposed the facts responsible for their mode of mechanism [43]. On the basis of the above study, the antioxidant activities have studied and analyzed the decrease in absorbance or scavenging effect of a stable free DPPH$^+$ as per standard procedure for the Pd(BLs)$_2$ [45, 46]. The percentage scavenging activity of Pd(BLs)$_2$ has been determined in a concentration-dependent mode in comparison with the DPPH$^+$ absorption at 517 nm [47–50]. The DPPH$^+$ free radical’s absorption at 517 nm with DMSO was 0.906 and for Pd(BLs)$_2$. From 50 to 250 $\mu$M with an interval of 50 $\mu$M, complexes have expressed a decrease in absorption (Figure 10) that characterised them as an antioxidant [51].

The 55.47, 28.812, and 24.24% for Pd2MBA, Pd3MBA, and Pd4MBA, respectively, inferred Pd2MBA, a strong antioxidant among them. Thus, the antioxidant activities of Pd complexes have inferred their significance in medicinal sciences.
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