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ONO pincer type of palladium(II) complexes of heterocyclic hydrazone: Synthesis, characterization and biological evaluation

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Abstract

Two new Pd(II) complexes of N'-(4-(diethylamino)-2-hydroxybenzylidene)furan-2-carbohydrazide have been synthesized and characterized by various spectral studies. The structure of one of the complexes has been determined by single crystal X-ray diffraction studies. DNA and protein binding affinity of the synthesized compounds were examined by UV-visible and fluorescence titration method. In addition, the *in vitro* cytotoxicity of the compounds were evaluated against A549 (lung cancer) and MCF7 (breast cancer) cell lines using the MTT assay method.

Keywords: Schiff base ligand, Pd(II) complexes, DNA/protein binding and Cytotoxicity.

Introduction

Transition metal pincer type complexes have been given a very special attention in organometallic and coordination chemistry.^[1,2] Interestingly, hydrazone ligands usually create a pincer type of coordination similar to the one that is observed in biological systems by way of coordination through oxygen and nitrogen atoms. It has also been demonstrated that the transition metal complexes of aryl hydrazones have not only been shown to function as antibacterial, antiviral and antifungal agents but also have exhibited kinetic stabilities of their coordination spheres very similar to biological environment.^[3-7] The hydrazones, in addition to offering rigidity in a conjugated system, have an NH unit that readily participates in hydrogen bonding in addition to being a site for protonation–deprotonation. Like for many other complexes, it has also been well-established that the formations of metal-hydrazone complexes are important in enhancing the biological activity compared to that of free hydrazones.^[8] In the process of design and synthesis of transition metal complexes as efficient anticancer drugs, a detailed knowledge on the interaction of such complexes with DNA and protein is considered as

essential.^[9-13] As far as the metal complexes are concerned, complexes derived from Pd(II) ion have been seen as a potential alternative to Pt(II) compounds as anticancer agents owing to structural and thermodynamic similarities between Pt(II) and Pd(II) complexes. Very recent investigations have in fact revealed that some of the Pd(II) complexes exhibit similar or even better cytotoxicity than the presently used cisplatin and its analogues.^[14-16] This might be due to the fact that Pd(II) complexes undergo aquation and ligand exchange reactions 10^5 times faster than the corresponding Pt(II) complexes^[17], which has resulted in a number of mixed-ligand Pd(II) complexes being tested as of antitumor drugs.^[18-20] Hence, during the course of our systematic investigations on the synthesis and biological studies of hydrazone complexes, we report herein the synthesis, characterization, crystal structure and biological activities of ONO pincer type Pd(II) complexes derived from N'-(4-(diethylamino)-2-hydroxybenzylidene)furan-2-carbohydrazide.

Experimental section

Materials and methods

All the reagents used were of analar or chemically pure grade. Solvents were purified and dried according to the literature procedure.^[21] The ligand and $[\text{PdCl}_2(\text{PPh}_3)_2]$ were prepared by following previously reported literature methods.^[22,23] Melting points of the complexes were recorded with the lab India melting point apparatus. Elemental analyses of the complexes were done by using Vario EL III CHNS instrument. IR spectra of the ligand and complexes were recorded in KBr pellets with a JASCO FT-IR 4100 instrument in the $400\text{--}4000\text{ cm}^{-1}$ range. The electronic spectra of the complexes were recorded in DMSO using a JASCO V-630 UV-Vis Spectrometer in the $200\text{--}800\text{ nm}$ range. The $^1\text{H-NMR}$ spectra were recorded in DMSO on a Bruker 400 MHz instrument with TMS as the standard. DNA binding, competitive binding fluorescence measurements, protein binding^[24], cytotoxicity experiments^[25] were carried out according to the reported procedures.

Crystallography

X-ray diffraction experiments on complex **1** were carried out at $100(2)\text{ K}$ on a Bruker APEX II diffractometer with CCD detector using Mo-K_α radiation ($\lambda = 0.71073\text{ \AA}$). Intensities were integrated in SAINT^[26] and absorption corrections based on equivalent reflections were applied using SADABS.^[27] The structure was solved using Superflip^[28,29], while X was solved using ShelXT^[30] and refined against F^2 in SHELXL^[31,32] using Olex2.^[33] All of the non-hydrogen

atoms were refined anisotropically while all of the hydrogen atoms were located geometrically and refined using a riding model. Crystal structure and refinement data are given in Table 1. Crystallographic data for complex **1** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 1585980. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax(+44) 1223 336033, e-mail: deposit@ccdc.cam.ac.uk].

Preparation of ligand

Preparation of N'-(4-(diethylamino)-2-hydroxybenzylidene)furan-2-carbohydrazide(H₂L):

It was prepared from 2-furoic hydrazide (1.26 g, 0.01 mmol) and 4-diethylaminosalicylaldehyde (1.9 g, 0.01 mmol). The mixture was refluxed for 6 h (30 cm³) of methanol, during which a yellow precipitate formed. The reaction mixture was then cooled to room temperature and the solid formed was filtered. It was then washed with methanol and dried under *vacuum*. Yield: 95 %. M.p.:133°C. Anal.Calc. for C₁₆H₁₉N₃O₃ (%): C, 63.77; H, 6.36; N, 13.94. Found: C, 63.71; H, 6.30; N, 13.89. Selected IR bands (cm⁻¹): 3434 (ν_{OH}), 1634 (ν_{C=O}), 1634 (ν_{C=N}), 3208 (ν_{NH}). λ_{max}, nm; 261, 356 (intra ligand transition). ¹H NMR (DMSO-*d*₆, δ ppm, *J* Hz, (Fig. S1)): 11.32 (s, 1H, -OH), 8.42 (s, 1H, C=N), 9.61 (s, -NH), 7.93 (s, 1H, (Ar-H)), 7.24-7.25 (d, *J* = 3.2 Hz, 2H, (Ar-H)), 6.34-6.36 (dd, *J* = 8.8 Hz, 1H, (Ar-H)), 2.54-2.50 (q, *J* = 15.6 Hz, 4H, -CH₂N), 1.09-1.11 (t, *J* = 6.8 Hz, 6H, -CH₃).

Synthesis of the complexes

[PdL(PPh₃)] (**1**)

0.043 g of H₂L (0.143 mmol) was dissolved in methanol (30 cm³) and [PdCl₂(PPh₃)₂] (0.100 g, 0.143 mmol) dissolved in hot dichloromethane (30 cm³) was added along with two drops of triethylamine. The mixture was heated under reflux for 5 h. The resulting solution was cooled, filtered and allowed to stand for three days at room temperature which afforded an orange colored compound. It was filtered, washed with petroleum ether (60-80 °C) to remove traces of free triphenylphosphine and dried under *vacuum*. Reddish brown colored single crystals suitable for X-ray diffraction studies were obtained on slow evaporation of a methanol/toluene solution of the compound. Yield: 81 %. M.p.:157°C. Anal.Calc. for C₃₄H₃₂N₃O₃PPd (%): C, 61.13; H, 4.83; N, 6.29. Found: C, 61.09; H, 4.78; N, 6.21. Selected IR bands (cm⁻¹): 1240 (ν_{C-O}), 1598 (ν_{C=N}). λ_{max}, nm; 268 (intra ligand transition), 411 (metal to ligand charge transfer transition). ¹H NMR (DMSO-*d*₆, δ ppm, *J* Hz, (Fig. S2)): 8.15 (s, 1H, C=N), 6.68-6.69 (dd, *J* = 3.2 Hz, 2H,

(Ar-H)), 6.19-6.22 (dd, $J = 9.2$ Hz, 1H, (Ar-H)), 7.28-7.72 (m, 18H, (Ar-H)), 3.22-3.28 (q, $J = 7.2$ Hz, 4H, $-\text{CH}_2\text{N}$), 1.02-1.05 (t, $J = 6.8$ Hz, 6H, $-\text{CH}_3$).

[PdL(AsPh₃)] (**2**)

This was prepared following a procedure similar to the one described for **1** from H₂L (0.038 g, 0.126 mmol) and [PdCl₂(AsPh₃)₂] (0.100 g, 0.126 mmol). The mixture was heated under reflux for 5 h, filtered and allowed to stand for three days at room temperature which afforded orange colored crystalline compound. Yield: 74 %. M.p.: 163 °C. Anal.Calc. for C₃₄H₃₂AsN₃O₃Pd (%): C, 57.36; H, 4.53; N, 5.90. Found: C, 57.31; H, 4.50; N, 5.86. Selected IR bands (cm⁻¹): 1241 ($\nu_{\text{C-O}}$), 1597 ($\nu_{\text{C=N}}$). λ_{max} , nm; 263 (intra ligand transition), 415 (metal to ligand charge transfer transition). ¹H NMR (DMSO-*d*₆, δ ppm, J Hz, (Fig. S3)): 8.15 (s, 1H, C =N), 6.03-6.75 (m, 6H, (Ar-H)), 7.30-7.73 (m, 15H, (Ar-H)), 3.25-3.34 (q, $J = 7.2$ Hz, 4H, $-\text{CH}_2\text{N}$), 1.03-1.08 (t, $J = 6.8$ Hz, 6H, $-\text{CH}_3$).

Results and discussion

Synthetic routes for the ligand and its Pd(II) complexes are shown in **Scheme 1**. The compounds are air stable and soluble in chloroform, dichloromethane, methanol, ethanol, dimethylformamide and dimethylsulfoxide.

Scheme 1. Preparation routes of the ligand (H₂L) and Pd(II) complexes (**1** and **2**).

Spectral characterization

The electronic spectrum of the ligand showed two bands in the region around 261-356 nm which have been assigned to intra ligand transition.^[34,35] In the spectra of the complexes **1** and **2**, the band around 263-268 nm has been assigned to intra ligand transitions and another band around 411-415 nm has been attributed to a metal to ligand charge transfer transition (MLCT).^[35] The IR spectra of the N'-(4-(diethylamino)-2-hydroxybenzylidene)furan-2-carbohydrazide (**H₂L**) and the corresponding complexes provided significant information about the metal ligand bonding. A strong band observed at 3434 cm⁻¹ in the free ligand due to phenolic -OH stretching had completely disappeared in the IR spectra of complexes **1** and **2** indicating deprotonation prior to coordination.^[36] A strong band in the region 1634 cm⁻¹ characteristic of the azomethine group in the free ligand has shifted to lower frequency 1598 and 1597 cm⁻¹ for **1** and **2** respectively indicating the coordination of

azomethine nitrogen atom to the palladium ion.^[36] Other characteristic bands due to triphenylphosphine and triphenylarsine were also observed in the regions at 1432, 1075 and 690-693 cm⁻¹ in all the complexes (**1** and **2**).^[37] The ¹H-NMR spectra showed in supporting information (Figure S1-S3) of the ligand (**H₂L**) and the corresponding complexes recorded in DMSO showed all the expected signals in the respective regions. A sharp singlet appeared at δ 11.32 ppm corresponding to the presence of hydroxy group in the free ligands which completely disappeared in complexes **1** and **2** confirming the involvement of phenolic oxygen in coordination.^[36,38] A singlet that appeared at δ 9.61 ppm due to the –NH proton for the free ligand has also completely disappeared in the spectra of complexes **1** and **2**, indicating the enolisation and subsequent deprotonation prior to the coordination of imine N atom.^[39] Two singlets and one doublet appeared at δ 8.42, δ 8.15 and δ 8.16 ppm corresponding to the presence of the CH=N azomethine proton in the free ligand, complex **2** and complex **1**, respectively. The aromatic protons present in the ligand and the complexes appeared at δ 6.19-7.90 ppm.^[40] A quartet observed around at δ 2.64-3.28 ppm has been assigned the CH₂N group of protons in ligand and complexes and a triplet observed at δ 1.02-1.13 ppm has been assigned to the terminal methylene group of protons.^[38]

X-ray crystallography

The molecular structure of complex **1** has been determined by single crystal X-ray diffraction study. Details of the data collection and the parameters of refinement process are given in Table 1. The structure of complex **1** with the atom numbering is depicted in Figure 1. The coordination geometry around the palladium ion is distorted square planar in which the ligand acts as adianionic tridentate forming a five-member and a six-member chelating ring. The heterocyclic hydrazone ligand is bonded to the palladium ion *via* both phenolate and enolate oxygen atoms and the imine nitrogen atom and the fourth coordination site is occupied by triphenylphosphine. The bond angles are 176.15(4)° for (N1-Pd-P1) and 174.74(5)° for (O1-Pd-O2).

Figure 1. Molecular structure of the complex **1** with atomic numbering scheme depicted.

Ellipsoids are depicted at the 50 % probability level.

Table 1.Crystal data and structure refinement for complex**1**.

Identification code	Complex 1
Empirical formula	C ₃₄ H ₃₂ N ₃ O ₃ PPd
Formula weight	667.99
Temperature/K	100(2)
Crystal system	Monoclinic
Space group	<i>P</i> 2 ₁ / <i>n</i>
<i>a</i> /Å	18.6468(3)
<i>b</i> /Å	8.2188(1)
<i>c</i> /Å	21.0064(5)
α /°	90
β /°	112.3125(8)
γ /°	90
Volume/Å ³	2978.28(8)
<i>Z</i>	4
ρ_{calc} /cm ³	1.492
μ /mm ⁻¹	0.717
F(000)	1368.0
Crystal size/mm ³	0.444 × 0.305 × 0.287
Radiation	MoK α (λ = 0.71073)
2 θ range for data collection/°	4.192 to 55.834
Index ranges	-24 ≤ <i>h</i> ≤ 24, -10 ≤ <i>k</i> ≤ 10, -27 ≤ <i>l</i> ≤ 27
Reflections collected	26599
R _{int} / R _{sigma}	0.0298/ 0.0268
Data/restraints/parameters	7128/0/381
Goodness-of-fit on F ²	1.036
Final R indexes [<i>I</i> ≥ 2 σ (<i>I</i>)]	R ₁ = 0.0243, wR ₂ = 0.0553
Final R indexes [all data]	R ₁ = 0.0303, wR ₂ = 0.0579
Largest diff. peak/hole / e Å ⁻³	0.43/-0.32

DNA binding studies

In order to determine the ability of any compound to act as a drug, it is important to determine the binding of the compound with DNA and this is usually done by electronic absorption titrations. Among the possible types of interaction, the intercalative mode of binding usually results in hypochromism along with or without a small red or blue shift due to the strong stacking interaction between an aromatic chromophore and the base pairs of DNA.^[41] But, for a compound that binds non-intercalatively or electrostatically with DNA this may result in hyperchromism.^[42-44] Hence, we measured the absorption spectra of the ligand and complex in the absence and presence of CT-DNA and the results are shown in Figure 2. Upon the incremental addition of DNA to the test compounds, the following changes were noticed. The nature of the shifts observed and the percentage of hypochromism in the absorption bands suggested that they interacted with DNA *via* intercalative mode of binding and complex **1** bound more strongly to DNA than the ligand and complex **2**.^[45] To compare quantitatively the binding strength of the compounds, their intrinsic binding constants (K_b) with CT-DNA were determined from the following equation.

$$[\text{DNA}]/[\varepsilon_a - \varepsilon_f] = [\text{DNA}]/[\varepsilon_b - \varepsilon_f] + 1/K_b[\varepsilon_b - \varepsilon_f]$$

where $[\text{DNA}]$ is the concentration of DNA in base pairs, ε_a is the extinction coefficient of the complex at a given DNA concentration, ε_f is the extinction coefficient of the complex in free solution and ε_b is the extinction coefficient of the complex when fully bound to DNA. A plot of $[\text{DNA}]/(\varepsilon_a - \varepsilon_f)$ *versus* $[\text{DNA}]$ gave a slope and an intercept equal to $1/[\varepsilon_b - \varepsilon_f]$ and $1/K_b[\varepsilon_b - \varepsilon_f]$ respectively (Figure 3). The intrinsic binding constant K_b is the ratio of the slope to the intercept. The values of intrinsic binding constants (K_b) were calculated as 1.4311×10^6 , 2.8331×10^6 and $2.5422 \times 10^6 \text{ M}^{-1}$ for the ligand and complexes respectively. The observed values of K_b revealed that the ligand and Pd(II) complexes bind to DNA *via* an intercalative mode. These values are very similar to the ones reported previously for the intercalative mode of various metallo intercalators.^[46,47] From the results obtained, it has been found that the ligand and the complexes exhibit almost similar binding affinity towards DNA, but complex **1** and **2** showed slightly better affinity. This may be due to the chelation of palladium ion with the heterocyclic hydrazone ligand.

Figure 2. Absorption titration of the ligand (A), complex 1 (B) and Complex 2 (C) with CT-DNA.

Figure 3. Plot of $[\text{DNA}] / [\varepsilon_a - \varepsilon_f]$ *versus* $[\text{DNA}]$.

Ethidium bromide fluorescence displacement study

The binding of any compound to DNA should be proved by another method and in this connection; the fluorescent emission of ethidium bromide can be made use of to see whether the compound binds to DNA through intercalation.^[48] This method is based on the principle that there will be a decrease in intensity of the fluorescence of EB bound to DNA as and when the EB is replaced by other compounds that bind to DNA. This kind of competitive binding of compounds to DNA with EB could provide rich information with regard to the DNA binding affinity of any compound for its drug activity. Hence, we carried out the measurement of fluorescence spectra of DNA-EB system upon increasing amounts of our test compounds and Figure 4 shows the results that we have obtained. The fluorescence quenching spectra illustrated that upon increasing the concentration of the compounds a hypochromism has been observed in the fluorescence intensity for the ligand and the complexes. The observed decrease in the fluorescence intensity clearly indicates that the EB molecules are displaced from their DNA binding sites and are replaced by the compounds under investigation.^[49] Further, the quenching data were analyzed according to the following Stern-Volmer equation,

$$I_0/I = K_q[Q] + 1$$

Where I_0 and I represent the emission intensities in the absence and presence of the compound, respectively, K_q is the quenching constant, and $[Q]$ is the concentration ratio of the compound. The K_q values have been obtained as the slope from the plot of I_0/I versus $[Q]$. The K_q values for ligand and complexes have been calculated as 4.12×10^3 , 6.85×10^3 and $6.02 \times 10^3 \text{ M}^{-1}$ respectively which show that the compounds bind to CT-DNA *via* the intercalation mode.^[50]

Figure 4. Fluorescence titration of the DNA-EB system of the ligand (A), complex 1 (B) and Complex 2 (C).

Figure 5. Stern-Volmer plot of the EB-DNA fluorescence titration for the compounds.

BSA protein binding studies

In determining the potential of a compound to act as a drug, it is not enough if we know about the DNA binding alone but it is equally important to know about its binding to protein as proteins are usually the carrier of the drug to the target. Bovine serum albumin (BSA) is a protein which has a characteristic fluorescence that can be used to quantify the binding of a compound to it. Usually, the quantum yield of fluorescence decreases as and when the test compound binds to BSA as a result of a variety of molecular interactions.^[51] BSA contains three fluorophores, *i.e.* tryptophan, tyrosine and phenylalanine, and the intrinsic fluorescence of BSA is mainly due to tryptophan alone and changes in the emission spectra of tryptophan are common in response to protein conformational transitions, subunit associations, substrate binding or denaturation. Therefore, we carried out the binding of the synthesized compounds to BSA by examining fluorescence spectra. **Figure 6** shows the fluorescence emission spectra of BSA after the addition of compounds in various concentrations. It can be seen from the spectra that when an increasing amount of the test compounds was titrated against a fixed concentration of BSA, the fluorescence intensity of BSA decreased gradually with a hypsochromic shift. The observed blue shift is mainly due to the binding of compounds with the active site in BSA^[52] which is an indication for interaction of the ligand and complexes with BSA. The fluorescence quenching observed can be described by the Stern-Volmer relation,

$$I_0/I = 1 + K_{sv}[Q]$$

Where I_0 and I are the fluorescence intensities of the fluorophore in the absence and presence of quencher, K_{sv} is the Stern-Volmer quenching constant and $[Q]$ is the quencher concentration. The K_{sv} values can be obtained from the slope of a plot of I_0/I versus $[Q]$ (Figure 7(A)). Further, when small molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules is represented by Scatchard equation.^[53,54]

$$\log[(F_0-F)/F] = \log K + n \log [Q]$$

where K and n are the binding constant and the number of binding sites, respectively. A plot of $\log[(F_0-F)/F]$ versus $\log [Q]$ [Figure 7(B)] can be used to determine the values of both K and n , the values calculated for the compounds are listed in Table 2. From the values of n , it is inferred that there is only one independent class of binding site on BSA that was available for the compounds under investigation and a linear relation between the binding constant and number of binding sites exists.

From the Table 2, the calculated value of n is around 1 for all of the compounds, indicating the existence of just a single binding site in BSA for all of the compounds. The values of K_{bin} and K_{SV} for all of the compounds suggested that the complexes interact with BSA more strongly than the ligand.

Figure 6. The fluorescence titration of BSA with the ligand (A), complex 1 (B) and Complex 2 (C).

Figure 7. Stern–Volmer (A) and Scatchard (B) plot of the compounds with BSA.

Table 2. Binding constant (K_{bin}), quenching constant (K_{SV}), and number of binding sites (n) for the interactions of compounds with BSA.

Compound	$K_{\text{bin}} (\text{M}^{-1})$	$K_{\text{KSV}} (\text{M}^{-1})$	n
1	4.28×10^2	5.4×10^3	0.79
2	5.30×10^2	6.95×10^3	0.98
3	5.43×10^3	6.19×10^3	1.01

The UV-visible absorption spectra of BSA with the ligand and complexes were measured in order to find the type of quenching that exists. A simple method to explore the type of quenching is UV–visible absorption spectroscopy. The dynamic quenching usually affected only the excited state of fluorophores and hence will not change the absorption spectrum. However, if the interaction is on the ground state (static), there will be a change in the absorption spectrum of the fluorophores. Hence, we measured the UV-vis spectra of BSA in the presence of the compounds (Figure 8) which showed that the absorption intensity of BSA was enhanced as the complexes and ligand were added with a little shift in the absorption maxima. ^[55]

Figure 8. UV absorbance spectra of BSA in the presence of the compounds.

Synchronous fluorescence spectra.

Fluorescence spectral studies of BSA on the interaction of any compound give information about the binding site and binding affinity of the compound. They do not give any information on the

micro environmental changes that take place during such interactions, however this is very important. A synchronous fluorescence spectrum usually provides information about the molecular environment in the vicinity of chromophore molecules in low concentrations under physiological conditions which has several advantages such as spectral simplification, bandwidth reduction and avoiding different perturbing effects.^[56] In synchronous fluorescence spectroscopy, the difference between the excitation (λ_{exc}) and emission (λ_{emi}) wavelength ($\Delta\lambda = \lambda_{emi} - \lambda_{exc}$) reflects the spectra of a different nature of chromophores. Large $\Delta\lambda$ values of around 60 nm are characteristic of tryptophan residues and a small $\Delta\lambda$ value of around 15 nm is characteristic of tyrosine.^[57] To explore the structural changes of BSA during the addition of the test compounds, we measured synchronous fluorescence spectra of BSA with added test compounds at both $\Delta\lambda = 15$ nm and 60 nm (Figure 9). Upon increasing the concentration of the compounds, it has been observed that the intensity of emission decreases without any change in the position of the wavelength. These experimental results indicate that the compounds affect the microenvironment of both the tryptophan and tyrosine residues more effectively during the binding process and synchronous measurements confirmed the effective binding of the ligand and complexes with BSA.^[58]

Figure 9. The synchronous fluorescence titration of BSA with the compounds.

Cytotoxicity

The progressive results obtained from the DNA and BSA binding studies for the ligand and their palladium(II) complexes have stimulated us to test their ability to inhibit cell growth and induce cell death in the selected human cancer cell lines. MTT assay were performed against two A549 (lung cancer) and MCF7 (breast cancer) cell lines to ascertain the cytotoxicity of the compounds. The results obtained have been analyzed by means of cell inhibition expressed as IC_{50} values and are shown in Table 3 and Figure 10. From the biological assays of the ligand and Pd(II) complexes, it is seen that the complexes exhibit better activities than that of the ligand against the cancer cells which indicate that the chelation of the ligand with the Pd(II) ion is responsible for the observed cytotoxic properties of the new complexes. The enhanced cytotoxic properties of the complexes over the ligand may be due to the extended planar structure induced by the conjugation resulting from the chelation of the heterocyclic hydrazone ligand to the palladium

ion.^[59] However, there is no substantial difference in the cytotoxic activities when triphenyl phosphine was replaced by triphenyl arsine in the complexes.

Table 3. The IC₅₀ values of the compounds

Compound	IC ₅₀ values (μM)	
	A549	MCF7
Ligand	59 ± 1.4	63 ± 1.3
Complex 1	36 ± 1.5	28 ± 1.1
Complex 2	39 ± 1.2	31 ± 0.9
Doxorubicin	15 ± 1.2	16 ± 1.3
Cisplatin	25 ± 2.1	18.7 ± 0.1

Figure 10. The IC₅₀ values of compounds are depicted against (A549) and (MCF7) cancer cell lines.

Acridine orange /ethidium bromide (AO/EtBr) staining method

To elucidates the apoptotic activity of synthesized compounds, apoptotic acridine orange /ethidium bromide (AO/EtBr)staining fluorescence microscopic analysis was carried out. Fluorescence microscopic images of A549 and MCF7 cancer cells in the absence of compounds (control) and in the presence of compounds (treated) are shown in Figure 11 which shows that the untreated-(a, b, c) A549 and MCF7 cancer cells (control) did not show any significant adverse effect compared to the compounds treated-(d, e, f) cancer cells. It can be seen that with the addition of compounds to the cancer cells, the green colour of cells are converted into yellow/red colour cells which is due to induced apoptosis and the nuclear condensation effect on the cells. The morphological changes examined by staining method suggested that the cell death mechanism was through apoptosis.

Figure 11. AO/EB stained A549-(A) and MCF7-(B)(a, b, c control and d, e, f are treated with the ligand and complexes) cells after 24 h incubation. The yellow or red color cells indicate early apoptotic cells.

Conclusion

An equimolar reaction of $[\text{PdCl}_2(\text{PPh}_3)_2]$ and $[\text{PdCl}_2(\text{AsPh}_3)_2]$ with N'-(4-(diethylamino)-2-hydroxybenzylidene)furan-2-carbohydrazide ligand resulted in the formation of two new palladium complexes. They were characterized by various spectral studies. Based on the spectroscopic and single crystal X-ray diffraction studies, a square planar geometry has been proposed for the Pd(II) complexes. The ligand coordinated to palladium in dibasic tridentate manner by using its phenolate and enolate oxygen atoms and the imine nitrogen atom. The fourth position is occupied by triphenylphosphine or triphenylarsine. CT-DNA and binding studies of the ligand and Pd(II) complexes suggested that the two complexes bind to CT-DNA more strongly than the ligand and the intrinsic fluorescence of BSA was quenched by a static quenching mechanism. From the cytotoxic studies of the compounds carried out against A549 (lung cancer) and MCF7 (breast cancer) cell lines, it has been inferred that the new palladium(II) complexes **1** and **2** showed better anticancer activity in both cancer cell lines compared to the free ligand. The strong binding affinity and cytotoxicity of the complexes is attributed to the extension of the π system of the intercalated ligand which leads to a greater planar area of the complexes.

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