



# Cd(II) Derivatives of Substituted Phenylacetic Acids, Synthesis, Spectroscopic Characterization and Binding Studies with DNA

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**Abstract:** The methoxy substituted phenylacetic acid (MeOPhA) and chloro substituted phenoxyacetic acid (ClPhA) were used to synthesized eight new Cd(II) based complexes. The nitrogen donor 2,2'- bipyridine (MeOPhA2, ClPhA2) and 1,10-phenanthroline (MeOPhA3, ClPhA3) were used as auxiliary ligands for the synthesis of mixed ligand complexes. These complexes were characterized by FT-IR and multinuclear NMR (<sup>1</sup>H and <sup>13</sup>C-NMR) spectroscopic techniques. The FT-IR spectra of the complexes showed characteristic COO<sup>-</sup> asymmetric and COO<sup>-</sup> symmetric vibrational bands indicating metal coordination through oxygen. Moreover, their difference, i.e., Δν reveal that the selected ligands are coordinated to the Cd(II) center in a bidentate manner. The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data recorded in deuterated solvents also supported successful synthesis in pure form as well as metal coordination through carboxylate group. The nature of the complex–DNA interaction was examined, and the impact of hetero ligand attachment on binding strength and reactivity was assessed using UV–visible spectroscopy. The obtained data confirmed the effective binding ability through partial intercalation and groove binding through spontaneous process for all the complexes.

**Keywords:** Mixed Ligands, Spectroscopic Techniques, Auxiliary Ligands, Surface Binding, Multinuclear NMR.

## 1. INTRODUCTION

Metal complexes have been used in medicinal industry since ancient times; however, their pharmacological significance was firmly recognized after Rosenberg's 1969 discovery of cisplatin's anticancer activity [1]. Cisplatin's distinct method of action, which involves covalent interaction with DNA, has been attributed to its exceptional therapeutic success. This interaction ultimately inhibits the growth of cancerous cell by blocking the mechanisms required for their replication [2]. DNA is regarded as the blueprint of life, controlling and regulating a wide range of cellular metabolic activities [3]. Many other anticancer drugs such as Actinomycin D and Doxorubicin exert their effect by binding with DNA [4-6]. Nitrogenous bases of DNA show distinct preferences for metal cations, general stability order for 3d transition series is

given as: M–guanine > M–adenine and M–cytosine > M–thymine [6, 7]. Chelation results in increase in drug absorption across cells by reducing metal ion polarity through orbital overlap and resonance. Hence, understanding these selective interactions of metal and DNA bases and the right selection of metal and ligands is necessary for developing advanced metallodrugs [8, 9]. Cadmium (Cd) is a d<sup>10</sup> metal belonging to group 12 of periodic table with zero crystal field stabilization energy. It has no strong geometric preference due to filled d orbitals and can easily be identified through spectroscopy [10]. This is, however, categorized as a highly toxic heavy metal due to its strong affinity for sulfhydryl groups in protein which results in oxidative stress, enzyme inhibition and tissue damage. Recent studies revealed that the toxicity of a metal is not a fixed property, it is influenced by various factors such as the ligand environment, oxidation state,

Received: November 2025; Revised: December 2025; Accepted: December 2025

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and coordination geometry [11, 12]. Egorova and Ananikov [13] highlighted the role of the metal in the living systems, which is intrinsically linked to the specific molecular form in which the metal exists. Thus, toxicity of Cd(II) can be reduced by its complexation with suitable oxygen and nitrogen donor ligands which stabilize the Cd(II) center and can direct its biomolecular interaction in a controlled way [14]. A variety of important functionalities are associated with Cadmium complexes such as anti-microbial, anti-cancer, catalytic and anti-bacterial properties [15, 16].

In coordination chemistry, the choice of ligand is crucial because it affects the coordination behavior, stability, geometry, and biological activity of the desired complex [17]. Carboxylic acids are organic ligands of choice on account of their favorable chemistry especially the versatile coordination ability [18]. Carboxylic acids can form complex and stable structures by coordinating with metals in many ways such as ionic, monodentate, and bidentate. In biological and catalytic processes, their coordination flexibility is crucial [19]. Utilizing these medicinally active ligands in metal complexation has become a developing trend to create more potent and focused therapeutic agents because carboxylic acids are essential structural elements of many already available therapeutic agents [8, 20]. Cadmium carboxylates display flexible coordination geometries due to the large ionic radius of  $\text{Cd}^{2+}$ , i.e., 109 pm [21]. Due to this flexibility, these complexes find their applications in bio-sensing, bio-imaging, nanomedicine and drug delivery [22, 23].

Naturally occurring phenylacetic acid and its derivatives are known for their bioactivity and significant contribution to improving the flavor and scent of food and cosmetic items [24]. 2-methoxyphenylacetic acid contains a methoxy group in addition to carboxylic group attached to phenyl ring. The presence of these strong electron-donating and coordinating groups significantly enhance its reactivity as well as metal binding capabilities. The commonly used NSAIDs like diclofenac etc., with an aromatic carboxylate group, exhibited significant pharmacological and coordinating properties. This chemical resemblance allows the formulation of metal based pharmacologically active compounds by the incorporation of active functional groups

[20, 25, 26]. 2,4-Dichlorophenoxyacetic acid contains a phenoxy oxygen atom in addition to carboxylate group offering versatile coordination modes thus making it suitable for forming stable metal complexes [27]. The synthesis of complexes with different donors and heterocyclic ligands is a current trend, inspired by biomacromolecules. Overall efficiency can be improved by using a heterocyclic donor as an auxiliary ligand and a carboxylate group as the main ligand.

N-donor heterocycles are regarded as stable and adaptable co-ligands because the lone pair on their  $\text{sp}^2$ -hybridized nitrogen [28, 29]. Both 2,2'-bipyridine and 1,10-phenanthroline are planar ligands having  $\text{sp}^2$  hybridized nitrogen as well as extended  $\pi$ -conjugation system enabling  $\pi$ - $\pi$  stacking and other non-covalent interactions in resulting complexes. The ligand 2,2'-bipyridine contains trans-oriented nitrogen atoms, mostly forms slightly strained cis complexes which show diverse electronic and biological activity [30]. Whereas, 1,10-Phenanthroline contains cis-oriented nitrogen atoms that favor bidentate chelation with metal centers, thus making it significant in bioinorganic and therapeutic chemistry [31].

According to data found in the literature, the overall effectiveness of the resultant complexes is greatly increased by the addition of active structural motifs including metal centers, carboxylate ligands, and heterocycles containing nitrogen. Numerous mixed ligand complexes based on substituted aromatic carboxylic acids such as methoxyphenylacetic and dichlorophenoxyacetic derivatives have been reported. Consistently, both ligand form structurally unique and biologically relevant heteroleptic metal complexes when coordinated with N-donor co-ligands [8, 18, 20, 32].

The present research project is an attempt to synthesize mixed ligand complexes of cadmium by using substituted phenylacetic acids and N-donor ligands and to evaluate their ability to bind with DNA. The FT-IR and multi-nuclear NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ ) were employed for their characterization.

## 2. MATERIALS AND METHODS

Reactant used in the synthesis like 2-methoxyphenylacetic acid (MeOPhA), 2,4-dichlorophenoxyacetic acid (ClPhA),

sodium bicarbonate, cadmium chloride, nitrogen donor ligands e.g. 2,2'-bipyridine and 1,10-phenanthroline were acquired from Sigma-Aldrich (USA) and were used as such. The solvents used during the synthesis, recrystallization and for NMR data collections include some organic solvents and n-hexane, etc., were of absolute purity and were acquired from Merck (Germany). They were utilized in all the experiments without any further purification processes. Gallen Kamp (UK) electrothermal apparatus was employed to find out the melting point of all complexes by using the capillary tubes. FT-IR Spectrophotometer of Thermo Nicolet-6700 was used to record FT-IR spectra of complexes in the range of 4000-400  $\text{cm}^{-1}$ . Multi-nuclear NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) spectra of ligands and complexes were taken by Bruker Advanced Digital instrument having frequency of 300 MHz at room temperature in deuterated dimethyl sulfoxide (DMSO). Chemical shifts and coupling constants were noted in parts per million (ppm) and Hertz (Hz), respectively. The UV-Visible spectrophotometer (Shimadzu 1800) served to record the absorption spectra of the complexes for DNA binding analysis.

## 2.1. Synthetic Protocols

### 2.1.1. Procedure for ligand's sodium salts

To prepare sodium salts (see scheme 1) the aqueous solution of sodium bicarbonate (3 mmol, 0.252 g) was added dropwise to the aqueous solution of each ligand, i.e., 2-methoxyphenylacetic acid (3 mmol, 0.498 g) and 2,4-dichlorophenylacetic acid

(3 mmol, 0.615 g) under continuous stirring. The mixtures were stirred maximum until neutralization at room temperature until. The solvents were then evaporated under reduced pressure to get the solid sodium salts, which were collected and stored in glass vials. This synthesis procedure for the sodium salt is in accordance with the previously reported method [33]. The scheme 2 represents the structure of ligands used in synthesis along with the numbering scheme for NMR interpretation.

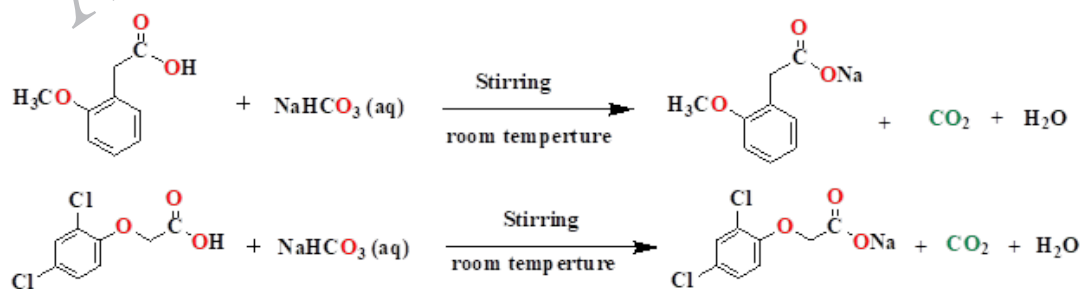
### 2.1.2. Synthesis of single ligand cadmium carboxylates

#### 2.1.2.1. Synthesis of MeOPhA1

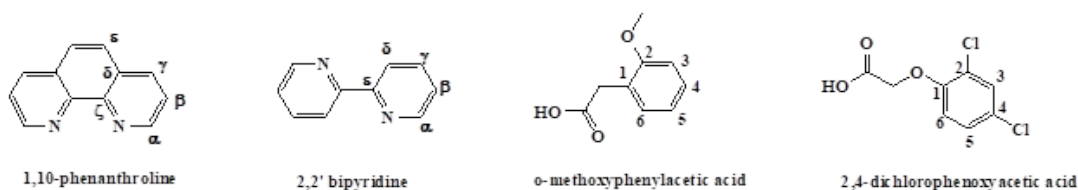
Under constant stirring, methanolic solutions of sodium salt of ligand MeOPhA (3 mmol, 0.564 g) were added into aqueous solution of cadmium chloride (1.5 mM, 0.275 g). The reaction mixtures were stirred for 5 hours at 50  $^{\circ}\text{C}$ , the resulting precipitates were obtained through filtration. They were washed with water to remove any impurity/residual reactants and was dried in air. The procedure is presented in Scheme 3.

#### 2.1.2.2. Synthesis of ClPhA1

The synthesis of the ClPhA1 was carried out by following the synthetic procedure as discussed for complex MeOPhA1. However, the sodium salt of ClPhA (3 mmol, 0.729 g) were added into aqueous solution of cadmium chloride (1.5 mM, 0.275 g) instead of MeOPhA. The product was also



Scheme 1: Synthesis of sodium salts of substituted phenylacetic acids.



Scheme 2: Numbering pattern for the ligands and nitrogen donor heterocycles.

processed in the same way and the synthetic route is presented in Scheme 3.

### 2.1.3. Synthesis of mixed ligand cadmium carboxylates

The sodium salt of ligand 2-methoxyphenylacetic acid (MeOPhA, 3 mmol, 0.564 g) and 2,4-dichlorophenoxyacetic acid (ClPhA, 3 mmol, 0.729 g) were dissolved separately in methanol. To this, an aqueous solution of cadmium chloride (1.5 mmol, 0.275 g) and bipyridine (1.5 mmol, 0.234 g) were added simultaneously for the synthesis of complexes MeOPhA2 and ClPhA2 respectively. The resulting mixtures were stirred for about 8 hours at 50 °C. The same procedure was used for the synthesis of complexes MeOPhA3 and ClPhA3 except that the addition of bipyridine was replaced by the phenanthroline (1.5 mmol, 0.270 g). The resulting solutions were filtered, extra solvents were removed through rotary evaporation, and the solid products were washed several times with water and dried in air. The obtained products were recrystallized from combination of appropriate solvents. Melting points were recorded for all the synthesized complexes. The synthetic route for complexes of both ligands and the corresponding NMR numbering scheme is presented in Scheme 4.

#### **Cd(MeOPhA)<sub>2</sub>: (MeOPhA1)**

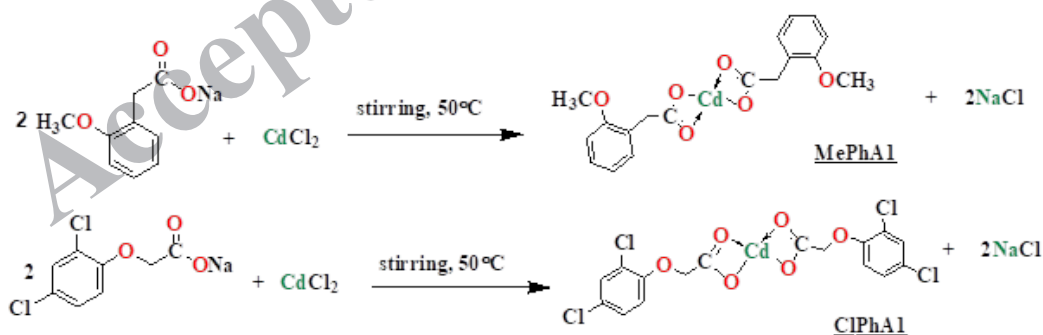
Solubility: Chloroform, DMSO, Methanol; M.P: 73-75 °C; % Yield: 78.1; FT-IR (cm<sup>-1</sup>): 1582 (COO<sub>asym</sub>), 1410 (COO<sub>asym</sub>), 172 (Δν), 526 (Cd-O); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, ppm): 3.20 (s, 2H, -CH<sub>2</sub>), 3.70 (br, 3H, -OCH<sub>3</sub>), 6.77-6.86 (m, 2H, H3, 5), 7.06-7.66 (m, 1H, H4), 7.09-7.15 (m, 1H, H6); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, ppm): 175.4 (C=O), 39.0 (-CH<sub>2</sub>), 55.6 (-OCH<sub>3</sub>), 126.7 (C1), 157.5 (C2), 110.6 (C3), 128.6 (C4), 120.1 (C5), 131.2 (C6).

#### **Cd(ClPhA)<sub>2</sub>: (ClPhA1)**

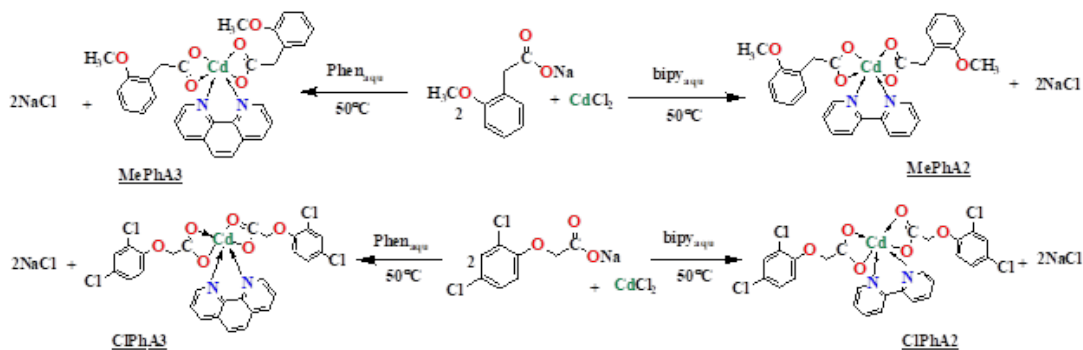
Solubility: DMSO, Ethanol, Methanol; M.P: 294-296 °C; %Yield: 78.3; FT-IR (cm<sup>-1</sup>): 1598 (COO<sub>asym</sub>), 1422 (COO<sub>asym</sub>), 176 (Δν), 460 (Cd-O); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, ppm): 4.28 (s, 4H, -OCH<sub>2</sub>), 7.47 (s, 2H, H3), 7.24-7.27 (d, 2H, H5, J = 9 Hz), 6.84-6.87 (d, 2H, H6, J = 9 Hz); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, ppm): 170.7 (C=O), 68.7 (-OCH<sub>2</sub>), 154.0 (C1), 129.2 (C2), 123.6 (C3), 127.9 (C4), 122.2 (C5), 115.4 (C6).

#### **Cd(MeOPhA)<sub>2</sub>(bipy): (MeOPhA2)**

Solubility: Chloroform, DMSO, Methanol; M.P: 68-70 °C, % Yield: 76.5; FT-IR (cm<sup>-1</sup>): 1560 (COO<sub>asym</sub>), 1386 (COO<sub>asym</sub>), 174 (Δν), 590 (Cd-N), 486 (Cd-O); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, ppm): 3.22 (s, 2H, -CH<sub>2</sub>), 3.70 (s, 3H, -OCH<sub>3</sub>), 6.77-6.86 (m, 2H,



**Scheme 3:** Synthesis of single ligand complex derived from substituted phenylacetic acids.



**Scheme 4:** Synthesis of mixed Cd(II) carboxylates derived from 2-methoxyphenylacetic acids.



H3, 5), 7.06-7.15 (m, 2H, H4, 6), 8.68-8.69 (d, 2H, H $\alpha$ ,  $J$  = 4.8), 7.44-7.47 (m, 2H, H $\beta$ ), 7.92-7.98 (m, 2H, H $\gamma$ ), 8.37-8.39 (d, 2H, H $\delta$ ,  $J$  = 8.1 Hz),  $^{13}\text{C}$  NMR (DMSO- $d_6$ , ppm): 175.7 (C=O), 39.1 (–CH $_2$ ), 55.6 (–OCH $_3$ ), 124.7 (C1), 149.7 (C2), 110.6 (C3), 126.8 (C4), 120.1 (C5), 128.4 (C6), 157.5 (C $\alpha$ ), 120.9 (C $\beta$ ), 131.1 (C $\gamma$ ), 137.8 (C $\delta$ ), 157.5 (C $\epsilon$ ).

#### **Cd(MeOPh) $_2$ (1,10-phen): (MeOPhA3)**

Solubility: Chloroform, DMSO, Methanol; M.P: 75-77 °C; % Yield: 73.7; FT-IR (cm $^{-1}$ ): 1570 (COO $_{\text{asym}}$ ), 1390 (COO $_{\text{sym}}$ ), 180 ( $\Delta\nu$ ), 607 (Cd-N), 517 (Cd-O);  $^1\text{H}$  NMR (DMSO- $d_6$ , ppm): 3.69 (br, 5H, –CH $_2$ , –OCH $_3$ ), 6.76-6.86 (m, 2H, H3, 5), 7.06-7.14 (m, 2H, H4, 6), 9.08-9.10 (dd, 2H, H $\alpha$   $J$  = 1.5 Hz, 4.2 Hz), 7.79-7.83 (m, 2H, H $\beta$ ), 8.52-8.55 (dd, 2H, H $\gamma$   $J$  = 1.5 Hz, 8.1 Hz), 8.02 (s, 2H, H);  $^{13}\text{C}$  NMR (DMSO- $d_6$ , ppm): 175.4 (C=O), 39.0 (–CH $_2$ ), 55.5 (–OCH $_3$ ), 126.7 (C1), 150.5 (C2), 110.6 (C3), 127.1 (C4), 120.1 (C5), 128.5 (C6), 157.5 (C $\alpha$ ), 124.0 (C $\beta$ ), 131.1 (C $\gamma$ ), 137.0 (C $\delta$ ), 128.9 (C $\epsilon$ ), 157.5 (C $\zeta$ ).

#### **Cd(ClPhA) $_2$ (bipy): (ClPhA2)**

Solubility: DMSO, Ethanol, Methanol; M.P: 125-127 °C; % Yield: 71.1; FT-IR (cm $^{-1}$ ): 1609 (COO $_{\text{asym}}$ ), 1419 (COO $_{\text{sym}}$ ), 190 ( $\Delta\nu$ ), 556 (Cd-N), 475 (Cd-O);  $^1\text{H}$  NMR (DMSO- $d_6$ , ppm): 4.25 (s, 4H, –OCH $_2$ ), 7.43-7.48 (m, 4H, H3, H $\beta$ ), 7.23-7.27 (dd, 2H, H5,  $J$  = 2.7 Hz, 9 Hz), 6.83-6.86 (d, 2H, H6,  $J$  = 9 Hz), 8.68-8.69 (d, 2H, H $\alpha$   $J$  = 3.9 Hz), 7.92-7.98 (td, 2H, H $\gamma$ ,  $J$  = 1.8 Hz, 7.8 Hz), 8.37-8.40 (d, 2H, H $\delta$ , 7.8 Hz);  $^{13}\text{C}$  NMR (DMSO- $d_6$ , ppm): 170.3 (C=O), 68.9 (–OCH $_2$ ), 154.0 (C1), 137.8 (C2), 124.6 (C3), 129.1 (C4), 123.4 (C5), 115.5 (C6), 149.7 (C $\alpha$ ), 120.8 (C $\beta$ ), 122.1 (C $\gamma$ ), 127.9 (C $\delta$ ), 149.7 (C $\epsilon$ ).

#### **Cd(ClPhA) $_2$ (1,10-phen): (ClPhA3)**

Solubility: DMSO, Ethanol, Methanol; M.P = 140-142 °C; % Yield: 72.8; FT-IR (cm $^{-1}$ ): 1588 (COO $_{\text{asym}}$ ), 1422 (COO $_{\text{sym}}$ ), 166 ( $\Delta\nu$ ), 584 (Cd-N), 461 (Cd-O);  $^1\text{H}$  NMR (DMSO- $d_6$ , ppm): 4.29 (s, 2H, –OCH $_2$ ), 7.45-7.46 (d, 2H, H3,  $J$  = 2.4 Hz), 7.21-7.25 (dd, 2H, H5,  $J$  = 2.4 Hz, 9 Hz), 6.85-6.88 (d, 2H, H6,  $J$  = 9 Hz), 9.08-9.10 (dd, 1H, H $\alpha$   $J$  = 1.5 Hz, 2.7 Hz), 7.80-7.84 (dd, 2H, H $\beta$  4.2 Hz, 8.1 Hz), 8.55-8.58 (dd, 1H, H $\gamma$   $J$  = 1.5 Hz, 8.1 Hz), 8.03 (s, 2H, H $\epsilon$ );  $^{13}\text{C}$  NMR (DMSO- $d_6$ , ppm): 171.1 (C=O), 68.8 (–OCH $_2$ ), 154.0 (C1), 137.3 (C2), 127.9 (C3), 128.9 (C4), 123.6 (C5), 115.5 (C6), 150.7 (C $\alpha$ ), 122.5 (C $\beta$ ), 129.2 (C $\gamma$ ), 127.1 (C $\delta$ ), 124.1 (C $\epsilon$ ), 145.6 (C $\zeta_{\text{phen}}$ ).

#### **2.1.4. DNA interaction study through UV visible spectroscopy**

In order to evaluate the ability of the synthesized complexes to interact with the DNA the binding experiments were performed. Here at first the solution of SS-DNA was prepared by dissolving 20 mg of the respective sodium salt in water and by stirring it for 24 hours. Concentration of DNA solution calculated by using Beer-Lambert was found to be 153  $\mu\text{M}$ . The absorbance of the resulting solution was noted at 259 nm to 260 nm and was adjusted at appropriate intensity, i.e., in between 0.9 to 1.3 a.u. The ratio of the absorbance at 260/280 was found to fall around 1.7, assuring the solution purity from any other interrupting proteins. Solutions of all the complexes were made in analytical grade ethanol. Concentration of test complexes was kept fixed and SS-DNA concentration was varied. Equivalent amount of SS-DNA was added into reference cell and sample cell to nullify the absorption of DNA. The complex-DNA solution was incubated for 5-7 minutes and then absorbance was recorded at room temperature [32-34].

### **3. RESULTS AND DISCUSSION**

#### **3.1. FT-IR Spectral Interpretation**

Infrared spectral analysis served as a crucial technique for confirming complex formation since observable shifts or disappearance of absorption bands indicate interactions between the ligand and metal ion. FT-IR data of all synthesized complexes is given in Table S1. Assignment of bands was made by comparison with spectra of free ligands and already reported similar data.

The FT-IR spectra of both free ligands MeOPhA and ClPhA consist of wide O–H stretching band between 3400 and 2700 cm $^{-1}$  region. After complexation, this band totally vanishes, demonstrating that the ligand is deprotonated [20, 35]. Similarly, both free ligands showed strong bands in the 1680-1740 cm $^{-1}$  region for C=O stretch and around 1240–1260 cm $^{-1}$  region corresponding to C–O stretching vibrations [33]. In the complexes, these strong vibrational bands were replaced by a new pair of bands, i.e.,  $\nu(\text{COO})_{\text{asym}}$  in the range of 1550-1610 and  $\nu(\text{COO})_{\text{sym}}$  in the range of 1370-1430 cm $^{-1}$  region. This is because electronic

density of carbonyl oxygen is pulled towards metal upon coordination thus the symmetry of C=O bond decreases and the strong C=O band replaced by two resonance stabilized COO<sup>-</sup> bands [33, 36, 37].

The mode of coordination of carboxylate ligand was decided by  $\Delta\nu$  ( $\nu_{\text{asym}} - \nu_{\text{sym}}$ ) according to the Deacon-Phillips description which they made after studying a big number of complexes [38]. The  $\Delta\nu$  values for all complexes were less than 200 cm<sup>-1</sup> which suggest that carboxylate ligand is coordinated through bidentate mode. In the fingerprint region, two new prominent bands appear in the 425–620 cm<sup>-1</sup> region due to Cd–N and Cd–O bonds which confirm the coordination of acid ligand and N-donor moiety to metal center. The similar finding has also been discussed by Singh *et al* [39] about the M–O and M–N bonds. All the heteroleptic Cd complexes showed strong vibrational bands in the region of 750–860 cm<sup>-1</sup> corresponding to C–H out-of-plane vibrations from the N-donor heterocyclic ligands [33, 40]. MeOPhA2 and ClPhA2 showed an intense band near 650 cm<sup>-1</sup> due to ring bending vibrations of bipyridine which confirmed the formation of both pyridine containing complexes [41].

### 3.2. <sup>1</sup>H NMR Spectroscopy

A 300 MHz spectrometer was used to record the <sup>1</sup>H NMR spectra of the ligands and their associated metal complexes in deuterated dimethyl sulfoxide (DMSO). <sup>1</sup>H NMR data of all complexes is given in Table S2 and S3.

NMR spectra of the free ligands showed O–H signals at 11–12 ppm that vanished in the spectra of complexes confirming deprotonation of acid ligands [20, 42]. All the other protons of ligands appeared in their characteristic regions, i.e., methoxy and aliphatic methyl proton, methylene proton and the aromatic protons. These protons showed negligible shift upon complexation indicating their non-involvement in metal coordination [33]. In the case of heteroleptic complexes, additional signals were observed for N-donor ligands. Four distinctive aromatic proton signals in the range of 7.1–9.1 ppm are seen in MeOPhA2 and ClPhA2 complexes containing 2,2'-bipyridine. The chemical shift values were assigned to the protons following the order: H<sub>α</sub> > H<sub>δ</sub> > H<sub>γ</sub> > H<sub>β</sub>. Similarly, four additional protons signal in the range of 7.1–8.8 ppm were spectra of complexes MeOPhA3 and

ClPhA3 containing 1,10-phenanthroline confirms its attachment. The chemical shift of proton was assigned the following order: H<sub>α</sub> > H<sub>γ</sub> > H<sub>δ</sub> > H<sub>β</sub>.

These signals shift to higher ppm values as compared to free ligand upon coordination indicating a decrease in electron density on the nitrogen atoms and consequent deshielding thus confirming the formation of heteroleptic cadmium carboxylates containing N-donor heterocyclic ligands. As the distance of proton from coordinating nitrogen increases, the effect of deshielding also decreases so only small shift in frequency on coordination [33, 43, 44].

### 3.3. <sup>13</sup>C NMR Spectroscopy

<sup>13</sup>C NMR helps in identification and quantification of different types of carbon atoms; methyl (CH<sub>3</sub>), methylene (CH<sub>2</sub>), methine (CH), aromatic carbons and carbons of N-donor ligands. It is a useful mean to directly observe a molecule's carbon structure. It provides important details regarding the hybridization states of individual carbon atoms, particularly those that are directly linked to a metal center [45]. <sup>13</sup>C NMR data of all complexes is given in Table S4 and S5.

<sup>13</sup>C NMR spectra of free ligands MeOPhA and ClPhA show resonance signal of C=O group at 172.3 ppm and 167 ppm respectively. Within spectra of complexes, this resonance signal was shifted toward a downfield (higher ppm) region which indicates the deprotonation of ligands and their coordination to the metal center. This deshielding effect occurs due to the electropositive nature of Cd(II), which withdraws electron density from the carboxylate group so it resonates downfield [33, 46]. Aliphatic methylene carbon in MeOPhA and ClPhA appearing at frequency 55 ppm–67 ppm in free form showed noticeable downfield shift in spectra of complexes. Aromatic carbons of MeOPhA and ClPhA ligand appeared in their respective regions in the spectra of metal complexes thus providing strong evidence of desired complexes formation. Appearance of five additional peaks in the spectra of complexes MeOPhA2 and ClPhA2 confirms the coordination of bipyridine to metal center and chemical shifts values were assigned in the following order C<sub>ε</sub> > C<sub>α</sub> > C<sub>δ</sub> > C<sub>γ</sub> > C<sub>β</sub>. The coordination of 1,10-phenanthroline is confirmed by six peaks in spectra of complexes MeOPhA3 and ClPhA3 and

assignment of chemical shift values was done in following order  $C_\alpha \geq C_\zeta > C_\delta > C_\gamma > C_\epsilon > C_\beta$  [20, 47].

### 3.4. DNA Interaction Studies

A drug's biological action is greatly influenced by how it interacts with DNA, which has an impact on vital cellular functions like transcription and translation. Understanding these interactions is an important field of study in medicinal chemistry. Here, UV-visible spectroscopy was used to observe the interaction of SS-DNA with synthesized complexes in an ethanolic solution, using an aqueous solution of DNA. The mode of interaction is revealed by variations in absorbance and wavelength [18]. The binding constant  $K_b$  ( $M^{-1}$ ) is used to measure the binding strength, whereas the Gibbs free energy ( $\Delta G$ ) is used to measure the spontaneity of interaction. Both parameters were calculated using Benesi- hildebrand equation [48].

$$\frac{A_0}{A-A_0} = \frac{\epsilon_G}{\epsilon_{H-G} - \epsilon_G} + \frac{\epsilon_G}{\epsilon_{H-G} - \epsilon_G} \times \frac{1}{K[DNA]} \quad (1)$$

A is the absorbance of complex in the presence of DNA and  $A_0$  is the absorbance of complex without DNA addition. For each complex, A and  $A_0$  are noted and  $A_0/A-A_0$  ratio is plotted on y-axis and inverse of DNA concentration is taken on x-axis.  $\epsilon_{H-G}$  and  $\epsilon_G$  represent the molar absorptivity of synthesized complexes without DNA and after DNA addition [42].

Binding constant  $K_b$  is calculated by taking intercept to slope ratio. Gibbs free energy is calculated for each complex by using Equation 2:

$$\Delta G = -RT \ln K_b \quad (2)$$

In homoleptic complexes MeOPhA1 and ClPhA1, hypochromism is observed with a minor blue shift after incremental additions of DNA and strong absorption bands at 271 nm and 284 nm respectively. Hypochromic effect is observed due to binding of partially filled  $\pi^*$  orbital of complex with  $\pi$  orbital of DNA hence the probability of excitation is getting less so decrease in absorbance [49]. Shoulder peak is also seen in both complexes due to n- $\pi$  transitions. Both homoleptic complexes bind to DNA by groove binding mode [50].

All heteroleptic complexes MeOPhA2, MeOPhA3, ClPhA2, ClPhA3 showed strong

absorption bands that appear at 278nm, 264 nm, 264 nm, and 282 nm, respectively. After incremental additions of DNA, hypochromism is observed along with a blue shift of 3 to 4 nm in  $\lambda_{max}$ . These complexes bind to DNA by groove binding. The interacting molecule creates a parallel stacking arrangement by occupying a location where it sits on the DNA chromophore's floor. This configuration produces a parallel interaction (at a 90° angle where transitions are restricted for forbidden states and permitted for upper states), which raises the energy needed for the transition and, consequently, the blue shift. Additionally, there is a slight hypochromic impact from this face-to-face position [8] The binding constant values measured for the homo and heteroleptic cadmium carboxylates with ligand o-methoxyphenylacetic acid were found to be in the order: MeOPhA3 > MeOPhA2 > MeOPhA1

The binding constant values measured for the homo and heteroleptic cadmium carboxylates with ligand 2,4-dichlorophenoxyacetic acid were found to be in the order:

ClPhA3 > ClPhA2 > ClPhA1

$K_b$  values for heteroleptic complexes are high because they contain intercalating agent 1,10-phenanthroline and 2,2'-bipyridine which strongly intercalate with DNA and provide more area of interaction thus increasing reactivity [34]. Homoleptic complexes have no such intercalating agents thus the value of binding constants is low. Negative Gibbs free energy shows the spontaneous nature of interaction with DNA [51]. The UV-Visible spectra and graphs showing DNA binding studies of all the synthesized complexes are given in Figure 1. The binding constant,  $\lambda_{max}$  and  $\Delta G$  values for all synthesized complexes are given in Table 1.

## 4. CONCLUSIONS

The synthesis of mixed ligand Cd(II) complexes by using the already bio-active moieties like substituted phenylacetic acid and nitrogen heterocycles was carried out over here. The synthesis was carried out by keeping in view their application as a drug which could target DNA, which is considered to be the main house of disease cause, propagation, its treatment and diagnosis. The ligand 2-methoxyphenyl acetic acid and 2,4-dichlorophenoxy acetic acid used as primary



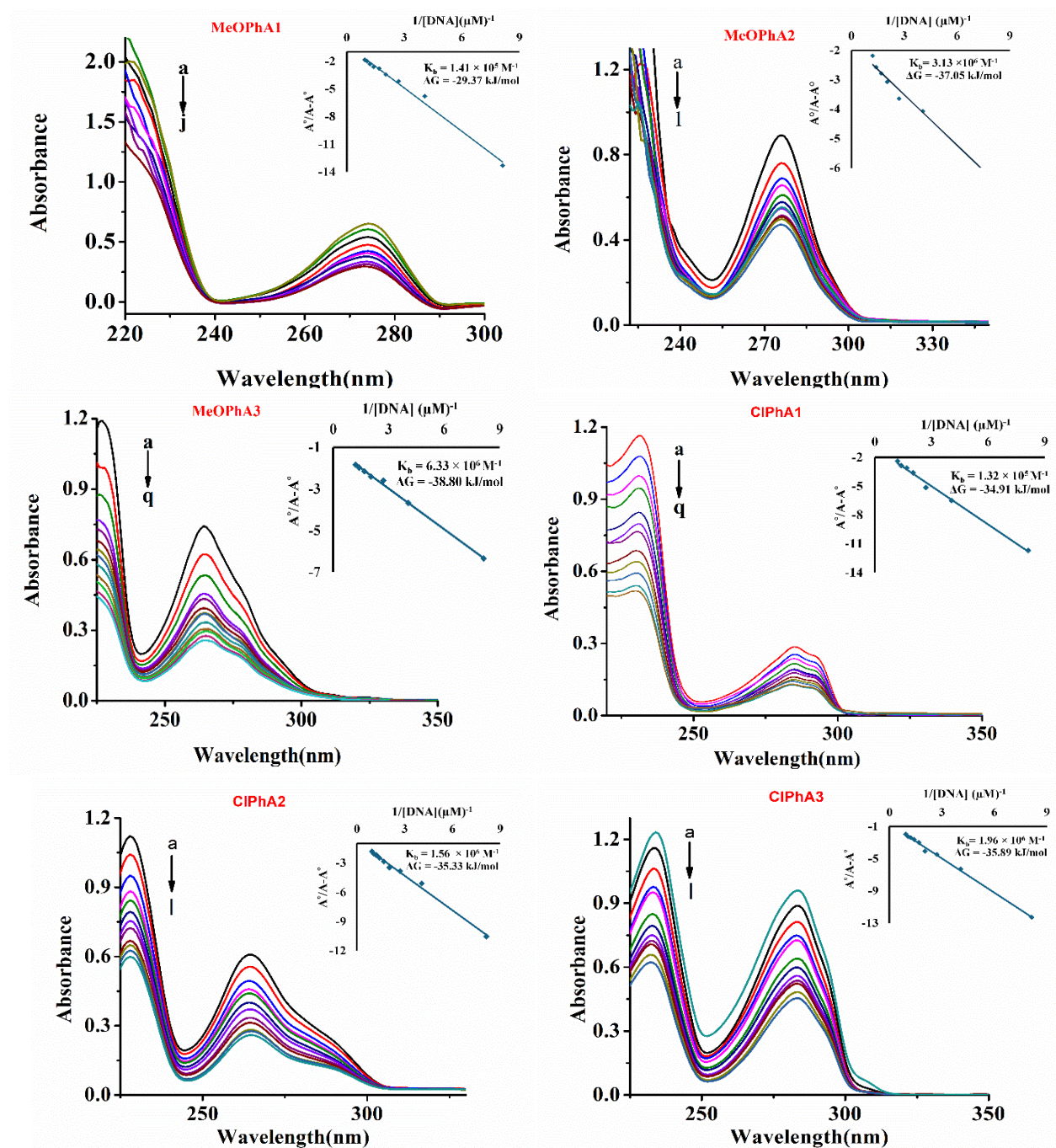


Fig. 1. Absorption spectra of complexes showing the effect of addition of DNA.

Table 1. Binding constant  $K_b$  and Gibbs Free energy  $\Delta G$  values for all synthesized complexes.

Complex	$\lambda_{\text{max}}$ (nm)	Binding Constant $K_b$ ( $\text{M}^{-1}$ )	Gibbs Free Energy $\Delta G$ (kJ/mol)	Mode of interaction
MeOPhA1	271	$1.41 \times 10^5 \text{ M}^{-1}$	-29.37 kJ/mol	Groove binding
MeOPhA2	278	$3.13 \times 10^6 \text{ M}^{-1}$	-37.05 kJ/mol	Groove binding
MeOPhA3	264	$6.33 \times 10^6 \text{ M}^{-1}$	-38.80 kJ/mol	Groove binding
CIPhA1	284	$1.32 \times 10^5 \text{ M}^{-1}$	-34.91 kJ/mol	Groove binding
CIPhA2	264	$1.56 \times 10^6 \text{ M}^{-1}$	-35.33 kJ/mol	Groove binding
CIPhA3	282	$1.96 \times 10^6 \text{ M}^{-1}$	-35.89 kJ/mol	Groove binding



and 2,2-bipyridine as well 1,10-phenanthroline posses structural and electronic characteristic which effectively tuned the geometric environment around Cd(II) center. The FT-IR data reveal the bidentate coordination mode adopted by the primary ligands. The  $^1\text{H}$  and  $^{13}\text{C}$  spectra reveal the presence of clear resonance signal attributed to proton and carbon of the complex under study. The DNA binding study through absorption spectroscopy indicate the success of the synthesized complexes. The planar moieties and other characteristics of the planar moieties founds to play a significant role in binding with DNA. The data indicate that such kind of structural design could provide significant help in the search for novel, effective therapeutic agents against diseases relevant to DNA.

## 5. DECLARATION OF COMPETING INTEREST

The authors have no conflict of interest.

## 6. ACKNOWLEDGEMENT

S.A. is grateful to the Pakistan Academy of Sciences and Quaid-i-Azam University, Islamabad for the financial assistance.

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