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DNA binding activity of new Nickel (II) mixed ligands complexes containing 1, 10-Phenanthroline or 2, 2′-Bipyridine based on Sulfanilic acid.

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ABSTRACT

Two new nickel complexes with mixed ligands of 1, 10-phenanthroline (**1**) or 2, 2′-bipyridine (**2**) based on sulfanilic acid in presence of 5-chloro salicylaldehyde were synthesized. The identity of the complexes was determined by CHNS analysis, FTIR, HNMR and ^{13}CNMR techniques. According to the characterization techniques, the structural geometries were suggested as distorted octahedral. The efficiency of complexes against cancer tumors was investigated by spectroscopic and physical methods via in vitro binding with DNA. The results revealed a strong intercalation binding between the complexes and DNA. The extent of binding constant (K_b) was determined and the complex **2** was recorded the higher value of binding strength (6.4559 x 10^7 M^{-1}) than **1** (6.1238 x 10^7 M^{-1}).

Keywords: Semicarbazone, Groove DNA binding, UV titration, Binding constant, Viscosity

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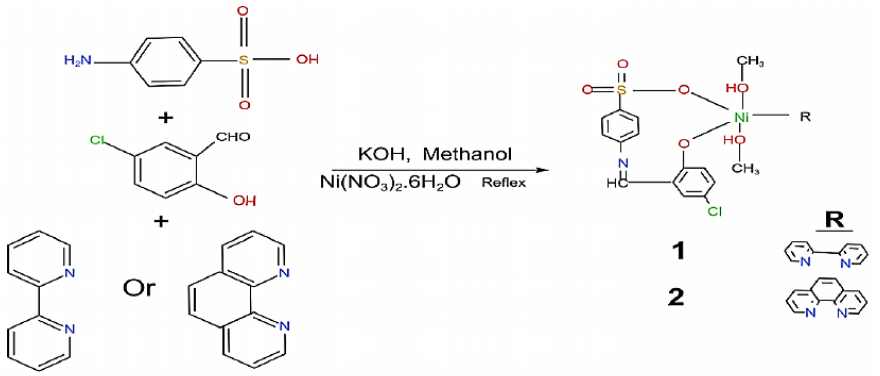
INTRODUCTION

Most anticancer and antiviral therapies are target DNA molecule [1], and because the site-specific binding properties of metal ions, the binding investigations between DNA and metal complexes supplies a new ways for the development of new chemotherapeutic drugs [2]. Essentially, the interaction between the metal complexes and double helix DNA achieves either by non-covalent way which includes external electrostatic, intercalative and groove binding [3], or by covalent way. Especially, the important among them is the intercalative binding that can make DNA duplex more stable, which an often affected by the ligand planarity, atom type of donor and the coordination geometry [4]. The most active anticancer drugs like cisplatin have limited usage due to its side-effects and cellular resistance [5]. So, the efforts to find other drugs with more-effective, less toxic and target-specific DNA binding ability are still searchers interest. One of the important interactions between the transition metal complexes and DNA is that based on multidentate aromatic ligands due to their possible applications as new therapeutic agents [4]. Nickel complexes have played an essential part in bioinorganic chemistry development. The divalent nickel ion forms stable complexes with a variety of chemical ligands and biomolecules. Nickel ions' distinctive features have been used to develop novel metal-based medications in the field of medicinal inorganic chemistry [6]. Ni (II) complexes of multidentate aromatic ligands revealed strong DNA binding with prominent anticancer/antiproliferative activities [7–9]. 2, 2′-Bipyridine and 1, 10-Phenanthroline are conjugated systems with excellent planarity, can easily slide into the adjacent base pairs of DNA and are potential antitumor agents [10]. The nucleolytic efficiency of 2, 2′-Bipyridine and 1, 10-Phenanthroline has received more attention due to their act as auxiliary ligands that lead to strengthen the binding ability of a complex through increasing the planarity of the molecule [11]. As far as we know, reports about the DNA-binding and cytotoxic properties of 2, 2′-Bipyridine and 1, 10-Phenanthroline based on sulfanilic acid complexes are rare, while the works on the 2, 2′-Bipyridine and 1, 10- Phenanthroline based on taurine complexes are easy to find [12,13]. Therefore, to develop efficacious metal–based chemotherapeutic molecular agents, herein we report the synthetic design of Ni (II) complexes containing 2, 2′-Bipyridine and 1, 10-Phenanthroline based on sulfanilic acid in presence of 5-chloro salicylaldehyde. The synthetic route of the complexes is shown in Scheme 1.



Scheme 1. Synthesis route of Ni (II) complexes

EXPERIMENTAL

Materials and instrumentation

All chemicals were purchased from Sigma Aldrich/Merck, and used without more purification. DNA obtained from human blood. FTIR spectra were recorded on a Shimadzu (FTIR-8400S, Japan) spectrometer using KBr pellets. Electronic spectra were recorded on a UV-Vis double-beam spectrophotometer using cuvettes of 1 cm path length (Spectroscan-80D, England). ^{1}HNMR and ^{13}CNMR spectra were recorded on a BRUKER 400 MHz spectrometer using DMSO-d_6 as solvent. Elemental analysis was performed on Vario EL Cube CHN analyzer.

DNA binding assay

The binding with DNA were performed in 6.3 mM Tris-HCl/50 mM NaCl buffer (pH = 7.2). DNA stock solution was prepared by dissolving a suitable amount of DNA in buffer solution (1 ml) at room

temperature and stored in refrigerator. DNA stock concentration was estimated by the UV absorbance at 260 nm using the known molar absorption coefficient of 6600 M^{-1} cm^{-1} [14]. The absorption titrations were performed using a known concentration of the ligands or complexes (50 μM) with increasing amounts of DNA from 10 μM to 100 μM. Each addition was left 10 min at 25 °C before was scanned from 230 nm to 600 nm.

Viscosity measurement

Viscosity measurements were performed using a Cannon Manning Semi-micro viscometer flooded in a thermostatic water bath at 37 °C. Flow times were manually measured with a digital stopwatch. From the observed flow times of the DNA-containing solutions (t), the viscosity values were calculated from the observed flow time of DNA-containing solutions (t) corrected for that of solvent mixture used (t_0), $\eta = t - t_0$. Viscosity data were given as $(\eta / \eta_0)^{1/3}$ versus [complex] / [DNA], where η and η_0 are the viscosity of the complex in the presence of DNA and the viscosity of DNA alone, respectively [15].

Synthesis of complexes

The complexes were prepared by the following general procedure in which a solution of Sulfanilic acid (0.1733g, 1 mmol) was dissolved in methanol (10 ml). To this, methanolic solution (10 ml) of 5-Chlorosalicylaldehyde (0.1566g, 1 mmol) was added. The reaction mixture was refluxed with stirring for about 1 h. To the resulting solution, nickel(II) nitrate hexahydrate (0.2908 g, 1 mmol) was added and the reaction mixture was stirred for 1 h, followed by addition of 2,2′-Bipyridine (0.1563 g, 1 mmol) (**1**) or 1,10-Phenanthroline (0.1982 g, 1 mmol) (**2**). The mixture was stirred for another 2 h at the same temperature. The resultant colored solution was filtered and kept at room temperature. Crystals were obtained after the evaporation of the mother liquor.

(1) Anal. Calc. for $C_{25}H_{24}O_6N_3SNi$: C, 54.28; H, 4.37; N, 7.60; S, 5.80, Found: C, 54.50; H, 4.60; N, 8.30; S, 6.30; IR(KBr) ($\nu_{max/cm^{-1}}$): 1433 (C=C), 725 (C-H), 1660 (C=O), 1598 (C=N), 3375 (O-H); ^{1}H NMR (DMSO-d_6, ppm): 3.07 (CH_3), 9.71 (OH), 7.79, 8.11, 8.55, 9.29 (H-aromatic of bipyridine), 6.32, 7.05 (H-aromatic of sulfanilic acid), 6.81, 7.58 (H-aromatic of salicylaldehyde), 8.85 (CH=N); ^{13}C NMR (DMSO-d_6,ppm): 40.98 (CH_3), 126.63, 141.88, 146.72, 150.00, 190.21 (C-aromatic of bipyridine), 111.11, 124.12, 170.16, 172.57 (C-aromatic of sulfanilic acid), 132.17, 154.91, 160.00, 179.61 (C-aromatic of salicylaldehyde), 184.67 (C=N).

(2) Anal. Calc. for $C_{27}H_{24}O_6N_3SNi$: C, 56.18; H, 4.10; N, 7.28; S, 5.55, Found: C, 56.90; H, 4.70; N, 7.90; S, 6.10; IR(KBr) ($\nu_{max/cm^{-1}}$): 1433 (C=C), 725 (C-H), 1660 (C=O), 1598 (C=N), 3375 (O-H), 3319 (N-H); ^{1}H NMR (DMSO-d_6, ppm): 3.07 (CH_3), 9.73 (OH), 7.71, 8.01, 8.56, 9.38 (H-aromatic of phenanthroline), 5.99, 7.05 (H-aromatic of sulfanilic acid), 6.33, 7.63 (H-aromatic of salicylaldehyde), 8.86 (CH=N); ^{13}C NMR (DMSO-d_6,ppm): 40.98 (CH_3), 132.76, 137.12, 146.87, 190.00, 194.93 (C-aromatic of phenanthroline), 111.12, 121.12, 165.12, 175.11 (C-aromatic of sulfanilic acid), 116.23, 152.71, 155.02, 160.00, 179.83 (C-aromatic of salicylaldehyde), 184.71 (C=N).

RESULTS AND DISCUSSION

Synthesis

The complexes were prepared by condensation of 4-aminobenzenesulfonic acid (sulfanilic acid) with 5-chloro-2-hydroxybenzaldehyde in presence of 2,2′-Bipyridine or 1,10-Phenanthroline with 1:1 molar ratio, using methanol as solvent.

The elemental analysis (CHNS) of the complexes was in closes consent with those calculated for the proposed formula. The complexes were identified by FTIR, ^{1}H NMR and ^{13}C NMR. All the compounds are air-stable and highly soluble in DMSO and DMF.

Infrared spectra

In IR spectra of the **1** and **2** complexes, the essential characteristic is the peak emergence at 1598 cm^{-1} which is supporting the formation of the C=N group and the stretching vibration band at 3375 cm^{-1} that attributed to O-H group which indicates the presence the solvent in coordination sphere. A band at

1433 cm^{-1} is attributed to ν(C=C) group, a stretching vibration of ν(C-H) group is emerged at 725 cm^{-1}, a band at 1660-1662 cm^{-1} is attributed to ν(C=O) group, and the IR band about 677-686 cm^{-1} is attributed to ν(Ni-O) group.

^{1}H and ^{13}C NMR spectra

^{1}HNMR spectral data of **1** and **2** are shown in Fig. 1. The singlet signal appeared at 3.07 ppm is attributed to CH_3 protons, the signals appeared at 6.32, 6.81 and 7.05 ppm are attributed to the protons of 9, 11; 15, 16; and 8, 12, respectively. The aromatic proton 18 and the proton 19 are in respective emerged at 7.58 ppm and 8.85 ppm. The signals appeared at 7.79; 8.11; 8.55 and 9.29 ppm are attributed to aromatic protons of 4, 4'; 3, 3'; 5, 5' and 6, 6', respectively. The singlet signal arise at 9.71 ppm is attributed to the proton of OH group.

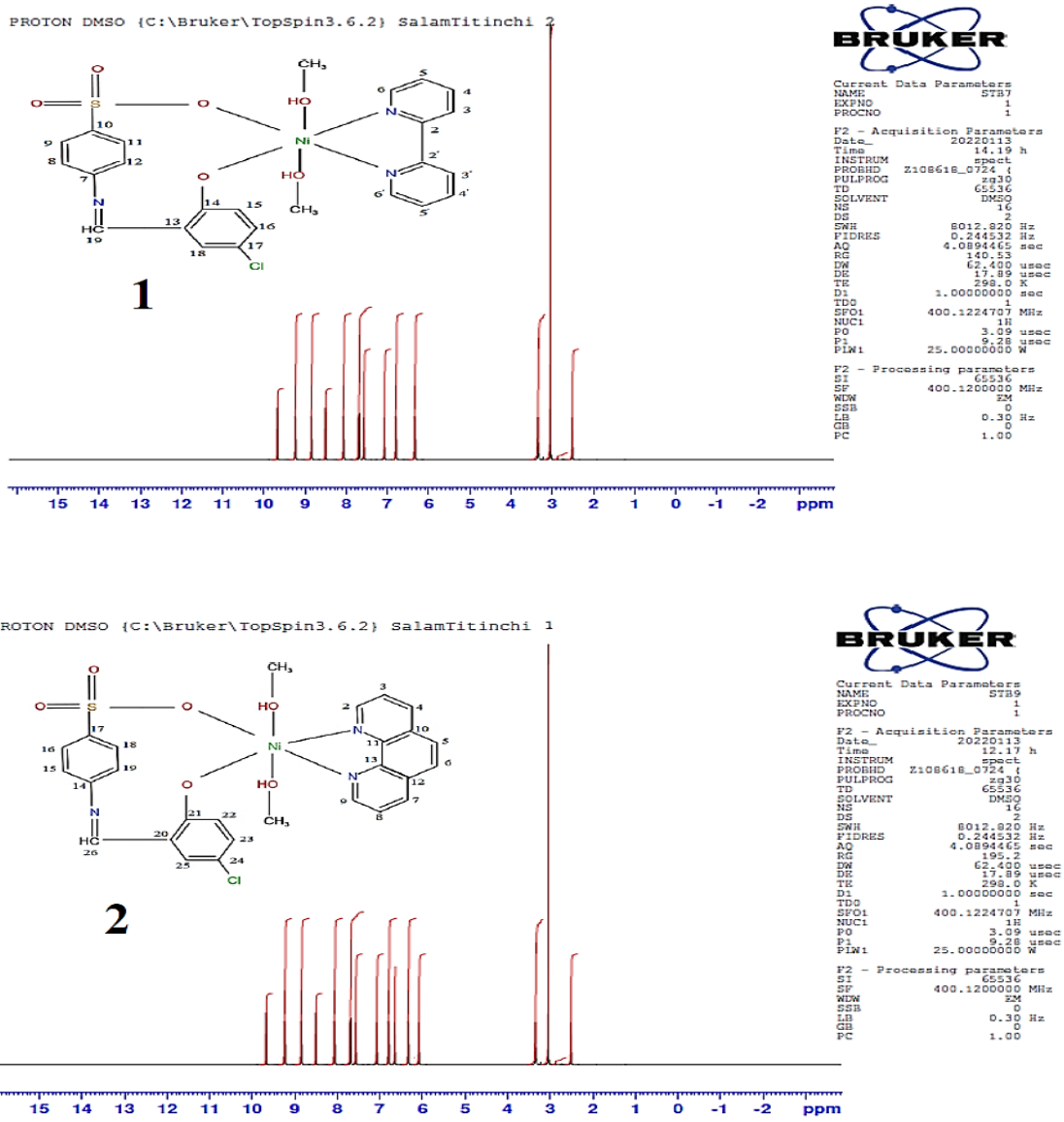


Fig. 1. ^{1}HNMR spectra of the complexes 1 and 2

In ^{13}CNMR of (Fig. 2), the signal appeared at 40.98 ppm is back to the carbon of CH_3 group, the carbon signal emerged at 111.11 ppm is attributed to 9 and 11, the carbons of 8 and 12 are emerged at 124.12 ppm. The aromatic carbons of 4, 4'; 3, 3'; 5, 5'; 6, 6' and 2, 2' are arising at 126.63; 141.88, 146.72; 150.0 and 190.21 ppm. The carbon signals of quaternary carbons of 7, 10, 14 and 17 are emerged at 172.57, 170.16, 179.61 and 160.0 ppm, respectively. The carbons of 18 and 19 are appeared respectively at 154.91 and 184.67 ppm.

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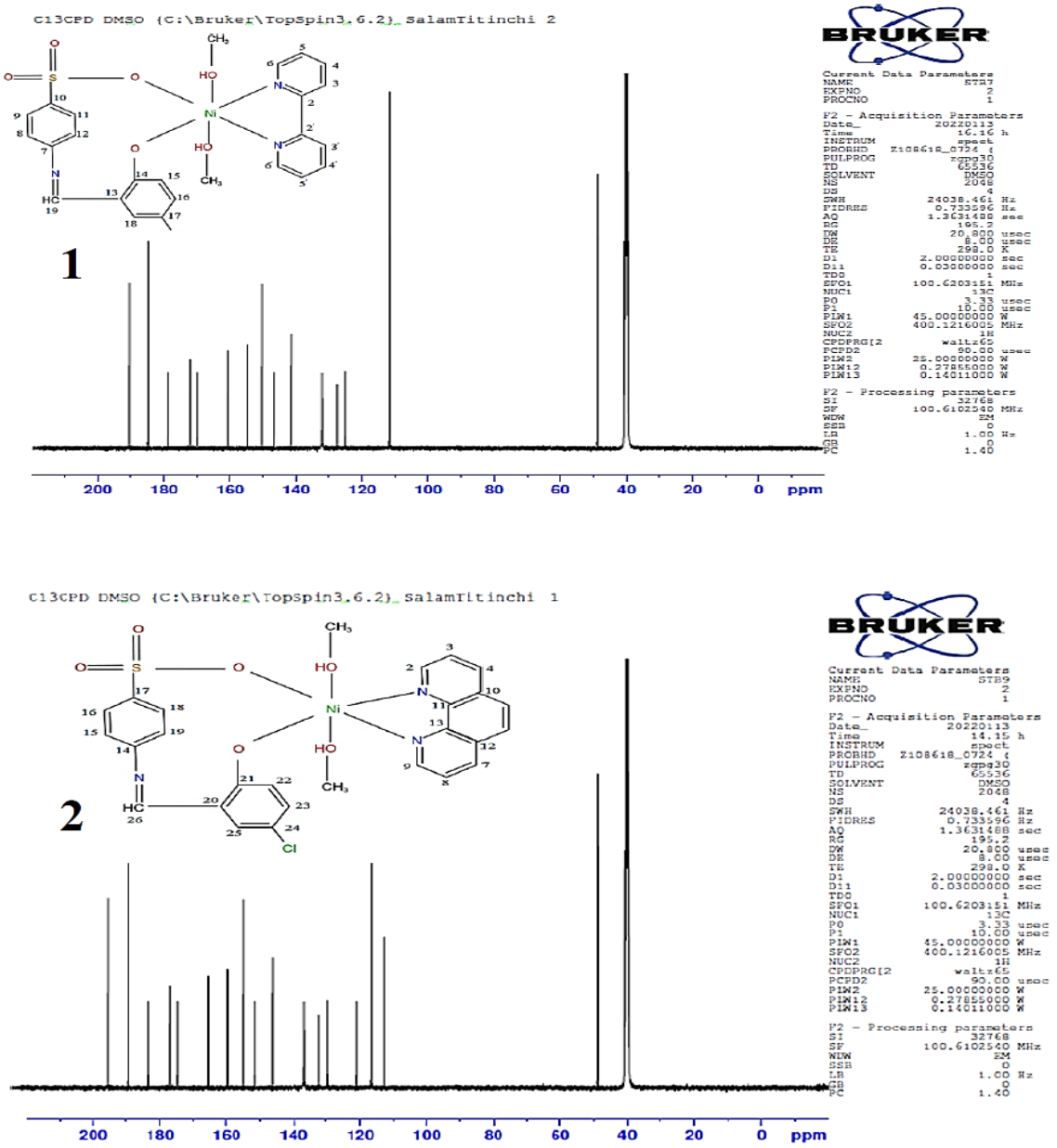

Fig. 2. ^{13}CNMR spectra of the complexes 1 and 2

Binding with DNA

Absorption spectroscopic studies

UV–Visible spectroscopy is the simplest and most frequently employed for studying both DNA stability and its interaction with small molecules. The method is carried out by monitoring the changes in the absorption properties of the drug or the DNA molecules. Usually, the interaction between the DNA and the drug is examined by the changing in the intensity of the maximum absorption band before and after the drug is added to the DNA [16–18]. The DNA show two absorbance bands at 260 and 280 nm and the intensity ratio between the two should be in the range of 1.8–1.9 to ensure that DNA is sufficiently free of protein [19].

The studies displayed that the small molecules can bind to DNA either by covalent bonding, like in complexes having ligands can be substituted with the nitrogen base of DNA [20], or by noncovalent interactions, like intercalation and electrostatic or groove binding [21].

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The samples were scanned from 200 nm to 600 nm. The complexes observed intense absorption band at high energy region to π -π* transition at 353 nm. To determine the possible interactions with DNA, a constant concentration 10 μM of complex was titrated with DNA in Tris-Buffer pH 7.2 at room temperature. The absorption spectra of the complexes in the absence and presence of DNA are shown in Fig. 3.

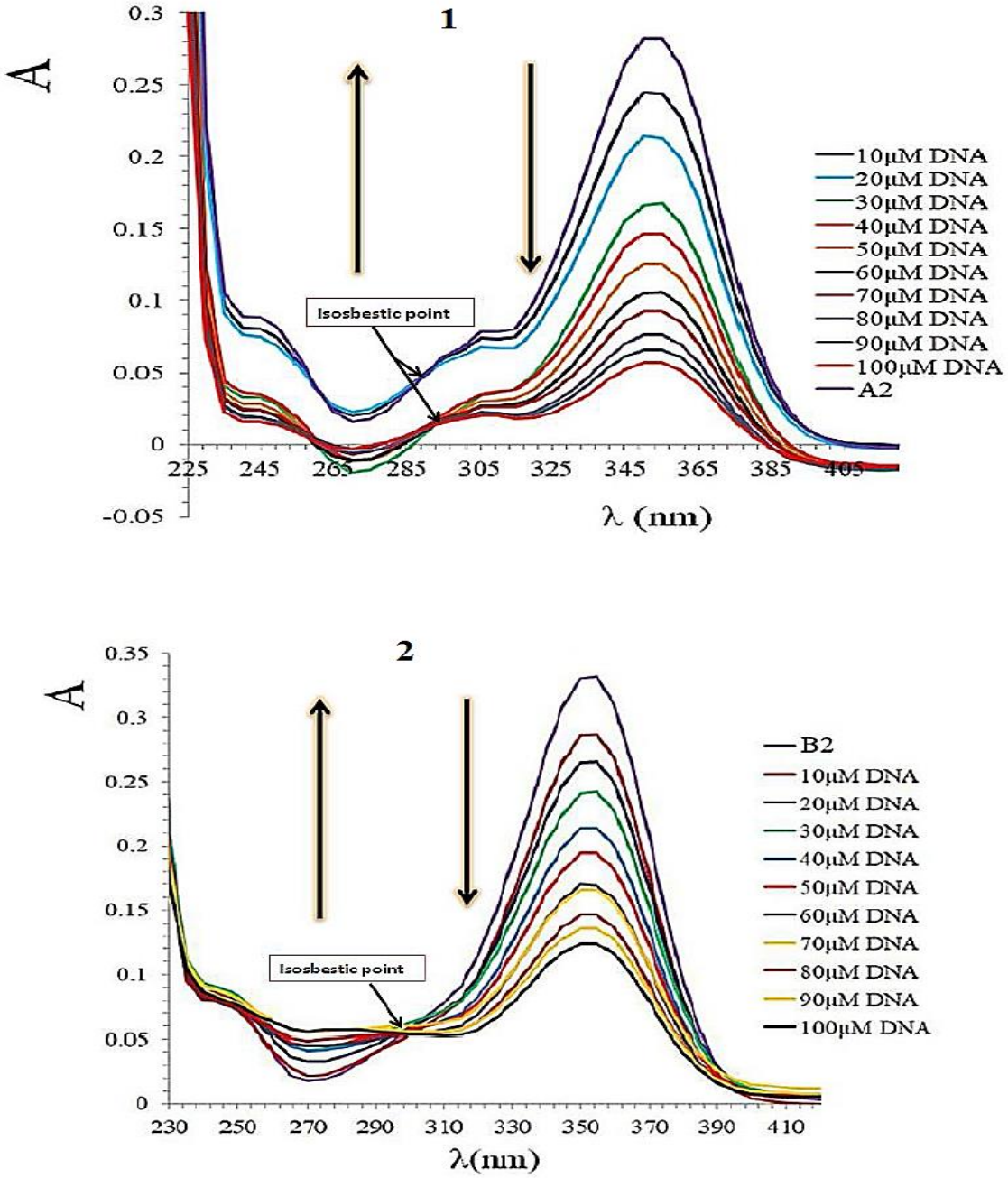

Fig. 3. Electronic absorption spectra of 1 and 2 in presence of DNA. The arrows show the changes in absorbance upon increasing amounts of DNA

Upon addition of DNA to the complexes, the absorption intensity at 353 nm decreases with increases the added amount of DNA (10 μM-100 μM). This spectral behavior indicates that the complexes are introducing an intercalation binding at all concentrations of DNA. The intercalative mode involving a stacking interaction between an aromatic chromophore and the base pair of DNA, the extent of the hypochromism is usually consistent with the strength of intercalative interaction [22]. Moreover, the addition of DNA results an isosbestic spectral changes, at 291 nm for **1** and at 297 nm for **2**. The isosbestic points confirm the interaction of complexes with DNA, which are suggested that chemical equilibria exist

between the bound complexes with DNA (products) and the free unbound DNA (reactants). Isosbestic points appeared at wavelengths in which the molar absorption coefficients of reactants and the products are the same.

The binding strength of the complexes was evaluated by calculating the intrinsic binding constant K_b using an equation (1) [23].

$$[DNA]/(\varepsilon a - \varepsilon f) = [DNA]/(\varepsilon b - \varepsilon f) + 1/(Kb\ (\varepsilon b - \varepsilon f)) \quad \ldots\ldots (1)$$

Where εa, εf, and εb are the apparent, free, and bound molar extinction coefficients, respectively. Kb is the equilibrium binding constant (in M–1) of complex binding to DNA. The binding constant is obtained by plotting [DNA]/(εa – εf) vs [DNA]. The plots are shown in Fig. 4. The obtained values of Kb are scheduled in Table 1.

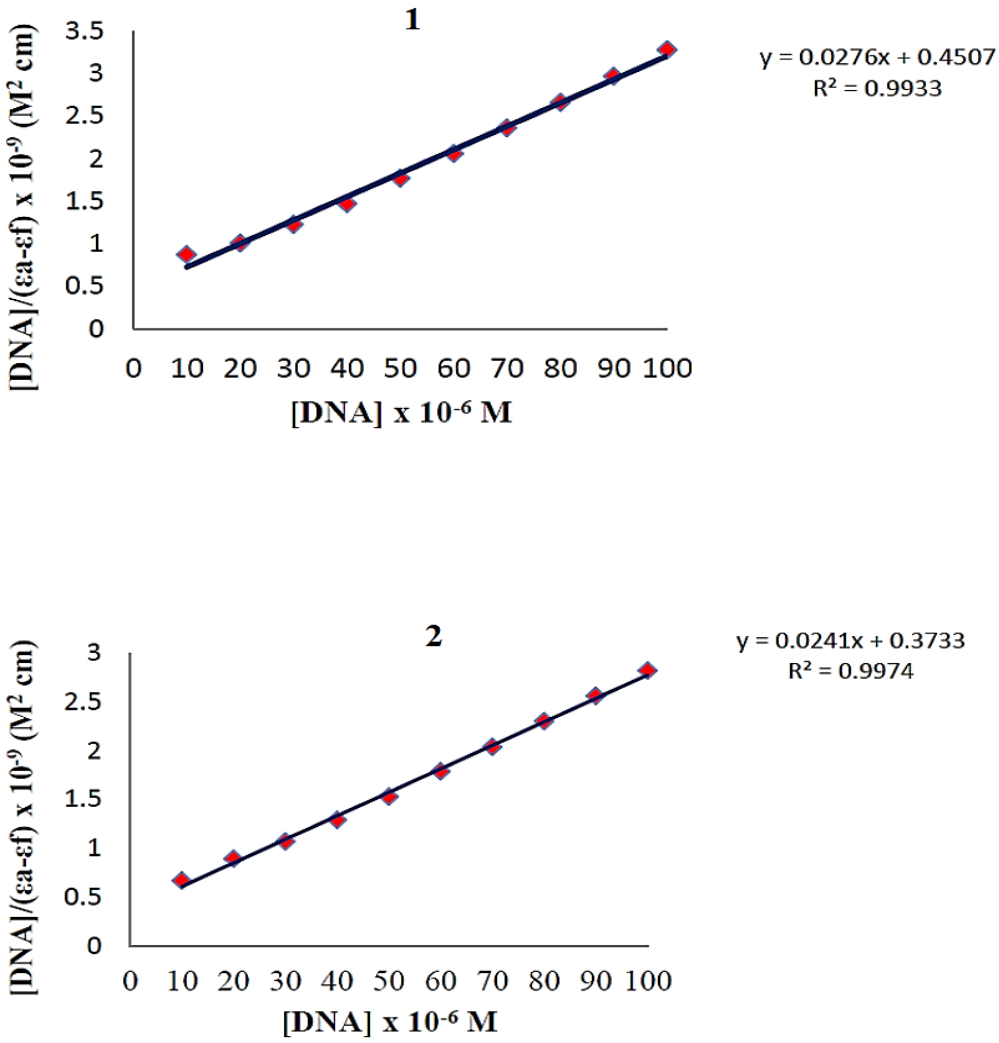

Fig. 4. Plot of [DNA]/(εa – εf) vs. [DNA] for the titration 1 and 2 with DNA

Table 1. Binding constants (M^{-1}) of complexes with DNA

Complex	K_b (M^{-1})
1	$6.1238X10^7$
2	$6.4559x10^7$

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The binding constant values show that the complexes are strongly bound to DNA in order: **2** > **1**. The binding strength of complexes with DNA depends on lipophilic properties which increase the affinity between the complex (drug) and DNA, this affinity depends on the lipophilicity extent of complexes. The extent lipophilicity of complexes depends on lipophilic properties provided by substituents that bounded with metal ion in coordination sphere. Accordingly, the complex **2** show the strongest binding with DNA than **1** due to their high lipophilicity extent compared to other complexes, and because the lipophilic properties of 1, 10-phenanthroline more than 2, 2'-bipyridine.

Viscometric studies

Viscosity measurement is a main method for confer support to the non-covalent binding modes of compounds with DNA, and provides a simple common means of differentiating DNA binding mode. The intercalation binding causes major conformational changes in DNA, affect DNA helix length, and lead to increases the viscosity of DNA solutions. The changes in viscosity of the complexes are shown in Fig. 5.

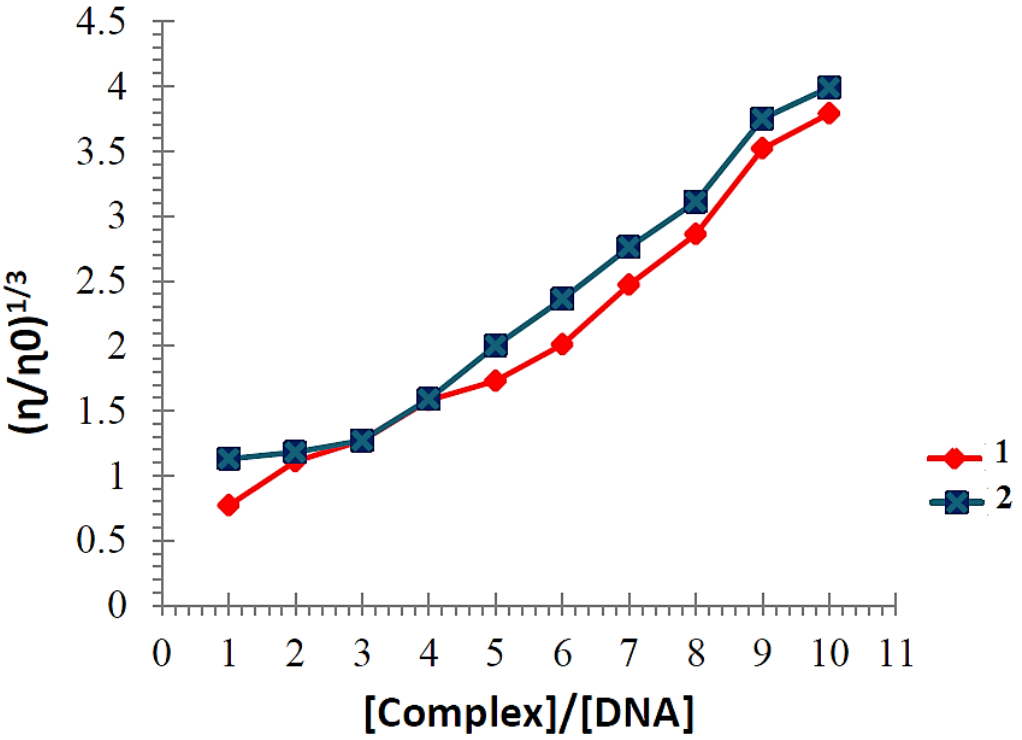

Fig. 5. Effect of increasing amounts of complexes on the relative viscosities of DNA in tris-HCl buffer solution

To further clarify the interaction modes of the complexes and DNA, the viscosity measurements were carried out. The plots of relative viscosity $(\eta/\eta_0)^{1/3}$ versus [DNA] illustrate a significant increase in the relative viscosity of DNA on increasing the concentration of complexes [24]. This result further suggests an intercalative binding mode of the complex with DNA. Thus, the viscosity measurements are consistent with the results of the electronic absorption titrations.

CONCLUSION

Two complexes of Ni(II); **1** and **2** with mixed ligands containing 1, 10-phenanthroline and 2, 2'-bipyridine in presence of 4-amino benzene sulfonic acid as based ligand. The activity of synthesized complexes against cancer tumor via interaction with DNA was investigated using spectroscopic and physical methods. The results are revealed the intercalation binding between the complexes and DNA. The obtained binding constants values are indicated that **2** complex is the strongest intercalator compared to the complex **1**. These finding indicate that1, 10-phenanthroline provides high lipophilic properties than 2, 2'-bipyridine and suggesting that the presence of phenanthroline molecule in coordination sphere increases the planarity of complex leads to ease the intercalate it in binary helix DNA.

Based on these findings, the synthesized complexes may promise new drugs could be potentially useful in chemotherapy.

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