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# Mononuclear Dioxomolybdenum(VI) semicarbazonato Complexes: Synthesis, Characterization And In Vitro DNA Binding Activity.

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**ABSTRACT**

In mole ratio 1:1, four molybdenum (VI) complexes (A2, B2, C2 and D2) were synthesized by the reaction of $MoO_2(acac)_2$ with semicarbazone ligands derived from 3-methoxy-2-hydroxybenzaldehyde (**A**), 5-chloro-2-hydroxybenzaldehyde (**B**), 2-hydroxybenzaldehyde (**C**) and 2-hydroxynaphthaldehyde (**D**). The synthesized ligands and complexes were structurally characterized by FTIR, $^{1}$HNMR, $^{13}$CNMR; furthermore the complexes were identified by EDX and SEM spectroscopic techniques. The anti-tumor activity of the ligands and their complexes was extracted by their ability to bind with DNA. The spectroscopic and physical methods were used to studying the binding activity. The spectroscopic method showed decreases in the absorbance with increases the DNA amount added to the ligand or complex. This behavior indicates the intercalation binding mode with DNA. On the other hand, the physical method was carried out by measurement the viscosity changes due addition the ligand or complex to DNA. The results revealed that the viscosity increases as the ligand or complex increases, this behavior confirms the intercalation binding which consistent with spectroscopic results. The binding constants ($K_b$) were calculated. The results exhibited a noticed binding strength for complexes compare to the ligands with DNA, which are follow the order **D**, **D2** > **B**, **B2** > **A**, **A2** > **C**, **C2**.

**Keywords:** Semicarbazon, Dioxomolybdenum(VI), Intercalation binding, Absorption titration, Viscosity

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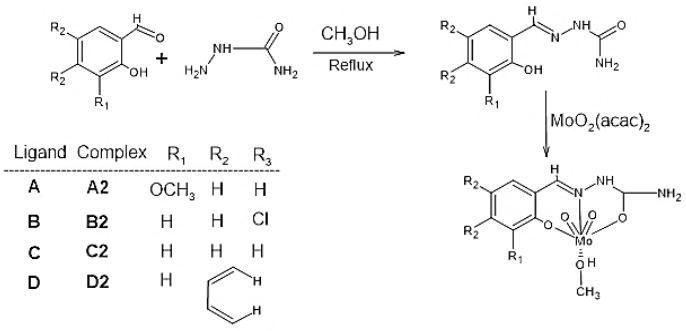
## INTRODUCTION

Semicarbazones have been target of interest to researchers due to their biological applications, such as anticancer, antioxidant, antifungal anticonvulsant, antiinflammatory, analgesic, and antibacterial agents [1]. Semicarbazones are of special interest to coordination chemistry, they form with many metals varied complexes of significant structural characteristics [2-4]. Semicarbazones as well as their complexes have important role in an industrial, pharmaceutical, and agricultural chemistry. The reported studies were observed that the metal complexes can be more active than the free ligands [5].

Mononuclear molybdenum complexes have been of wide interest due to their presence in the active sites of containing enzymes. Mo(VI) is reported as model active site for such mononuclear oxidoreductase and hydroxylase enzymes [6-9]. Mo(VI) complexes have shown satisfactory results regarding the biological activity specAtrum. Among of these complexes, the ligands coordinated in tridentate mode with Mo(VI). The chemistry of semicarbazones, containing O/N/O donor sets that used as polydentate chelating ligands to prepare complexes containing the $MoO_2^{2+}$ core. Mo(IV) complexes continue to be developed as structural and functional models for molybdenum-containing cofactors [10]. Also, many thiosemicarbazone-based cis-dioxomolybdenum(VI) complexes have been prepared and examined as catalysts in processes as oxygen atom transfer and epoxidation [11]. It is also reported that these kind of molecules have been tested for biological purposes in terms of their activities, such as antitumor, antioxidant and enzyme inhibion [12-14].

Here, we report the synthesis, spectral characterization of dioxomolybdenum(VI) complexes of substituted salicylaldehyde semicarbohydrazone as ONO-donor ligand binding to the molybdenum through oxygen atom, hydrazinic nitrogen atom and phenolic oxygen atom (Scheme 1). In this report, we have investigated the binding ability of dioxomolybdenum(VI) with DNA.




| Ligand | Complex | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|
| A | A2 | $OCH_3$ | H | H |
| B | B2 | H | H | Cl |
| C | C2 | H | H | H |
| D | D2 | H | | |

**Scheme 1. Synthetic route of ligands and complexes**

## EXPERIMENTAL

### Materials and instrumentation

All the 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. EDX was performed to determine the elemental composition using the acceleration electron beam energy to excite the X-ray is 18 kV.

### DNA binding assay

The binding with DNA were performed in 6.3 mM Tris-HCl/50 mM NaCl buffer (pH = 7.2). Stock solution of DNA was prepared by dissolving a suitable amount of DNA in buffer solution (1 ml) at room temperature and stored in refrigerator. The concentration of DNA stock solution was determined by the UV absorbance at 260 nm using a known molar absorption coefficient that of 6600 $M^{-1}$ $cm^{-1}$ [15]. 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. Viscosity was determined 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 $\eta$ and $\eta_0$ are the viscosity of the complex in the presence of DNA and the viscosity of DNA alone, respectively [16].

**Synthesis of ligands**

In general, the ligands were prepared as follows: to a methalonic solution (20 ml) of semicarbazide hydrocloride (1g, 8.96 mmol), a corresponding aldehyde was added, that of 2-hydroxy-3-methoxybenzaldehyde (1.3632 g, 8.96 mmol); 5-chloro-2-hydroxybenzaldehyde (1.4027 g, 8.96 mmol); 2-hydroxysalisalaldehyde (1.0941 g, 8.96 mmol) or 2-hydroxy- naphthaldehyde (1.5427 g, 8.96 mmol). The mixture was refluxed with stirring for 2h. The product then was filtered, washed with ethanol, and air-dried. The products were assigned as **A** ($C_9H_{11}O_3N_3$), **B** ($C_8H_8O_2N_3Cl$), **C** ($C_8H_9O_2N_3$) and **D** ($C_{12}H_{11}O_2N_3$), respectively.

**Synthesis of complexes**

The molybdenum complexes were prepared as follows: $MoO_2(acac)_2$ (2.9209g, 8.96 mmol) was added to an ethanolic or methalonic solution (10 ml) of corresponding ligand that of **A** (1.8726 g , 8.96 mmol); **B** (2.0025 g, 8.96 mmol); **C** (1.73 g, 8.966 mmol); and **D** (2.0518 g , 8.96 mmol). The mixture was refluxed for 2 h and then filtered. The filtrate was left to stand at room temperature, and the product was formed after some days. The obtained complexes are assigned **A2**, **B2**, **C2** and **D2**, respectively.

## RESULTS AND DISCUSSION

**Characterization**

The ligands considered in this study were prepared in good yield by condensation of semicarazide with 2-hydroxybenzaldehyde, 3-methoxy-2-hydroxybenzaldehyde, 5-chloro-2-hydroxybezaldehyde and 2-hydroxy- naphthaldehyde, in 1:1 molar ratio, using methanol or ethanol as solvent. The complexes were synthesized in 1:1 molar ratio by adding a suitable amount of $MO_2(acac)_2$ to the methanolic or ethanolic solution of a certain ligand, the reaction mixture was leaved under reflux for 2h before the product was collected. The compounds were characterized by FTIR, $^1$H NMR, $^{13}$C NMR. Moreover, the complexes are further identified by EDX and SEM techniques. All the compounds are air-stable and highly soluble in DMSO and DMF.

**Infrared spectra**

In general, the ligands of **A**, **B**, **C** and **D** exhibited very similar IR features. The important feature in the IR spectra of the ligands is the band appears about 1597-1604 $cm^{-1}$ indicating the formation of the C=N group. The bands at about 3450 $cm^{-1}$ to 3479 $cm^{-1}$ are attributed to the stretching vibration of υ (O-H) group, the bands at about 3277 $cm^{-1}$ to 3298 $cm^{-1}$ are corresponding to υ (N-H) group, and the bands at about 1670 $cm^{-1}$ to 1689 $cm^{-1}$ is attributed to υ (C=O) group. These results are consistent with the literature [17].

The IR spectra of complexes show two sharp bands at about 938 $cm^{-1}$ and 902 $cm^{-1}$ assigned to antisymmetric and symmetric vibrations, respectively, of the Mo=O groups in *cis*–$MoO_2^{+2}$ core. The IR bands of semicarbazone ligands have shifted to lower or higher frequencies upon chelation with $MoO_2^{+2}$. The stretching C–O band has been upward shifted to appear in the region 1560-1568 $cm^{-1}$. The υ (C=N) underwent a change in frequency and intensity, lead to appeared at 1586-1590 $cm^{-1}$, indicating coordination of azomethine nitrogen [18].

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

The ligands and their molybdenum complexes have exhibited $^{1}$H and $^{13}$C NMR spectra are consistent with their suggested structures (Figs. 1 and 2). In $^{1}$H NMR spectra of **A**, The singlet signal that appears at 3.820 ppm is attributed to $CH_3$ protons, the broad signal appears at 6.993 ppm is attributed to the protons of NH group, the signals that emerge at 6.779, 6.989 and 7.386 ppm are attributed to aromatic protons nominated at 5, 6, and 4, respectively. The singlet signal that arises at 9.360 ppm is back to the protons of OH groups. In $^{1}$HNMR spectral data of **B**, the broad signal appears at 6.514 ppm is attributed to the protons of NH group, the signals that emerge at 6.973, 7.386 and 7.734 ppm are attributed to aromatic protons nominated at 4, 3, and 6, respectively. The singlet signals that arise at 8.360 and 9.360 ppm are back to the proton nominated at 7 of HC=N and the proton of OH group, respectively. In $^{1}$HNMR spectral data of **C**, the signals that emerge at 6.475, 6.863, 7.206 and 7.769 ppm are attributed to aromatic protons nominated at 3, 4, 5 and 6, respectively. The singlet signal arises at 9.569 ppm is back to the proton of OH group. Whereas, In $^{1}$HNMR spectral data of **D**, the signals that emerge at 7.238, 7.375, 7.540, 7.937 and 8.419 ppm are attributed to aromatic protons nominated at 6, 7, (5, 8), 4 and 3, respectively. The singlet signals arise at 8.929 and 9.569 ppm are back to the protons of HC=N and OH groups, respectively.

In $^{13}$CNMR of **A**, the signal appears at 56.12 ppm is attributed to the carbon of $CH_3$, the aromatic carbons assigned at 5, 4 and 6 appear at 109.37, 115.64 and 121.54, respectively. The quaternary carbons assigned at 1, 2, 3 and 8 appear at 140.38, 148.43 and 157.49 ppm, respectively. Whereas, the signal emerges at 126.72 ppm is attributed to the carbon assigned at 7 of C=N group the signal appears at 56.12 ppm is attributed to the carbon of $CH_3$, the aromatic carbons assigned at 5, 4 and 6 appear at 109.37, 115.64 and 121.54 ppm, respectively. The quaternary carbons assigned at 1, 2, 3 and 8 appear at 140.38, 148.43 and 157.49 ppm, respectively.

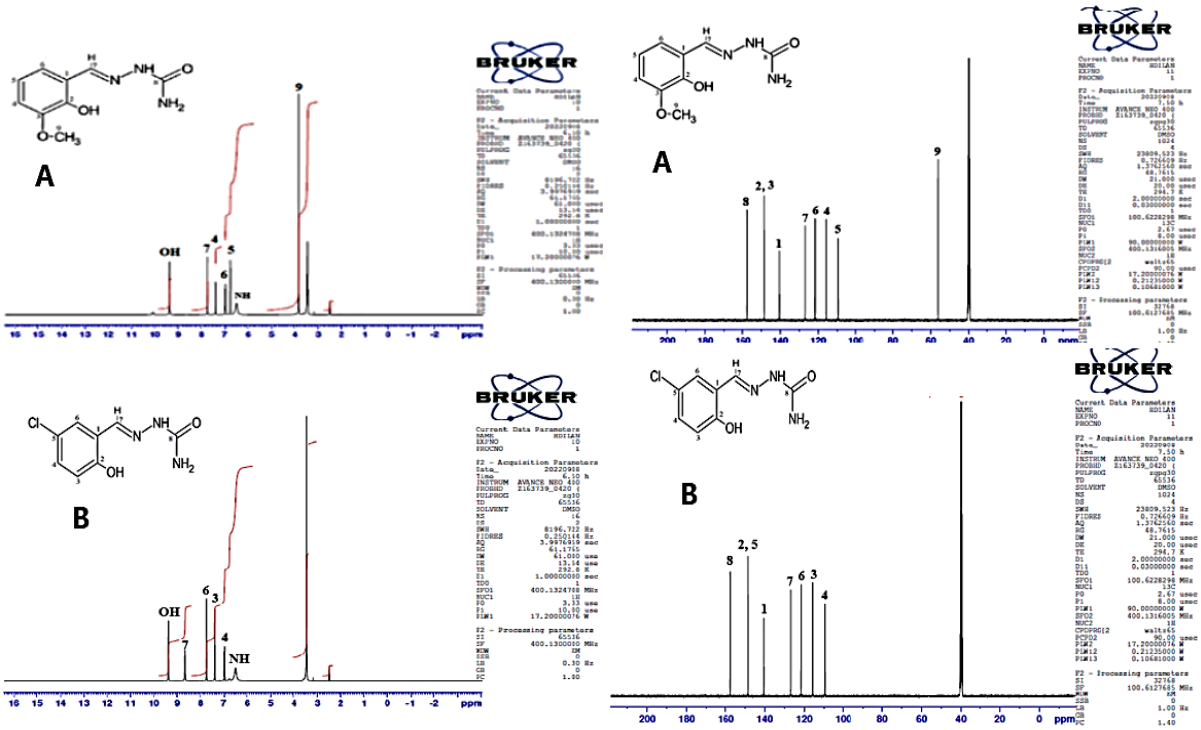


**Figure 1: $^{1}$HNMR (Left) and $^{13}$CNMR (Right) spectra of the ligands A and B**

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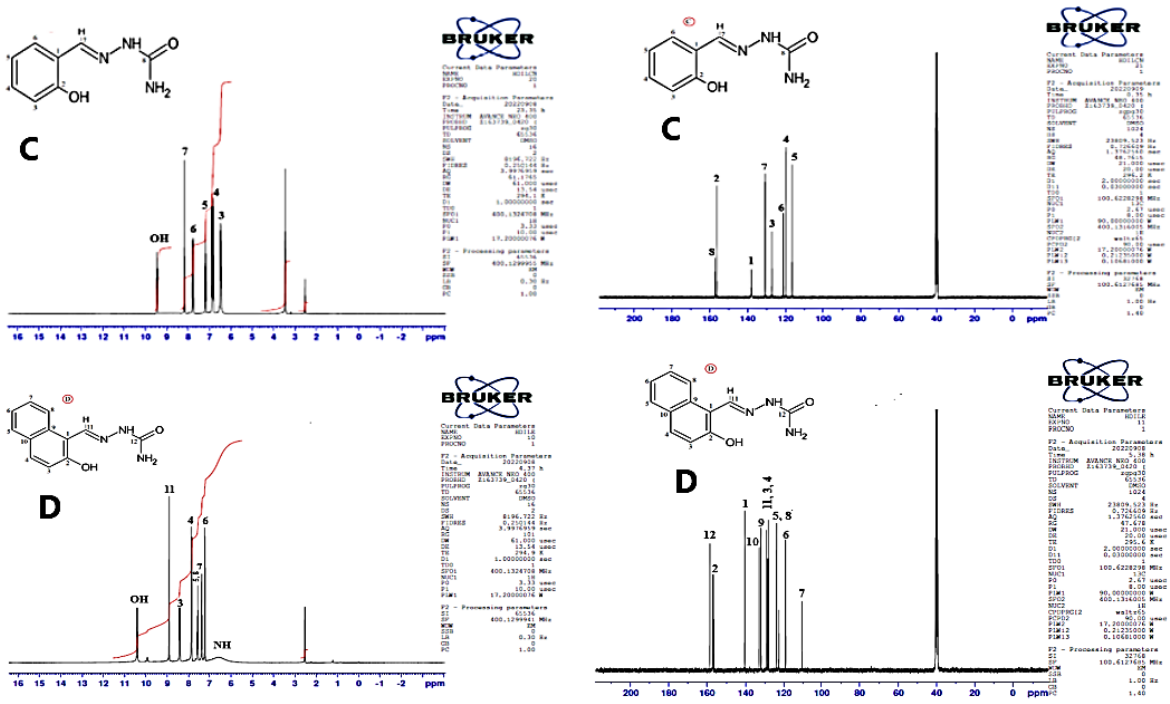


**Figure 2: $^{1}$HNMR (Left) and $^{13}$CNMR (Right) spectra of the ligands C and D**

Whereas, the signal emerges at 126.72 ppm is attributed to the carbon assigned at 7 of C=N group. In $^{13}$CNMR of **B**, the aromatic carbons assigned at 4, 3 and 6 appear at 109.55, 115.82 and 121.68, respectively. The quaternary carbons assigned at 1, 2, 5 and 8 appear at 140.97, 149.06 and 158.11 ppm, respectively. Whereas to the signal emerges at 127.02 ppm is attributed to the carbon assigned at 7 of C=N group. In $^{13}$CNMR of **C**, the aromatic carbons assigned at 5, 4, 6, 3, 1 and 2 appear at 116.42, 119.69, 121.06, 127.14, 137.98 and 156.28 ppm, respectively. The quaternary carbon of C=O group assigned at 8 appears at 157.15 ppm. Whereas, the signal emerges at 130.65 ppm is attributed to the carbon assigned at 7 of C=N group. While In $^{13}$CNMR of **D**, the aromatic carbons assigned at 7, 6, 5, 8, 4, 3, 9, 10, 1 and 2 appear at 110.28, 118.96, 122.52, 123.74, 127.97, 128.43, 131.80, 131.89, 140.43 and 156.39 ppm, respectively. The quaternary carbon of C=O group assigned at 12 appears at 158.28 ppm. Whereas, the signal emerges at 129.16 ppm is attributed to the carbon assigned at 11 of C=N group.

The NMR spectra of complexes (Figs 3 and 4) can be distinguishable by the appearance new signals that attributed to the protons of solvent that participates in $MO_2^{+2}$ coordination sphere.

In $^{1}$HNMR spectral data of **A2**, **B2** and **D2** show the methanol solvent signal that attributed to $CH_3$ protons at 2.281 ppm, 2.352 ppm and 3.427 ppm and, the solvent OH group at 8.982 ppm, 9.602 ppm and10.482 ppm, respectively. In $^{1}$HNMR spectral data of **B2**, the signals appear at 1.228 and 3.394 ppm are attributed to the ethanol solvent $CH_3$ and $CH_2$ protons appointed at 10 and 9, respectively. While the singlet signal emerges at 9.403 ppm is attributed to solvent OH group. In $^{13}$CNMR of **A2**, **B2** and **D2** show the carbon methanol solvent signal that attributed to $CH_3$ carbon at 57.01 ppm, 52.21 ppm and 49.08 ppm, respectively. In $^{13}$CNMR of **B2**, the signals appear at 26.32 and 58.10 ppm are attributed to the solvent $CH_3$ and $CH_2$ carbons, respectively. All the other NMR signals were numbered and back to their related signals in spectrum, which are shifted up or down field due the coordination with $MoO_2^{+2}$ core.

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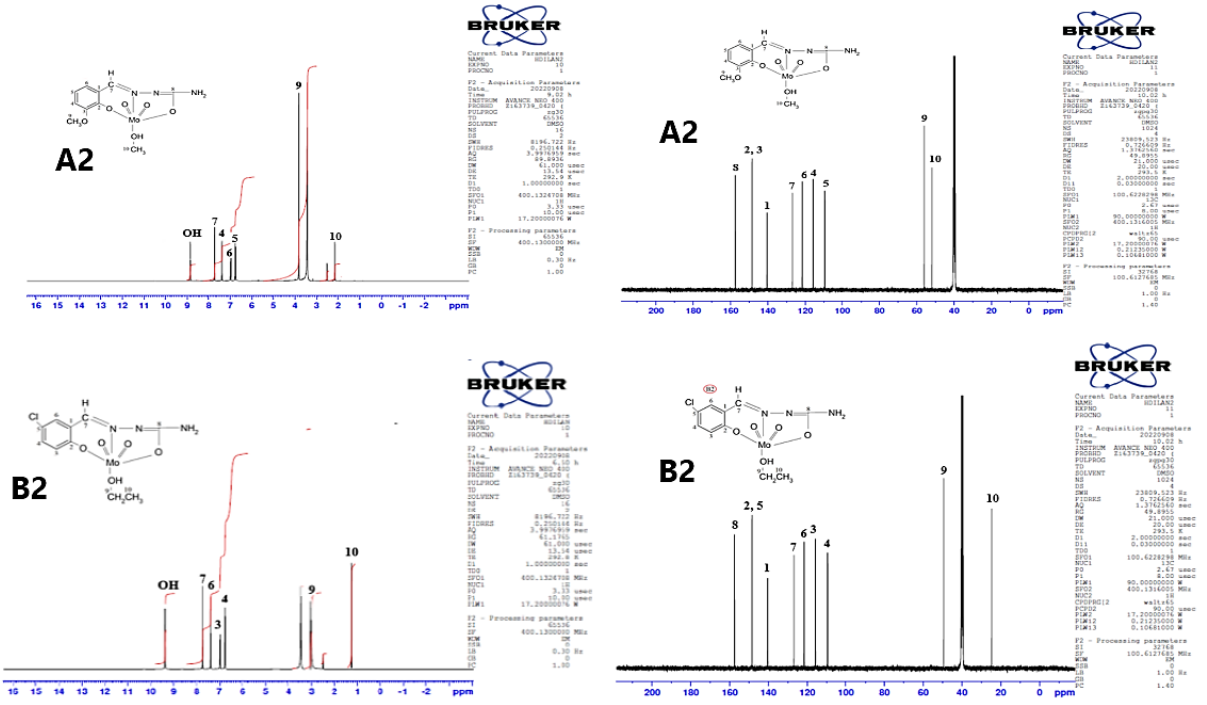


**Figure 3: $^{1}$HNMR (Left) and $^{13}$CNMR (Right) spectra of the complexes A2 and B2**

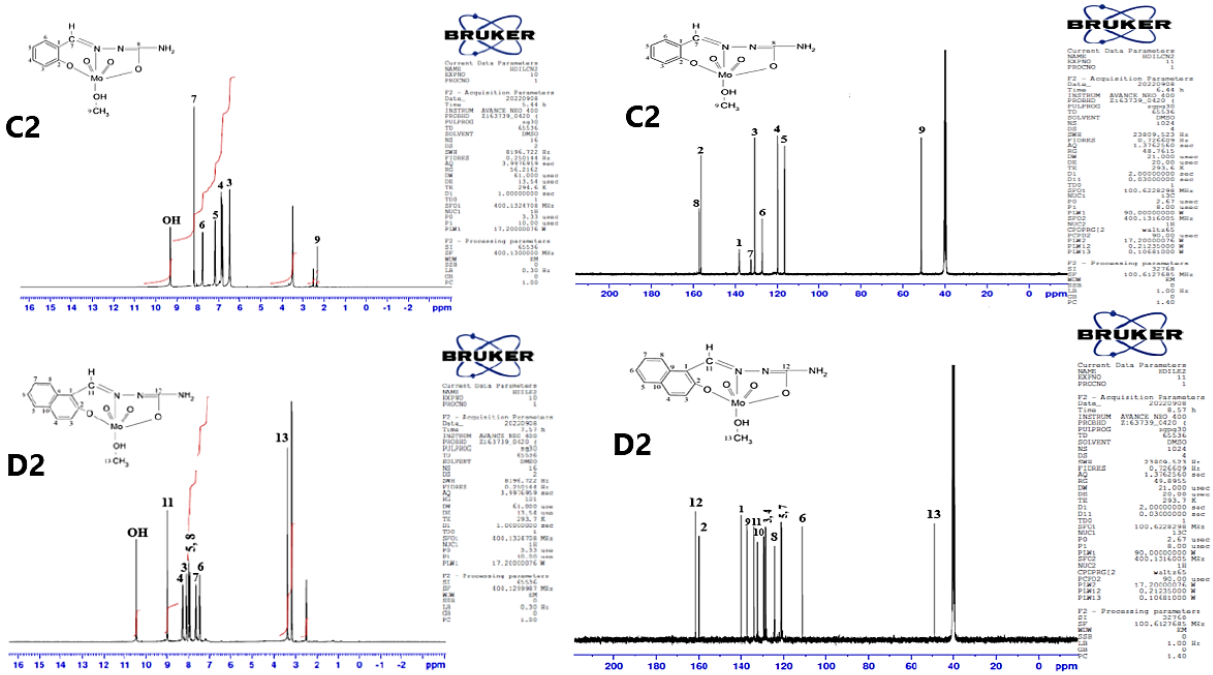


**Figure 4: HNMR (Left) and $^{13}$CNMR (Right) spectra of the complexes C2 and D2**

**EDX analysis**

The ligands coordination with molybdenum was confirmed by EDX technique. The results revealed that the ligands are successfully complexation with MO2+2 as shown in fig. 5.

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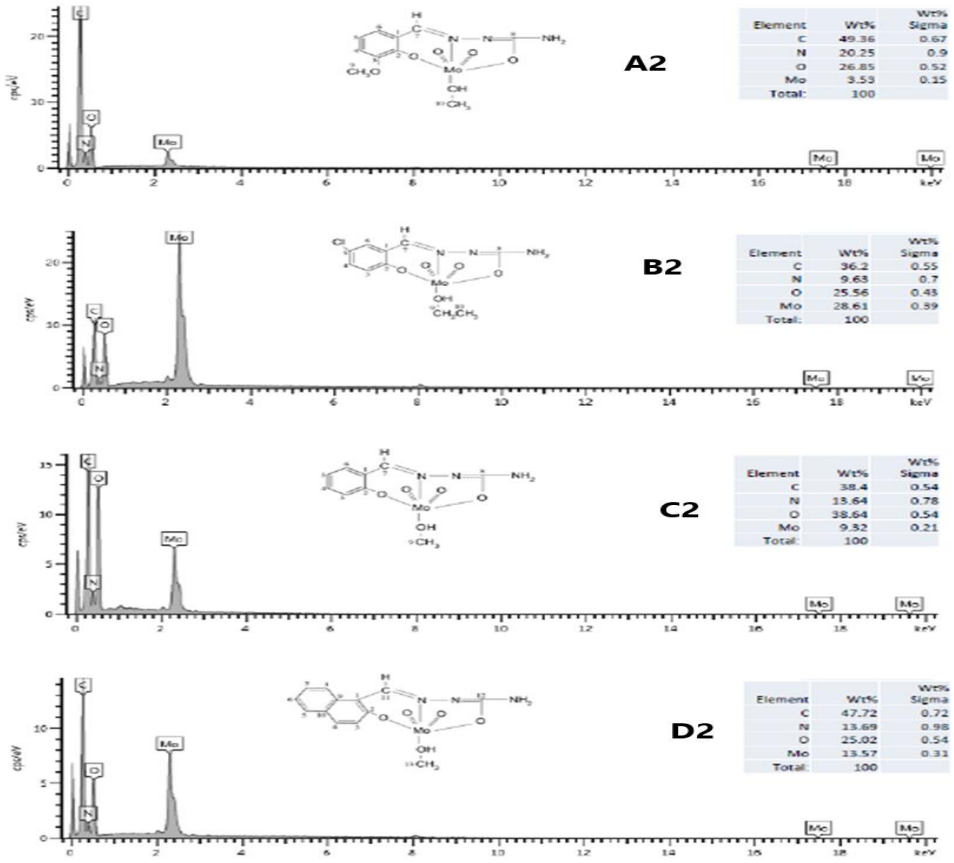


**Figure 5: EDX analysis of the complexes**

**Scanning Electron Microscopy (SEM) analysis**

The surface morphology and crystalline structure of complexes are examined using scanning electron microscopy, the visual data show totally different morphology for complexes as shown in Fig. 6.

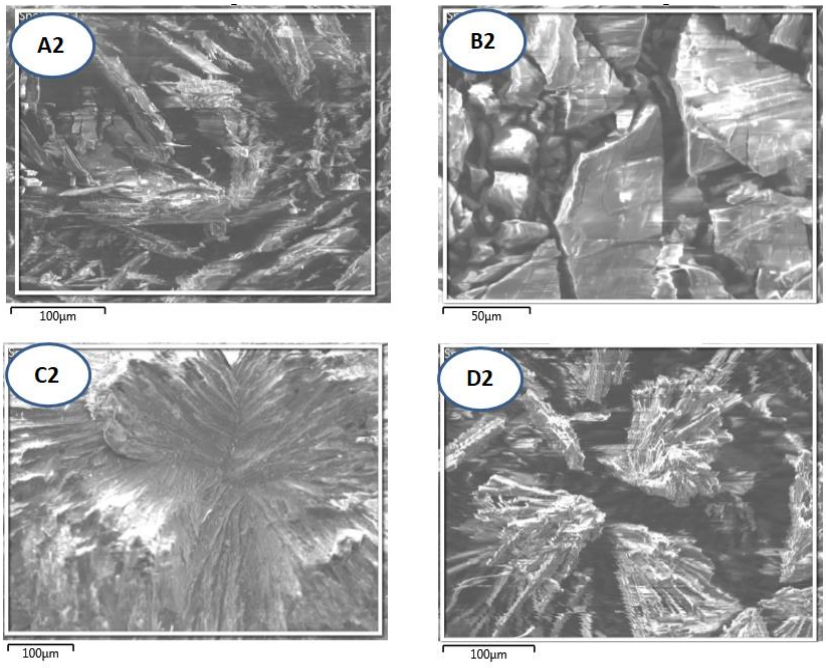


**Figure 6: SEM images of complexes**

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**Binding with DNA**

The efficiency of DNA binding with the complexes with DNA was monitored using spectroscopic and physical methods.

**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 molecule is added to the DNA [19]. 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.

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].

The samples were scanned from 200 nm to 600 nm. All the ligands and the complexes show intense absorption band at high energy region to π -π* transition at 310 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 Figs. 7 and 8.

Upon addition of DNA to the ligands or complexes, the absorption intensity at 310 nm decreases with increases the added amount of DNA (10 μM-120 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].

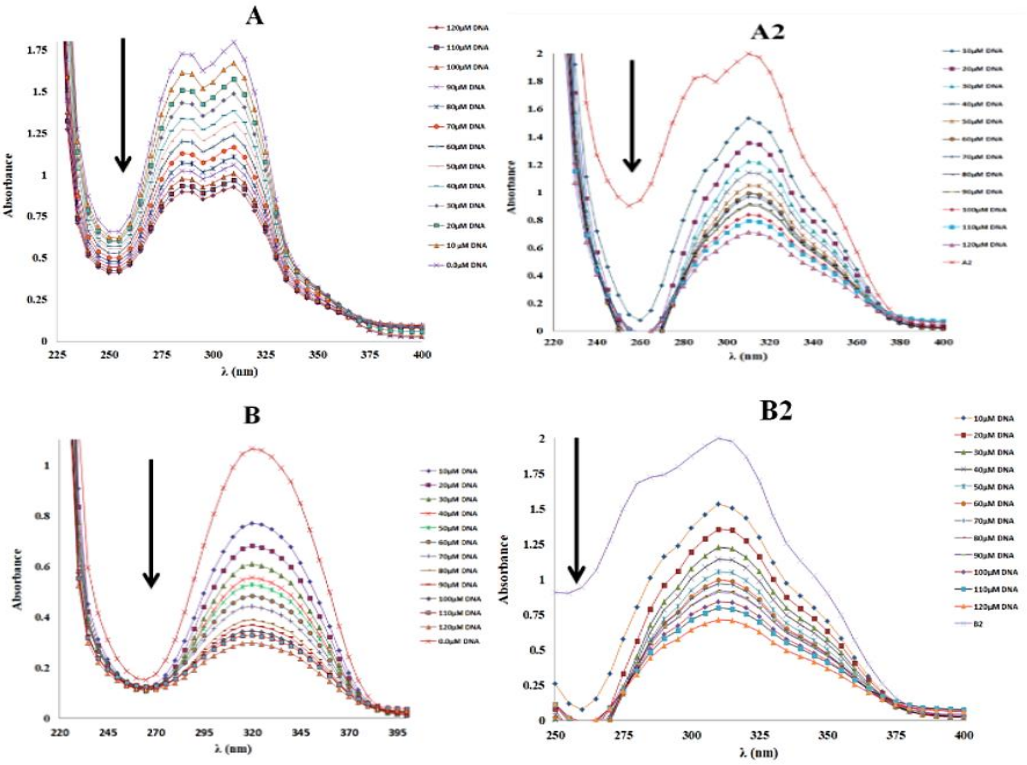


**Figure 7: Electronic absorption spectra of A and B (Left), and their complexes A2 and B2 (Right) in presence of DNA. The arrows show the changes in absorbance upon increasing amounts of DNA.**

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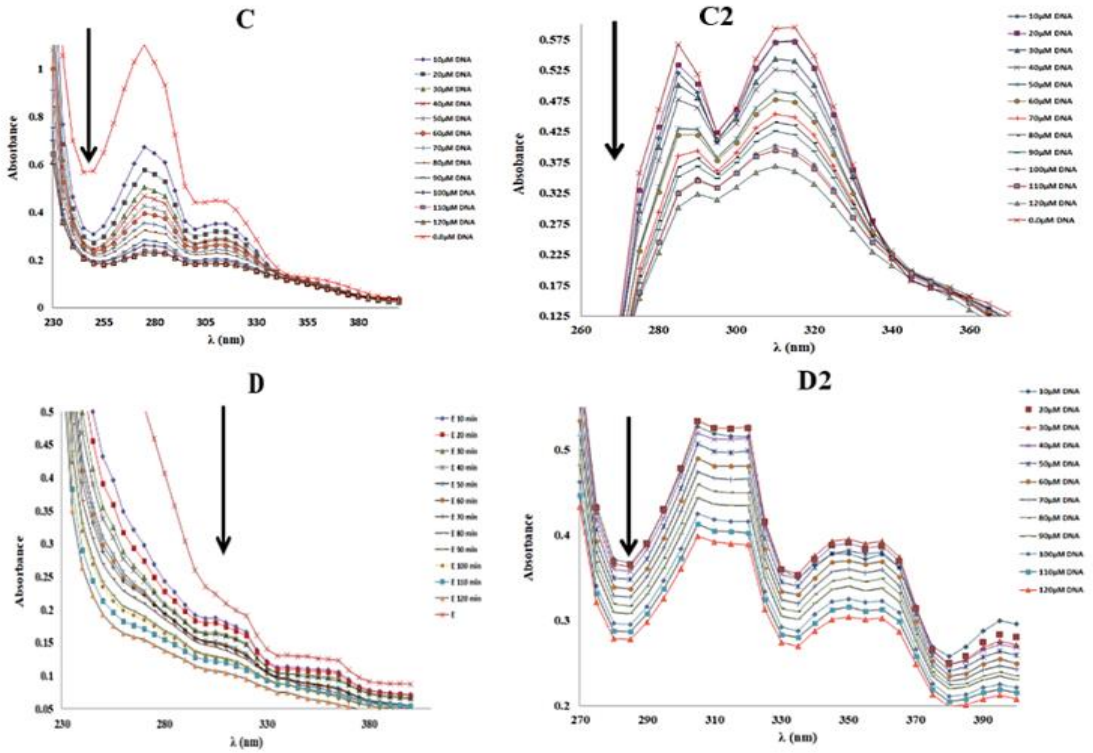


**Figure 8: Electronic absorption spectra of C and D (Left), and their complexes C2 and D2 (Right) in presence of DNA. The arrows show the changes in absorbance upon increasing amounts of DNA.**

The binding strength was evaluated by calculating the intrinsic binding constant *Kb* 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 $\varepsilon a$, $\varepsilon f$, and $\varepsilon b$ 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 $[DNA]/(\varepsilon a - \varepsilon f)$ vs $[DNA]$. The obtained values of *Kb* are scheduled in Table 1.

**Table 1: Binding constants ($M^{-1}$) of ligands and complexes with DNA**

| Ligand | *Kb* ($M^{-1}$) | Complex | *Kb* ($M^{-1}$) |
|---|---|---|---|
| **A** | $5.87 \times 10^4$ | **A2** | $7.85 \times 10^5$ |
| **B** | $8.44 \times 10^4$ | **B2** | $8.25 \times 10^5$ |
| **C** | $5.29 \times 10^4$ | **C2** | $7.16 \times 10^5$ |
| **D** | $8.87 \times 10^4$ | **D2** | $9.55 \times 10^5$ |

The binding constant values show that the binding strength of ligands is improved when they coordinated with $MO_2^{+2}$ and are in order: D2 > B2 > A2 > C2, as exposed in Scheme 3.2.

The binding strength of compounds with DNA depends on lipophilic properties which increase the affinity between the compound and DNA, this affinity depends on the lipophilicity extent that possess by ligands before and after coordination. The extent of lipophilicity depends on lipophilic properties provided by substituents that located in the ligand and that binding with metal ion in coordination sphere. Accordingly, the ligands A, B and D have substituent group in respective of $OCH_3$, Cl and $C_4H_6$ compared to C. Therefore, the binding strength attributed to these substituents, which increases the lipophilic properties in order; $C_4H_6$ > Cl > $OCH_3$, so the ligand D slides easily between the nitrogenous bases compared to other ligands following by the ligand B, A and then C.

The complexation of ligands with $MO_2^{+2}$ increases the binding strength which due to the presence additional $CH_3$ group from the participating solvent molecule in coordinating field of Mo like in A2, C2 and D2, or $C_2H_5$ like B2.

On the other hand, the binding strength depends on the geometrical conformation changes that take place upon complexation. All the complexes show the distorted octahedral geometry and geometrical conformation changes that accompany this geometry are provide an extent of planarity that participate with increasing the binding strength because it increases an intercalation through the nitrogeneous bases of DNA.

**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. 9. 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.

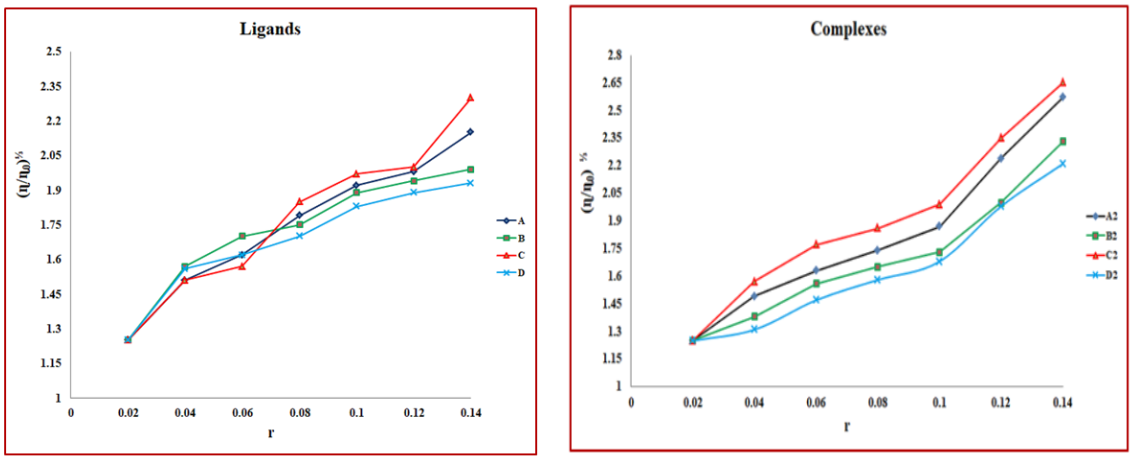


**Figure 9: Effect of increasing amounts of ligands and complexes on the relative viscosities of DNA in tris-HCl buffer solution.**

## CONCLUSIONS

Four dioxo-molybdenum complexes of A2, B2, C2 and D2 were prepared from the condensation MO2(acac)2 with ligands derived from semicarbazide with 3-methxy salicyaldehyde (A), 5-chloro salicyaldehyde (B), salicyaldehyde (C) and 2-hydroxy naphthaldehyde (D). The activity of synthesized ligands and complexes against cancer tumor via interaction with DNA was investigated using spectroscopic and physical methods. The results are revealed the intercalation binding with DNA. The obtained binding constants values are indicated that D and D2 are the strongest intercalators compared to other. The binding strength of the other complexes was revealed in order B2 > A2 > C2. The binding strength of the ligands was revealed in order D > B > A > C.

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

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