2-(2-(2-Hydroxybenzyliden) Hydrazinyl)-2-Oxo-N-(Pyridine-2-Yl) Acetamide Complexes: Synthesis, Characterization and Biological Studies

Yasmeen Gaber Abou El-Reash, Rania Zaky* and Mahmoud Abbas Yaseen
Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt

Abstract

2-(2-(2-hydroxybenzyliden) hydrazinyl)-2-oxo-N-(pyridine-2-yl) acetamide complexes of Ni(II) and Co(II) prepared. The proposed structures proved based on elemental, DFT, and spectral analysis. The DNA binding affinity and MIC activity against Gram-positive, Gram-negative bacteria, pathogenic C. albicans and A. flavus fungal strain tested.

Keywords: Hydrazones; DFT; DND; Potentiometry

Introduction

Hydrazones are versatile ligands and possessing an azomethine -NHN=CH- proton groups. They can be described by the following general structure R,C = NNR. Hydrazone ligands and their metal complexes were attracted many authors because of their varied applications in biological, pharmaceutical, analytical, catalytic [1-5] and industrial fields. Hydrazones and their coordination compounds play important roles in treatment of different diseases. Hydrazones considered as a significant class of compounds with wide importance due to their various biological applications such as anti-convulsant, analgesic, anti-inflammatory, antidepressant, antimalarial, antiplatelet, antitumor, antimicrobial, antiviral, antitumor, antidiabetic, vasodilator, anti-HIV, anthelminthic, and trypanocidal activities [6-14]. This work targets the synthesize and characterization Co(II) and Ni(II) complexes of 2-(2-(2-hydroxybenzyliden) hydrazinyl)-2-oxo-N-(pyridin-2-yl) acetamide (H$_2$L). The geometry and modes of chelation of complexes were discussed based on the resulted (DFT) quantum calculations, the magnetic moment, the different spectroscopic methods (H and $^{13}$C-NMR, UV-visible, IR, EI-mass). Moreover, the thermal decomposition steps were studied and both the kinetics and thermodynamic factors were determined using Coats-Redfern and Horowitz-Metzger models. In addition, potentiometric titrations were done in 50% DMSO-water mixture at various temperatures (298, 308 and 318 K) respectively. Moreover, their Minimum inhibitory concentration (MIC) and DNA-binding affinity assay were tested.

Experimental Methods

Instrumentation

(C, H and N) percent presented in the prepared ligand (H$_2$L) and complexes were detected using a Perkin–Elmer 2400 series II analyzer, while chloride and metal contents determined using standard methods reported previously [15]. A thermogravimetric analyzer (TGA-50H) from Shimadzu, Japan, used for both thermogravimetric (TGA) and differential thermal analysis (DTA) measurements with a heating rate of 10°C/min on at temperature range (20-800°C) and nitrogen flow rate of 15 ml/min. A Sherwood Magnetic Balance was utilized to measure the magnetic susceptibility of solid complexes. A Mattson 5000 FTIR spectrophotometer was used to analyze the prepared ligand and complexes under range of (4000-400 cm$^{-1}$) in KBr discs. While; the electronic spectra of complexes (in DMSO solution) was recorded using a Perkin Elmer Lamda 25 UV/Vis Spectrophotometer. $^1$H, $^{13}$C-NMR measurements were done on Mercury and Gemini 400 MHz spectrometer at room temperature in d$_6$-DMSO. pH meter HANNA-8519, Italy used in all pH- metric measurements.

Synthesis

Preparation of ligand (H$_2$L)

Preparation of ethyl 2-oxo-2-(pyridin-2-ylamino) acetate: Ethyl 2-oxo-2-(pyridin-2-ylamino) acetate were prepared by adding of diethyl oxalate (1 mmol) dissolved in xylene to 2-amino pyridine (1 mmol) dissolved in xylene with stirring followed by reflux with stirring for 3 hr. Let the resulted product to cool then filtered off, washed by ether and at the end dried over anhydrous calcium chloride in a vacuum desiccator. The product is yellow color powder with m.p. (180°C).

Preparation of 2-hydrazinyl-2-oxo-N-(pyridin-2-yl) acetamide: 2-hydrazinyl-2-oxo-N-(pyridin-2-yl) acetamide were prepared by adding of hydrazine hydrate (1 mmol) dissolved in xylene to ethyl 2-oxo-2-(pyridin-2-ylamino) acetate (1 mmol) dissolved in xylene with stirring followed by reflux with stirring for 3 hr. Let the resulted product to cool then filtered off, washed by ether and at the end dried in a vacuum desiccator over anhydrous calcium chloride. The resulted ligand is a yellow color powder with m.p (195°C).

Preparation of ligand (H$_2$L): 1:1 molar ratio of 2-hydrazinyl-2-oxo-N-(pyridin-2-yl) acetamide and 2-hydroxybenzaldehyde (salicylaldehyde) were mixed in a hot ethanolic solution with few drops of glacial acetic acid. The mixture was refluxed for 4 h under magnetic stirring. The formed products were separated by filtration, and then recrystallized from ethanol absolute. Finally, the resulted ligand was dried for 36 h in a vacuum desiccator, then investigated by TLC, elemental analysis (C, H and N), and spectroscopic methods (IR, UV-Vis., $^1$H NMR, $^{13}$C NMR and EI-mass).

Preparation of solid complexes: All the complexes were prepared by refluxing 1 mmol of ligand under investigation with 1 mmol of the metal salt, NiCl$_2$.6H$_2$O, and CoCl$_2$.6H$_2$O in an ethanolic solution on a water bath for 2-3 h. The resulting solid complexes filtered off, washed several times with absolute ethanol and finally dried.

*Corresponding author: Rania Zaky, Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt, Tel: +20502383781; E-mail: rania.zaky@yahoo.com

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Molecular modelling

Cluster calculations were evaluated using DMOL3 program [16] in Materials Studio package [17]. This program designed for the calculations of density functional theory (DFT) over a large scale. Moreover, DFT method was applied to calculate the semi-core pseudopods (dssp) by using the double numerical basis sets plus polarization functional (DPN). Delley et al. revealed that the DNP basis sets are more precise than Gaussian basis sets of the same size [18,19]. Lately, the RPBE basis sets are the best exchange-correlation functional [20,21]. It utilized for the determination of both the exchange and correlation effects of electrons based on the generalized gradient approximation (GGA). The geometric design predicted without any symmetry restriction.

Results and Discussion

Infrared and $^1$H, $^{13}$C NMR, mass spectra of $\text{H}_2\text{L}$ and its metal complexes

The Infrared spectrum of $\text{H}_2\text{L}$ (Structure 1) shown seven peaks at 3345, 3320, 3266, 1708, 1672, 1607 and 785 cm$^{-1}$ which attributed to $\nu$(OH) [8,26], $\nu$(NH), $\nu$(C=O). $\nu$(C=O)$_2$ [27] $\nu$(C=O)$_2$, $\nu$(C=O)$_3$, $\nu$(C=O)$_4$. The $^1$H-NMR spectrum of $\text{H}_2\text{L}$ and its metal complexes $\text{M}^+\text{H}_2\text{L}$ was removed under vacuum, and 200 µl of the DNA/methyl green solution were added to all tubes. All samples were incubated for 24 h in the dark at ambient temperature, and then the absorbance values for the samples were evaluated at 642.5-645 nm. Reading values were corrected per the initial absorbance of the untreated standard [25].
The $^{13}$C NMR spectrum of $\text{H}_2\text{L}$ was recorded in DMSO (Figure 3). The signals for the (C=O)$_2$, (C=O)$_2$, and (C=N) were displayed at downfield position (158.6, 162.7), and (149.8), respectively [27,28].

In the IR spectra of Ni(II) and Co(II) complexes (Structures 2 and 3), $\text{H}_2\text{L}$ behaves as binegative tetradentate via (C=N)$_2$, both (C=O) enolized with deprotonation and (OH) phenolates. This suggestion indicated by:

i) $\nu$(C=N) shifted to a lower wavenumber.

ii) $\nu$(C=O) disappeared with simultaneous appearance of new bands attributed to $\nu$(C=N') and $\nu$(C-O) [29-32].

iii) $\nu$(OH) shifted to a higher wavenumber.

iv) New bands appeared at (513 and 541) and (454 and 459) cm$^{-1}$ which attributable to (M-O) and (M-N) [26], respectively.

### Magnetic properties and electronic spectra

The functions for all the spectral bands for the prepared ligand and its complexes in DMSO and the magnetic moments are compiled (Table 1). The ligand ($\text{H}_2\text{L}$) showed two main absorption bands at 32787 and 27473 cm$^{-1}$ assigned to $\pi-\pi^*$ and one obvious band at 25510 cm$^{-1}$ attributed to $\pi-\pi^*$ of both C=O and C=N groups [33,34].

For $\text{[Co(L)(H}\_2\text{O)}])\cdot2\text{H}_2\text{O}$ complex, two bands were observed at 14084 and 17136 cm$^{-1}$ assignable to $^3T_1(F)\rightarrow^1A_2(F)(\nu_v)$ and $^3T_1(F)\rightarrow^1T_2(P)(\nu_v)$ transitions respectively, which agree with the high spin octahedral Co(II) [35,36]. Moreover, the ligand field parameters, $D_q$, $B$ and $\beta$ (964, 916 and 0.99) can be considered as an evidence for the proposed geometry. As well, the value of the magnetic moments ($\mu_{\text{eff}}=5.04$ BM) was consistent with the proposed octahedral geometry.

Furthermore, in $\text{[Ni(L)(H}\_2\text{O)}])\cdot2\text{H}_2\text{O}$ complex; two bands appeared at 17391 and 26667 cm$^{-1}$ that are assignable to $^3A_2(F)\rightarrow^3T_1(F)(\nu_v)$ and $^3A_2(F)\rightarrow^1T_2(P)(\nu_v)$ transitions. These transitions are distinguishing for the octahedral Ni(II) complexes [37] and the calculated ligand field factors, $D_q$, $B$ and $\beta$ (1067, 762 and 0.73) and magnetic moments value ($\mu_{\text{eff}}=3.3$ BM) support the supposed geometry. The position of $\nu_v$(7902 cm$^{-1}$) was calculated theoretically [28].

### Thermogravimetric studies

The TGA and DTA curves for the decomposition of Ni(II)-complex were depicted in Figures 4 and 5. The obtained results approved the proposed formulae. Where, the complex decomposed in three main...
steps. The primary step implied losing hydrated water molecules at 40-
76°C, followed by the losing coordinated water at 76-200°C. Then, the
deligation began at a temperature range of 200-800°C and at the end
metal oxide was formed.

**Kinetic data:** The kinetic parameters were calculated by using
non-isothermal methods of decomposition steps. The rate of degradation
(da/dt) is a linear function of rate constant (k) and function of
conversion (α) and can be expressed as follow [a]:

\[
\frac{d\alpha}{dt} = k(T)f(\alpha)
\]  

(1)

k can be calculated by the Arrhenius equation:

\[
k = Ae^{(-E/RT)}
\]  

(2)

Where R is the gas constant, E is the activation energy and A is the
pre-exponential factor.

By substituting Eq. (2) into Eq. (1):

\[
\frac{d\alpha}{dt} = A \int_{0}^{\infty} e^{-E/RT} d\alpha
\]  

(3)

When the temperature varied by a constant and controlled heating
rate, \(\Phi=dT/dt\), the change in degree of conversion which is a function of
temperature dependent also on time of heating. Therefore, Eq. (3) becomes:

\[
\frac{d\alpha}{dt} = A \int_{0}^{\infty} e^{-E/RT} d\alpha
\]  

(4)

By integrating Eq. (4):

\[
g(\alpha) = \int_{0}^{\infty} f(\alpha) \frac{d\alpha}{e^{E/RT}} = \frac{A}{\Phi} e^{E/RT} \int_{0}^{\infty} f(\alpha) e^{-E/RT} d\alpha
\]  

(5)

Where \(g(\alpha)\) is the integrated form of the conversion dependence
function? The right-hand side integral of Eq. (5) known as temperature
integral; has no closed form solution and can be evaluated by Coats-
Redfern (CR) method (Figure 6) [38] and the approximation method
of Horowitz-Metzger (HM) (Figure 7) [39].
Table 2: Kinetic Parameters evaluated by Horowitz-Metzger and Coats-Redfern equations for Ni(II) and Cu(II) complexes of H$_2$L.

| Compound                        | Step | Mid Temp. (K) | Method | E$_a$ | A | $\Delta H^*$ | $\Delta S^*$ | $\Delta G^*$ |
|---------------------------------|------|---------------|--------|-------|----|--------------|--------------|--------------|
| [Ni(L$_2$)(H$_2$O)$_2$]2H$_2$O   | 1st  | 334.08        | HM     | 203.82| 1.09 $\times 10^{23}$ | 201.04      | 0.32915      | 91.08        |
|                                 |      |               | CR     | 200.87| 3.85 $\times 10^{23}$ | 198.09      | 0.32053      | 91.01        |
|                                 | 2nd  | 457.82        | HM     | 26.02 | 1.43 | 22.21        | -0.24548     | 134.60       |
|                                 |      |               | CR     | 17.82 | 2.18 $\times 10^{21}$ | 14.01       | -0.26112     | 133.56       |
|                                 | 3rd  | 599.12        | HM     | 859.55| 2.49 $\times 10^{23}$ | 854.57      | 1.15437      | 162.96       |
|                                 |      |               | CR     | 848.81| 2.91 $\times 10^{23}$ | 843.83      | 1.13652      | 162.92       |

Table 3: The association constants of H$_2$L in 50% (v/v) DMSO-water, 0.05 M KCl and at different temperature.

| Ligand | 288 K | 308 K | 318 K |
|--------|-------|-------|-------|
| H$_2$L | pK$_a$ | pK$_b$ | pK$_a$ | pK$_b$ | pK$_a$ | pK$_b$ |
|        | 9.95  | 6.94  | 9.20  | 6.76  | 9.11  | 6.24  |

From the obtained results:

i) All kinetic parameters ($E_a$, $A$, $\Delta H^*$, $\Delta S^*$ and $\Delta G^*$) for all prepared solid complex were calculated by CR and HM method (Table 2). Both methods gave comparable values.

ii) For all complexes; decomposition stages fitted better when ($n=1$) suggesting a 1st-order decomposition process. Other $n$ values (eq. 3 and 4) did not show better correlations.

iii) The value of $\Delta G$ increases for complexes because while going from one decomposition step to another; the rate of H$_2$L removal will be lower [40,41]. This can be due to, the rigidity of remaining complex after the explosion of one or more H$_2$L molecules.

iv) The values of the entropy ($\Delta S^*$) for the decomposition steps of complexes show that the activated fragments have more ordered (negative values) or disordered (positive values) structure than the undecomposed complexes and/or the decomposition reactions are slow [38].

v) The positive value of $\Delta H^*$ means the endothermic nature of the decomposition processes.

Generally, the values of stability constants decrease with increasing the number of H$_2$L atoms attached to the metal ion [42,43]. Therefore, an opposite effect may occur during the decomposition process. Hence, the rate of removal of the remaining H$_2$L will be lower than that of the rate before the explosion of H$_2$L.

**Potentiometric studies**

**Proton-ligand system:** Irving-Rossotti equation used to calculate the average number of protons associated with the ligand ($n_1$) at different pH-values.

\[ \log a_{\text{H}^+} = Y + \frac{(V_0 - V_1)(N_0 + E^0)}{(V_0 + V_1)\pi C_{\text{H}^+}^{01}} \]

Where $Y$ is the number of ionizable protons in the ligand atom, $C_i$ is the initial ligand concentration, $V_0$ and $V_1$ are the volumes of NaOH needed to adjust the pH in the ligand mixtures, respectively. $V_0$ is the initial volume of the mixture (25 ml), and $N$ and $E$ are the concentration of the alkali and the free acid, respectively. The formation curves plotted between $n_1$ and pH for the proton-ligand systems (Figure 8). It is found that $n_1$ values extend between 0 and 2 indicating that the ligand has two dissociable hydrogen ions of NH$_2$ and NH$_3$ respectively. Also, the dissociation constants, pK$_a$ and pK$_b$ can be obtained directly from these curves by interpolation at $n_1 = 0.5$ and 1.5, respectively. The values recorded in Table 3. Inspection of the table reveals that pK$_b >$ pK$_a$ at the same temperature. In addition, the pK$_a$ values decrease with increasing temperature indicating that acidity increases with decreasing temperature.

**Metal-ligand system:** In the present work, metal ions under study were titrated against NaOH solution. n and pL values were evaluated by Irving and Rossotti.

\[ \log pL = \log \left( \frac{1 + K_1[H^+]}{1 + K_2[H^+]} \right) = \log \left( \frac{1 + K_1[H^+]}{1 + K_2[H^+]} \right) \times \frac{V_0 + V_1}{V_0} \]

The formation of both ML and ML$_2$ type of complexes was...
confirmed by the value of (n) that was found to be ~ 2. The calculated stability constant values summarized in Table 4.

**Distribution curves:** The different protonated forms as H$_2$L, HL$^-$ and L$^2$-$^-$ were detected during the titration processes. The distribution curves of H$_2$L displayed in Figure 9 at 298°K as a symbolic example. It is obvious that all protonated species have a wide protonation range between pH 5-13. While increasing the pH results in losing protons of ligands and conversion to the other forms. The percentage of H$_2$L, HL$^-$ and L$^2$-$^-$ forms are above 95% and the free ligand (L) formed at pH 9.8 and reached its maximum at pH 13.2.

The formed Co(II) complexes formulated as (ML$_2$, ML) were pH dependent. The distribution curves resulted from the calculations shown in Figure 9 at 298°K. Co(II) complexes are formed after pH 4.9, while the intermediate complexes are formed between pH 1.3-5.0.

**The thermodynamic parameters:** The corresponding thermodynamic parameters (standard free energy change, $\Delta G^\circ$ and the standard entropy change, $\Delta S^\circ$) for dissociation and metal complex formation were calculated via the following equations:

$$\Delta G^\circ = -2.303 RT \log K$$

$$\Delta G^\circ = \Delta H^\circ - T \Delta S^\circ$$

The thermodynamic functions are recorded in Tables 5 and 6 and Figures 10 and 11.

Checking these, values show that:

**a) For proton-ligand system:**

i) The positive values of $\Delta G^\circ$ indicate a non-spontaneous dissociation processes.

ii) The negative values of $\Delta S^\circ$ are due to increasing the order because of solvation process. This may be explained as the number of bound of solvent molecules accompanying with the undissociated ligand being less than those accompanying the dissociated form.

iii) The positive values of $\Delta H^\circ$ indicate the endothermic nature of the dissociation of ligand in aqueous solution. The process accompanied by heat absorption indicating the favorability of the process at higher temperatures.

**b) For metal-ligand system:**

i) The negative values of $\Delta G^\circ$ indicate that the spontaneous nature of the chelation process.

ii) The positive values of $\Delta S^\circ$ are owing to the decreasing the order due to solvation process.

iii) The positive values of $\Delta H^\circ$ indicate that the chelation process is endothermic and accompanied by consuming of heat and the chelation process is favorable at higher temperatures.

**Molecular modelling**

The molecular structures of H$_2$L and its complexes shown in Structures 1-3 an insight to the data in Tables 7-13 reveals the following remarks:

i) The bond angles of the hydrazone moiety of H$_2$L changed slightly upon coordination; the largest change affects in H...O(21)-C(17)-C(18), C(18)-C(12)-N(11), C(12)-N(11)-N(10), N(11)-
Table 7: Selected bond lengths (Å) of H$_2$L in using DFT-method from DMOL$^3$ calculations.

| Bond                  | Length (Å) |
|-----------------------|------------|
| O(24)-Ni(22)         | 1.46       |
| N(10)-N(10)          | 1.235      |
| C(9)-C(8)            | 1.524      |

Table 8: Selected bond angles (°) of H$_2$L in using DFT-method from DMOL$^3$ calculations.

| Bond                  | Length (Å) |
|-----------------------|------------|
| C(14)-O(21)          | 1.151      |
| C(9)-C(8)            | 1.516      |
| N(7)-C(8)            | 1.295      |

Table 9: Selected bond lengths (Å) of [Co(L)(H$_2$O)$_2$]H$_2$O in using DFT-method from DMOL$^3$ calculations.

| Bond                  | Length (Å) |
|-----------------------|------------|
| O(24)-Co(22)-Co(23)  | 87.047     |
| O(24)-Co(22)-O(19)   | 164.27     |
| O(24)-Co(22)-N(11)   | 102.689    |
| O(24)-Co(22)-O(20)   | 152.47     |
| O(24)-Co(22)-N(12)   | 77.207     |
| O(24)-Co(22)-C(12)   | 120.74     |
| C(14)-O(21)-Co(22)   | 118.651    |
| C(14)-O(21)-C(13)    | 133.076    |
| C(14)-O(21)-C(12)    | 109.233    |
| C(14)-O(21)-C(13)    | 125.807    |
| C(14)-O(21)-C(12)    | 107.35     |
| C(14)-O(21)-C(13)    | 117.763    |
| C(14)-O(21)-C(12)    | 114.546    |
| C(14)-O(21)-C(13)    | 111.336    |
| C(14)-O(21)-C(12)    | 120.987    |

Table 10: Selected bond angles (°) of [Co(L)(H$_2$O)$_2$]H$_2$O in using DFT-method from DMOL$^3$ calculations.

| Bond                  | Length (Å) |
|-----------------------|------------|
| C(14)-O(21)          | 1.518      |
| C(9)-C(8)            | 1.516      |
| N(7)-C(8)            | 1.151      |

Table 11: Selected bond lengths (Å) of [Ni(L)(H$_2$O)$_2$]H$_2$O in using DFT-method from DMOL$^3$ calculations.

| Bond                  | Length (Å) |
|-----------------------|------------|
| O(24)-Ni(22)-O(23)   | 90.077     |
| O(24)-Ni(22)-N(10)   | 90.57      |
| O(24)-Ni(22)-N(11)   | 90.57      |
| O(24)-Ni(22)-O(20)   | 91.861     |
| O(24)-Ni(22)-O(19)   | 91.09      |
| O(24)-Ni(22)-O(20)   | 88.237     |
| O(24)-Ni(22)-O(19)   | 115.507    |
| O(24)-Ni(22)-O(19)   | 122.718    |
| O(24)-Ni(22)-O(19)   | 123.787    |
| O(24)-Ni(22)-O(19)   | 105.608    |
| O(24)-Ni(22)-O(19)   | 111.993    |
| O(24)-Ni(22)-O(19)   | 121.756    |

Table 12: Selected bond angles (°) of [Ni(L)(H$_2$O)$_2$]H$_2$O in using DFT-method from DMOL$^3$ calculations.
N(10)-C(9), O(14)-C(9)-N(10), O(14)-C(9)-C(8), O(13)-C(8)-N(7) and C(9)-C(8)-N(7) angles. The bond angles in ligand are reduced or increased on complex formation because of bonding [44].

ii) The bond angles in metal complexes are quite near to an octahedral geometry predicting dsp² or spd³ hybridization in all complexes [38].

iii) All the active groups in taking part in coordination have bonds longer than that already exist in the ligand moiety like (C-O) in H₂L and (C-O) phenolic C=N(phenethyl). This is referred to the formation of the M-N bond which makes the C-N bond weaker because of coordination via N atom of (C=N) [45].

iv) The bond lengths of C(9)-N(10) and C(8)-N(7) become slightly longer in complexes as the coordination takes place via N atoms of -C=N-C=N- group that is formed on deprotonation of OH group in all complexes [44].

v) The bond distance of (CO) in H₂L that participate in coordination becomes longer due to the formation of the M-O bond which makes the C-O bond weaker [46] while the phenolic (C-O) that participates in complexes will become longer on coordination.

vi) The bond angles of ligand moiety containing atoms of coordination will be changed in all complexes due to the formation of the N-M-O chelate ring [47].

vii) The arrangement of complexes based on M-O and M-N bond lengths indicates that; the M-N and M-O in Co(II) complex have greater strength than in Ni(II) complex.

viii) The low HOMO energy values indicate the weak electron donating ability of molecules. LUMO energy indicate the ability of molecules to receive electrons [44].

ix) The overlap happens between both HOMO and LUMO is an important factor in all reactions. This can be indicated from the large values of molecular orbital coefficients. So, the orbitals that have the highest molecular orbital coefficients in the ligand can be considered as the sites of coordination. In addition; the energy gap (E_{HOMO}-E_{LUMO}) is a significant stability index that supports the characterization of both kinetic stability and chemical reactivity for the studied molecules [48].

Where molecules with smaller gap are more polarized and known as soft molecule that are more reactive than hard ones because they offer electrons easily to the acceptor. In case of ligand (H₂L); the energy gap is small also due to the groups that enter conjugation. This indicates that charge transfer easily, which influences the biological activity of the molecule [49].

x) DFT method illustrates both the chemical reactivity and site selectivity for all molecular systems. The energies of frontier molecular orbitals (E_{HOMO}, E_{LUMO}), band gap describes the ultimate charge transfer happens within molecules, chemical potential (µ), electronegativity (χ), global softness (S) and global electrophilicity index (ω) are listed in, the inverse value of the global hardness (η) [50,51] are listed in, the inverse value of the global hardness is designed as the softness (s) as follows:

\[
\sigma = 1/\eta
\]

### Biological activity

**Minimum Inhibitory Concentration (MIC):** Ligand and their complexes were evaluated for their antibacterial activity against *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. subtilis*) as an example of Gram-positive bacteria, *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) as examples of Gram-negative bacteria and against a pathogenic *Candida albicans* (*C. albicans*) and *Aspergillus flavus* (*A. flavus*) fungal strain. Antimicrobial and Antimycotic Activities in terms of MIC (µg/mL) in Table 14. The fungicide Colitrimazole and the bactericide Ampicillin were used as references to compare the potency of the tested compounds under the same conditions (Figure 12).

H₂L is the most potent compared with reference compounds against all bacterial and fungal stain. On the other hand, Co(II) complex shows no activity against *E. Coli*. While, it has lowest activity towards *P. aeruginosa*, *S. aureus*, *B. Subtilis*, *C. Albicans* and *A. flavus* with MIC 500, 375, 125, 375 µg/mL, respectively. Ni(II) complex exhibited moderate activity against all bacterial and fungal stain.

The prepared compounds can be arranged according to its activities towards *E. coli*, *C. albicans* and *A. flavus* as follows:

**DNA-binding affinity assay:** It was noticed that; methyl green binds in a reversible manner with polymerized DNA [25], and prepared
complexes are stable in neutral pH medium, though free methyl green declines. In this study, buffer used for displacement reactions and after incubation for 24 hours, complete loss of methyl green absorbance was noticed. The dislodging of methyl green from DNA by studied compounds and the ability to bind to DNA was measured colorimetric. Where; the displacement was detected by the decrease in absorbance at 630 nm [52].

The prepared compounds were exhibited high affinity to DNA as shown in Table 15 and represented graphically in Figure 13, which was confirmed by keeping the DNA-complex at the origin or by migrating for short distances. The most active compounds were, H$_{2}$L and Ni(II)-complex with IC$_{50}$ 27.8 ± 1.7 and 31.6 ± 2.1 µg/ml, respectively. The obtained results agree with the antimicrobial and antifungal screening data. This suggests that; binding with DNA may be contributed to the biological activity of these compounds against bacterial and fungal infections.

IC$_{50}$ values (mean ± SD, n=3–5 separate determinations), exemplify the concentrations needed for a 50% decrease in the initial absorbance the DNA/methyl green solution.

**Conclusion**

The hydrazone derived from the condensation 2-hydrazinyl-2-oxo-N-(pyridin-2-yl) acetamide to 2-hydroxybenzaldehyde (salicylaldehyde) and its Co(II) and Ni(II) complexes were produced. IR spectra suggest that the H$_{2}$L and its Co(II) and Ni(II) complexes were produced. H$_{2}$L and its metal complexes.

Table 15: DNA/methyl green IC$_{50}$ µg/ml of H$_{2}$L and its metal complexes.

| Compound          | IC$_{50}$ µg/ml |
|-------------------|-----------------|
| H$_{2}$L          | 27.8 ± 1.7      |
| Co(II)-complex    | 69.1 ± 3.8      |
| Ni(II)-complex    | 31.6 ± 2.1      |

Figure 13: DNA/methyl green IC$_{50}$ µg/ml of H$_{2}$L and its metal complexes.

The prepared compounds were exhibited high affinity to DNA as shown in Table 15 and represented graphically in Figure 13, which was confirmed by keeping the DNA-complex at the origin or by migrating for short distances. The most active compounds were, H$_{2}$L and Ni(II)-complex with IC$_{50}$ 27.8 ± 1.7 and 31.6 ± 2.1 µg/ml, respectively. The obtained results agree with the antimicrobial and antifungal screening data. This suggests that; binding with DNA may be contributed to the biological activity of these compounds against bacterial and fungal infections.

IC$_{50}$ values (mean ± SD, n=3–5 separate determinations), exemplify the concentrations needed for a 50% decrease in the initial absorbance the DNA/methyl green solution.

**Conclusion**

The hydrazone derived from the condensation 2-hydrazinyl-2-oxo-N-(pyridin-2-yl) acetamide to 2-hydroxybenzaldehyde (salicylaldehyde) and its Co(II) and Ni(II) complexes were produced. IR spectra suggest that the H$_{2}$L coordinates as binegative tetratadrate via (C=N)$_{2}$ both (C-O)$_{2}$ enolized with deprotonation and (OH)$_{2}$ physisorbed. The proposed geometries of isolated complexes were proved using DFT. The pH-mentrically at different temperatures in 50% DMSO-water mixture was applied to estimate the dissociation constant of the ligand and the stability constants of the Co(II) metal ions. H$_{2}$L shown the highest DNA binding affinity and minimum inhibitory concentration (MIC) activity than complexes.

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