Synthesis, DFT calculation, DNA-binding, antimicrobial, cytotoxic and molecular docking studies on new complexes VO(II), Fe(III), Co(II), Ni(II) and Cu(II) of pyridine Schiff base ligand

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Abstract

A new H2L ligand (6, 6′-(pyridine-2, 6-diylbis(azan-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene)bis(2-ethoxyphenol) was obtained from the reaction of 2, 6- diamino pyridine and 3- ethoxy salicyaldehyde in 1:2 molar ratio and fully characterized using elemental analyses and spectroscopic tools. The reaction of a ligand with the different metal salts yields five new complexes CuL, CoL, NiL, FeL, and VOL. The new complexes were identified according to the results of elemental analyses, IR and UV–vis spectra, magnetic moment, molar conductance and thermal analyses (TGA). From the conductivity data, it is deduced that all complexes are non-electrolytes. FT-IR spectra displayed that the Schiff base ligand coordinated to the metal ions in a manner with ONNO donor sites, the nitrogen atom of the pyridine ring, the nitrogen of one azomethine group and two oxygen atoms of the two phenolic OH groups. The results of electronic spectra and magnetic susceptibility confirmed octahedral geometry of FeL complex and square pyramidal geometry for CoL, CuL and VOL and square planar for NiL. The electronic structure of H2L ligand and its complexes were investigated theoretically at the DFT-B3LYP-311 G** level of the theory. FT-IR spectra displayed that the Schiff base ligand coordinated to the metal ions in a manner with ONNO donor sites, the nitrogen atom of the pyridine ring, the nitrogen of one azomethine group and two oxygen atoms of the two phenolic OH groups. The results of electronic spectra and magnetic susceptibility confirmed octahedral geometry of FeL complex and square pyramidal geometry for CoL, CuL and VOL and square planar for NiL. The electronic structure of H2L ligand and its complexes were investigated theoretically at the DFT-B3LYP-311 G** level of the theory. FT-IR spectra established the involvement of pyridine nitrogen in the coordination process. The presence of coordinated water molecules inside the coordination sphere of the complexes CoL, CuL and FeL are supported by TGA studies. The antimicrobial activities of the ligand and its complexes were determined against two Gram-positive bacteria (B. subtilises and S. aureus) and two Gram-negative (E. coli and P. vulgaris) and two fungus (Candida albicans and Aspergilla’s fumigates). The results showed that the complexes behaved as better antimicrobial agents than the ligand, VOL complex shows exceptional antimicrobial efficacy. The order of increasing the antibacterial and antifungal potency is H2L < NiL < CuL < CoL < FeL < VOL. The binding of the complexes with CT-DNA was followed using electronic absorption, viscosity and gel-electrophoresis measurements. These studies confirmed that the complexes bind to CT-DNA through a groove binding mode with certain affinities (Kb = 6.25 × 10^5, 5.50 × 10^5, 3.20 × 10^5, 2.50 × 10^5 and 1.52 × 10^5 for CuL, FeL, VOL, NiL, and CoL respectively). Moreover, cytotoxic effect against hepatocellular carcinoma cell line (HEP-G2) was screened. The IC50 values of the ligand and complexes suggest that the compounds possess very good cytotoxic activity and follow the order: CuL > FeL > VOL > NiL > CoL > H2L. These results strongly agree with results of binding constant of CT-DNA with different metal complexes.

1. Introduction

Over the years, the chemo-coordination of Schiff bases resulting from carbaldehyde has received more and more attention. This is mainly due to its wide application in the fields of pharmaceutical industry [1, 2]. Many research
papers reported that Schiff bases and their complexes possess exceptional antibiotic [3], antimicrobial [4], anticancer [5], and biological activities [6, 7].

Heterocyclic compounds, such as pyridine and related compounds are potent ligands due to the existence of one or more ring nitrogen with a localized pair of electrons. Their efficacy as pathogens of bacteria and fungi were enhanced once coordinated with metal ions [8]. Hodnett et al. [9] have pointed out that the carbonyl part of the Schiff bases takes an important part in antitumor activity. They have also observed that antitumor activity is elevated by the insertion of an electron withdrawing group in the benzene ring near to the azomethine group such as methoxy and ethoxy groups. For this reason, the coordination chemistry of Schiff bases based on carbaldehyde and their complexes has drawn plenty of attention [10, 11]. Among the metal complexes, vanadium, iron, cobalt, nickel and copper complexes have been shown to exhibit significant biological activities [12]. Since large number of anti-cancer drugs could cause damage to the DNA and influence the proliferation, transfer, and diffusion of cancer cells, there is an urgent demand to develop new chemothapeutic drugs [13, 14]. Several papers have reported that the DNA binding modes could be classified into intercalation, groove-force or external electrostatic [15].

Therefore, the motivation behind the present research is to fill the void left in this area due to the lack of studies related to Schiff base ligands resulting from 2, 6-diaminopyridine and 3-ethoxysalicylaldehyde, this has promoted us to shed light on a new tetradentate Schiff base ligand H2L, namely (6, 6'-pyridine-2, 6-diylbis (azan-1-yl-1-ylidene)bis(methan-1-yl-1-ylidene)bis(2-ethoxyphenol). The new ligand was prepared by a reaction of 2, 6-diaminopyridine which was chosen as a heterocyclic amine ligand with 3-ethoxysalicylaldehyde which contains electron withdrawing ethoxy groups. Considering the coordination with metal ions improved markedly the biological activities, new complexes of VO(II), Fe(III), Co(II), Ni(II), and Cu(II) had been synthesized and identified with physicochemical and spectral approaches. The structures of the new compounds are confirmed via thermal and DFT studies.

2. Experimental

2.1. Instrumentation
The new compounds were characterized using the following instruments; FTIR Shimadzu (8300) which was used to plot the spectra of all new compounds as KBr disc, Bruker Advance DPX-500 spectrometer was used to record 1H NMR and 13C NMR spectra, UV–visible spectra, and DMF was measured on a UV–vis spectrophotometer. The CHNM elements were analyzed using PerkinElmer 240c. Shimadzu TG-60H thermal analyzers were used to follow the thermal degradation of the compounds. Magnetic susceptibility measurements were conducted using a Gouy balance [16, 17]. Mass spectra were recorded using DI analysis Shimadzu Qp-2010 plus.

2.2. Synthesis of the new pyridine based H2L ligand
H2L was obtained by the addition of (1 mmol, 0.10 g) of 2, 6- diamino pyridine dissolved in absolute ethanol to (2 mmol, 0.32 g) of 3-ethoxysalicylaldehyde in an absolute ethanol (25 ml). The mixture refluxed for 1 h and kept to cool (cf scheme 1), during which a yellowish green solid compound was separated. It was then filtered, washed, recrystallized from diethyl ether and dried in vacuum.

2.3. Synthesis of the new metal complexes
The new 1:1 complexes were obtained by the addition of (1 mmol, 0.405 g) of the H2L ligand dissolved in (25 ml) of ethanol to (1 mmol) from different metal salts [CuCl2·2H2O (0.17 g), CoCl2·6H2O (0.23 g), NiCl2·6H2O (0.23 g), FeCl3·6H2O (0.27 g), VO(AC)2 (0.265 g)] in 25 ml of ethanol in presence of 1 mmol of triethylamine (cf scheme 1). The resulting mixture is heated for 1–4 h. After that the solution was evaporated overnight. The residue has precipitated, washed well with 20 ml of ethanol and dried instantly under vacuum.

2.4. Computational methods
All the calculations including geometry optimization were performed using the Gaussian 09 W program [18].

2.5. Antibiotic screening of the new compounds
The antibacterial bioassay of the new compounds was screened against different bacteria species (Bacillus subtilis (+ve), Escherichia coli (-ve) and Staphylococcus aureus (+ve)). The agar method dilution was used as described in [19, 20]. The antifungal activity was also studied against (Candida albicans, Aspergillus flavus and Trichophyton Rubrum) in well diffusion methods. Using potato dextrose agar as environment details are depicted in [19–21].
2.6. CT-DNA Binding analysis of the new compounds
Tris–HCl buffer (60 mM, pH = 7.2) used for dissolving protein free CT-DNA by sonication and stored at 4°C.

2.6.1. Electronic spectroscopy
The concentration of the new complexes was kept constant, whereas CT-DNA concentration was changed. A proper amount of CT-DNA added to the new complexes solutions for eliminating the absorption of CT-DNA itself. The intrinsic binding constant ($K_b$) estimated the $[\text{DNA}] / (\varepsilon_a - \varepsilon_b)$ plot versus $[\text{DNA}]$ regarding following relation [21, 22]:

$$
\frac{[\text{DNA}]}{(\varepsilon_a - \varepsilon_b)} = \frac{1}{[\text{DNA}]} \frac{1}{\varepsilon_b - \varepsilon_f} + \frac{1}{K_b \left(\varepsilon_b - \varepsilon_f\right)}
$$

Whereas $[\text{DNA}]$ the molar concentration of CT-DNA, in base pairs $\varepsilon_b, \varepsilon_a$ and $\varepsilon_f$ are the extinction coefficients of free apparent and fully bound complex, respectively. ($\Delta G^\circ_b$) The Gibbs standard free - energy was calculated using the equation (2) [21, 22]

$$
\Delta G^\circ_b = -RT \ln K_b
$$

2.6.2. Viscosity experiments
Oswald micro viscometer is utilized to perform viscosity experiments at ambient temperature. In addition, stopwatch was used to measure the fluidity times at varied concentrations of the as-prepared metal-imine complex from 10 to 60 μM, making the DNA concentration unchanged at 50 μM [22, 23]. The measured data was plotted as $(\eta/\eta^\circ)$ as ordinates versus [complex]/[DNA] as abscissa where $\eta$ is the CT-DNA viscosity in the existence of the new metal complexes and $\eta^\circ$ is the CT-DNA viscosity that is isolated. The relation $\eta = (t - t^\circ)$ is utilized for determination of viscosity values.

2.6.3. Gel electrophoresis
The DNA binding products were tested by agarose gel electrophoresis method. The stock solution of complexes was prepared by dissolving 20 mg of the compounds in 20 ml of DMF. The sample (25 μg ml$^{-1}$) was added to the isolated DNA of Calf-thymus (CT-DNA) and incubated at 37 ± 1°C for 1 h and then 30 μl of DNA sample (mixed with bromophenol blue dye at a 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with a standard DNA marker in TBE buffer (50 mM Tris base, pH 7.5; 1 mM EDTA/1 L) and finally
Table 1. Analytical and physical data of the new H2L Ligand and its new Cu(II), VO(II), Fe(III), Co(II) and Ni(II) complexes.

| Compound (Empirical formula) | M. wt. (g) | (M. p) °C | \( \Lambda m \) (\( \Omega \cdot \text{cm} \)) \(^{-1} \) cm \(^{-1} \) mol \(^{-1} \) | \( \mu_{\text{eff}} \) B.M. | Elemental analyses calculated% | Elemental analyses found% |
|-----------------------------|-----------|-----------|---------------------------------|----------------|-------------------------|-------------------------|
| H2L(C23H2O4N3)              | 405.00    | 165       | 0.00                            | 0.01           | 68.14                  | 68.20                   |
| CuL(C23H2O4N3Cu)            | 502.50    | >300      | 5.69                            | 2.01           | 54.92                  | 54.85                   |
| VO(C23H2O4N3V)              | 469.9     | 295       | 4.43                            | 1.80           | 58.73                  | 58.68                   |
| FeL(C23H2O4N3Fe)            | 548.3     | >300      | 12.67                           | 5.35           | 50.34                  | 50.38                   |
| NiL(C23H2O4N3Ni)            | 461.6     | 270       | 6.45                            | 3.34           | 59.79                  | 59.70                   |
| CoL(C23H2O4N3Co)            | 497.9     | >300      | 8.11                            | 3.81           | 55.43                  | 44.49                   |

loaded onto the agarose gel, then a constant electricity (60 V) was applied for 45 min. Finally, the gel was removed and stained with 20 micrograms ml \(^{-1} \) of ethidium bromide for 10-20 min. The bands obtained was observed under UV light using a transilluminator then photographed with DMC-LZ5 Lumix Digital Camera to determine the extent of DNA binding as compared with standard DNA marker [20—22].

2.7. Molecular docking

Dell Precision™ T3600 Workstation [Intel Xeon E5-1660 3.3 GHz, 16 GB, 1600 MHz DDR3, ECC RDIMM 1 TB, 7200 RPM, 1 GB NVIDIA Quadro 2000, Windows 7 Professional, 64 Bit], Molecular Operating Environment (MOE) package version 2016.08 and x-ray Crystal Structure of a B-DNA dodecamer d(CGCGAATTCGCG)\(_{2}\) running 3°–5° direction, PDB ID: 1BNA with 1.9 Å resolution were utilized to make docking studies. Depending on MOE, the DNA structure was opened then, hydrogen atoms were inserted and thus, energy optimization is accomplished. Systematic conformational research was applied on resulting model with RMS gradient of 0.01 kcal per mol with the utilization of parameters in the position finder tool in MOE software. The Schiff base ligand (H2L) and its metal complexes) were established in Chem Bio Draw Ultra 12.0. In MOE, the imine ligand and its desired complexes which synthesized for docking by these steps: (i) atoms of hydrogen were inserted, (ii) conformational search was performed on the compounds and (iii) energy minimization was applied on the best conformers by the MMFF94 force field. Also, energy minimization which has the steepest algorithm was utilized thus, running of conjugate gradient way, until an RMS gradient of 0.000 01 kcal per mol A \(^{-1} \) has been reached. For more docking studies, a database of the metal-ligand complexes was accomplished. Based on MOE 2016.08, the standard protocol was applied.

2.8. Anticancer activity

\[
IC_{50} (%) = \frac{\text{control OD}_t - \text{compound OD}_t}{\text{control OD}_t} \times 100
\]

Survival fraction = Optical density (treated cell)/Optical density (control cell). The concentration needed to offer 50% growth inhibition is referred to IC\(_{50}\). Eventually, the results are matched with the gained utilizing vinblastine as an antitumor agent.

3. Results and discussion

3.1. Elemental analysis and molar conductivity measurements

The five VO(II), Fe(III), Co(II), Ni(II) and Cu(II) new complexes of the N\(_2\)O\(_2\) ligand (scheme 1) separated due to the reaction between metal ions and H2L ligand with molar ratio (1:1)(M:L). The ligand and its complex are completely dissolved in hot DMSO and DMF. The empirical formula, melting points, elemental analysis, and the values of molar conductance of H2L ligand, and their complexes are presented in (table 1). It was found that all complexes have molar conductivity values of 4.43–12.67 \( \Omega \cdot \text{cm} \) \(^{-1} \) mol \(^{-1} \) cm\(^{2} \), and these small values confirm that the new complexes are non-electrolytes [24, 25]. The analytical data and proposed stoichiometry of the new complexes are in a good agreement.

3.2. \(^{1}H\)-NMR spectra and \(^{13}C\)NMR spectra of H2L ligand

Proton \(^{1}H\)-NMR spectrum of H2L (cf figure 1) shows a sharp characteristic peak at 9.55 (s) ppm corresponds to azomethine proton. A singlet corresponding to one proton is found at 10.24 ppm because of OH proton of the ligand. The protons of the aromatic moiety appear as a sharp multiple signal in 6.32–7.45 ppm. A single peak at 6
3.28 (s, 1H) (s, 3H) is referred to [–OC2H5] group. In addition, the 13CNMR spectra present the peaks at different values of δ as follow: azomethine group (CH = N) at δ 144 ppm and aromatic carbon atoms (17 CH-Ar) at (δ 108–137 ppm). Also, carbon atoms of the two ethoxy groups are at (δ 41–39 ppm) (OC2H5) as illustrated in figure S10 is available online at stacks.iop.org/MRX/7/065401/mediainline.

### 3.3. FTIR spectra

FTIR spectral data of both the H2L imine and its complexes were displayed in (table 2). The spectrum of H2L ligand displays an intense band at 1634 cm⁻¹ referred to ν(C=N) vibration of the imine ligand for the C=N group. The appearance of a band at 1588 cm⁻¹ which is characterized by ν(C=N) in pyridine ring of the ligand was shifted to lower wavenumber (1572–1520) cm⁻¹ in a case of CuL, FeL, CoL, NiL, and VOL complexes. In all complexes there is a shift to lower frequency of ν(C=N) band from 1634 cm⁻¹ to 1595–1620 cm⁻¹ confirming a coordination of azomethine group of the H2L imine to metal ions [26]. FeL, CoL, and CuL complexes exhibited a broad band at range 3420–3472 cm⁻¹ which has been attributed to stretching frequencies of coordinated water molecules [27]. Moreover, the new medium band appears at 976–967 cm⁻¹ in CuL, FeL and CoL complexes which could be assigned to the coordinated water molecules. The appearance of the new band in all complexes in the range 429–490 cm⁻¹, which is not found in spectrum of the free ligand, due to M-N bond. The second band at the range 511–573 cm⁻¹ could be addressed to the new M-O band. These results are confirmed by using DFT calculations. The most important infrared frequencies of the ligand H2L and its complexes listed in (table 2).

### 3.4. Magnetic susceptibility and reflectance spectral studies

The diffuse reflectance spectral data for the complexes, positions of bands and their transitions as segments are of importance as they are highly dependent on the geometry of the molecule. The magnetic moment values, μeff, measured for all complexes at room temperature (298 K) and recorded in the solid state are given in table 1.

The spectrum of Cu(II) complex shows broad bands at 12 466, 15 750 and 19 945 cm⁻¹. These bands are generally completely consistent with a five-coordinate geometry for Cu(II) complexes [28–30]. The spectrum
also shows a band at 30 452 cm$^{-1}$ which may be assigned to ligand to Cu charge transfer band. The magnetic moment value of 2.01 B.M. is indicative of a trigonal bipyramidal structure. The diffuse reflectance spectrum of the Co(II) complex displayed three bands at 13 050, 15 786 and 17 494 cm$^{-1}$. The fourth band at 21 978 cm$^{-1}$ is attributed to the charge transfer band. The value of the magnetic moment of the complex is 3.81 B.M., which is low compared with those observed for tetrahedral or octahedral complexes, and which supports the octahedral coordination geometry around Ni(II) ion was suggested according to the observed bands [30, 32]. Also, the presence of two bands at 22 132 and 23 872 cm$^{-1}$ were assigned to LMCT. The value of magnetic moment (3.71 B.M.) falls in the range reported for tetrahedral Ni(II). The diffuse reflectance spectrum of the Fe(III) complex exhibits a band at 21 357 cm$^{-1}$, which may be assigned to the $^{6}A_{1g} \rightarrow T_{2g}(G)$ transition of octahedral geometry of the complex [25]. The $^{6}A_{1g} \rightarrow 5T_{1g}$ transition appears to be split into two bands at 17 143 and 12 879 cm$^{-1}$. The observed magnetic moment of the Fe(III) complex is 5.35 B.M. Thus, the complex formed has octahedral geometry involving $d^{5}$ sp$^{3}$ hybridization in Fe(III) ion [33]. The spectrum also shows a band at 24 805 cm$^{-1}$ which may attribute to ligand to metal charge transfer.

The electronic spectra of the vanadyl complex VOL exhibits three bands in the region 13 334–13 444 cm$^{-1}$, 15 990–16 890 cm$^{-1}$ and 21 742–22 102 cm$^{-1}$ and 22 471 cm$^{-1}$. These spectra are like those of the other five coordinated oxovanadium complexes involving nitrogen donor atoms. The bands observed are assigned to the transitions $^2B_1 \rightarrow ^2E_2$, $^2B_2 \rightarrow ^2B_1$ and $^2B_2 \rightarrow ^2A_1$, respectively [24]. One more band is observed in the region 28 356–29 223 cm$^{-1}$, which may be due to charge transfer transition. The magnetic moment of oxovanadium (IV) complexes were found in the range 1.70–1.85 B.M. which corresponds to single electron d$^1$ system [21]. The magnetic moment of our VOL complex is 1.80 B.M. which agrees with the previously reported results [21]. The electronic spectra and magnetic moment clearly indicated square-pyramidal geometry for the VOL complex. (1.2 B.M) suggest the square stereochemistry of the complex. These results are in excellent agreement with those achieved earlier.

3.5. Mass spectral studies

Mass spectra has been increasingly used for elucidation of the molecular structures of many complexes. The electron impact spectrum of the complex FeL figure 2(A), confirms the proposed formula by showing a peak at 548.05 g mol$^{-1}$ m/z corresponding to complex moiety (C$_2$H$_2$O$_7$N$_3$CoFe), calculated atomic mass 548.3. 487.82, 452.54, 411.53, 387.4, 347.07, 292.42, 193.02, 132.3 m/z may be assigned to various fragments. Their intensity gives an idea of the stability of the different fragments. The electron impact spectrum of the complex VOL figure 2(B), confirms the proposed formula by showing a peak at 469.73 m z$^{-1}$ corresponding to complex moiety (C$_2$H$_2$O$_7$N$_3$V), calculated atomic mass 469.90. The series of peaks in the range of 419.23, 401.37, 362.95, 295.99, 248.77, 221.21 79.10, 163.32, 139.42, 127.16, 113.96, 93.98 g mol$^{-1}$ m/z may be assigned to various fragments of the complex.

3.6. Powder XRD

The crystal lattice parameters of the complexes VOL and NiL were measured by recording X-ray diffractogram (figures 3(a), (b)) in the range 20$^\circ$ to 80$^\circ$ 2θ value. XRD patterns of the VOL and NiL complexes are shown in figures 3(a) and (b) and show well defined crystalline and sharp peaks indicating that the samples were crystalline in phase. The appearance of crystallinity in the metal–Schiff base complexes is due to the inherent crystalline nature of the metallic compounds. The crystal parameters and the corresponding d-values were obtained. The data indicates the monoclinic crystal system for VOL complex, space group P121/c 1(14), $\beta = 90.901^\circ$, $d = 4$, $a = 8.0598$ Å, $b = 15.1237$ Å, $c = 19.2297$ Å. Triclinic P–1(2) crystal system was indicated for NiL complex, $\alpha = 96.391^\circ$, $\beta = 108.324^\circ$, $\gamma = 108.478^\circ$, $d = 2$, $a = 12.711$ Å, $b = 13.656$ Å, c = 16.069 Å.

3.7. Stability range of complexes

The pH profile (stability versus pH) figure 4 shows typical dissociation curves and a high stability range of pH (4.5–10.0) for the new complexes. The results indicate that the synthesized complexes are more stable compared to the free ligand. Consequently, the desired pH range for various applications such as biological or catalysis is pH (4.5–10.0).

3.8. TGA Studies

The thermal properties the complexes were characterized on the basis of TG and DTG methods within a temperature range from room temperature to 600$^\circ$C. Thermal analysis is considered as an important technique to explore the new complexes thermal stability and to decide if the water molecules are in a formula for complexes inside the coordination sphere of the central metal ion or as lattice water [8, 34]. The TG curve of
[Cu(L)(H$_2$O)]·H$_2$O chelate displayed four degradation steps starting from 21 °C to 608 °C. DTG curve gives maximum peak temperature at 90 and 248 °C. First step at 21 °C–136 °C is accompanied by a loss of one water molecule of hydration and a mass loss of 4.06% (calc. 4.08%). Second step occurred at temperature range of 136 °C–209 °C and corresponded to the elimination of one coordinated water molecule with mass loss of 3.44% (Calc. 3.58%). A loss of one ethyl alcohol molecule C$_2$H$_5$OH fragment with mass loss of 9.66% (calc. 8.96%) is found at the range of 209 °C–261 °C. The fourth stage occurred at range 261 °C–438 °C which has a weight loss amount of 58.05% (Calc. 59.50%) which can be pointed to C$_{21}$H$_{17}$NO fragment loss of the ligand. The total mass loss amounted to be 74.18% and CuN$_2$O$_2$ residue was left (cf table 3 and figure 5(b)). The TG degradation curve of [Fe(L)(H$_2$O)Cl]·2H$_2$O displayed four steps in the range 45 °C–614 °C. The first step occurred at a temperature range of 45 °C–148 °C and the mass loss of 6.01% (calc. 6.57%) were noticed, which attribute to loss of two water
aqua molecules. The second step took place at the temperature range 148 °C–173 °C and is accompanied by a loss of 3.76% (calc. 3.51%), which is due to the loss of one coordinated water molecule. The third decomposition step happened at 173 °C–288 °C and is pointed to the breaking down of C9H11O fragment with a mass loss of 24.16% (calc. 24.62%). Weight loss amounted to 7.07% (calc. 6.92%) at a temperature range 288 °C–403 °C could be assigned to a loss of one coordinated HCl. Last stage at 403 °C–611 °C includes loss of a C2H5ON fragment with a mass of 10.14% (calc. 10.76%). DTG curve gives maximum peak temperatures at 147 and 205 °C. (cf table 3, scheme 2 and figure 5(a)). The [Co(L)(H2O)].H2O complex decomposed in three steps at the range of 41 °C–603 °C. A range of 41 °C–217 °C and a mass loss of 7.30% (Calc. 7.23%) was detected and attributed to loss of one aqua water molecule and one of lattice water molecule. In second decomposition step (T = 217 °C–438 °C) a loss of two ethoxy groups C4H10O2 fragment was lost and its mass has a value of 17.84% (calc. 18.08%). The third step occurred at a range of 438 °C–601 °C and was accompanied by the loss of C2H5N fragment of a mass loss of 20.03% (calc. 20.89%). DTG curve [Co(L)(H2O)].H2O complex gives four maximum peaks temperatures at 106 °C, 273 °C, 360 °C and 553 °C (cf table 3).

### 3.8.1. Kinetic aspects

Table 3 displayed the thermo-kinetic parameters of degradation steps for the three Cul, FeL, CoL metal chelates. From the data obtained, the following comments are concluded:
The reactants are less ordered than the activated complexes as implied from the negative values of entropy. The decomposition processes are endothermic as shown from positive $\Delta H^*$ values. The degradation reactions are slow as indicated by the high positive values of the free activation energy. There is an immediate relation among ($A$) and ($E^*$) for acquired metal chelates. Comparatively low estimations of ($A$) had been showing slow process of pyrolysis reaction. Higher positive estimations of ($E^*$) had been demonstrated the procedures that

**Figure 5.** TGA and DTGA curves for thermal degradation of (a) FeL and (b) CuL.

**Scheme 2.** Thermal decomposition steps of FeL complex from ambient temperature to 900 °C at heating rate of 10 °C min⁻¹.
included changes in the mechanical potential energy, translational, vibrational and rotational states for metal chelates \(^{30}\).

Regarding the results of IR, the electronic spectroscopy, magnetic measurement, elemental analysis, and molar conductance, proposed structures for these complexes, were suggested (cf scheme 3).

3.9. Molecular DFT calculation

3.9.1. DFT calculation of ligand L and VOL

The lowest-energy configurations for the optimized structures of (L) and its complex [VOL] were displayed in (figure 6(a)). The five-coordinate pyramidal structure of vanadium metal with O5 taken an axial position and atoms (N1, O1, O2, N3) is nearly in a one plane with deviation of 12.33°. Due to the chelation, the bite angle (N1-V-N3 (63.38°) is less than 90°. The bond angles in the square are ranging from 63.38° to 97.82°, (table 4). Along with the metal of complex formation, the effect on distances between the donor atoms within coordination is highly decreased. The distances between N1- - - - N3- - - -, N1- - - - O1 and O1- - - - O2 (in the free ligand) are lowered from 2.401, 4.920 and 9.838 Å to 2.188, 2.783 and 2.878 Å respectively, (table 1). As the result of coordination of both N1 and N3, the angle N1-C1-N3 also decreased in complex, 103.9° compared with that in the free ligand, 120.1°.

The optimized structures of [CoL(H2O)] complex as the minimum energy configurations were demonstrated in (figure 6(b)). The cobalt metal is a five-coordinate pyramidal structure with O5 of coordinated water occupied in the axial position. The deviations of the atoms N1, N3, O2, and O1 from almost one plane are −0.604°. The chelation was the main reason for the bite angle (N1-Co-N3 (68.10°) to be lowered than 90°. Moreover, the angle O5-Co-O1 (73.78°) is lower than O5-Co-N3 (112.0°) due to H-bonding O5-H- - - O1(cf table 5). Upon the formation of the complex, the distances between the donor group of atoms within the coordination are highly decreased. The distances between N1- - - - N3, N1- - - - O1 and O1- - - - O1 in free ligand are reduced from 2.401, 4.920 and 9.838 Å to 2.191, 2.991 and 2.944 Å for the complex, respectively (table 8). Upon coordination of both N1 and N3, the angle of N1-C8-N3, 104.8° is lower in complex compared to free ligand, 115.7°. Due to the formation of hydrogen bond between coordinated water and O1 of ligand, the bond length of O5-H22, 0.996 Å, is longer than that of O5-H23, 0.976 Å.
3.9.2. DFT calculation of ligand L and NiL:
Optimized structures of the complex \( \text{NiL} \) as the lowest energy configurations exhibit in (Figure 6(c)). The characterization of the nickel atom is a four-coordinate square planar in a geometric distortion. The deviation of the atoms N3, O2, O1, and N1 in almost one plane is 5.810°. Upon chelation, the bite angle N1-Cu-N3 (66.94°) is lower than 90°. The bond angles in the square are ranging from 66.94° to 111.2°, Table 7. Moreover, upon complex formation, the distances between donor atoms involving in the coordination are greatly decreased. The distances between N1--N3, N1--O1 and O1--O2 decreased from 2.401, 4.920 and 9.838 (in free ligand) to 2.174, 2.991 and 2.664 Å (in the complex), respectively, (Table 6). Due to coordination of both N1 and N3, the angle N1-C1-N3, 103.2°, is lower in complex compared to free ligand, 120.1°. The HOMO, LUMO and

| Type of bond | Bond length (Å) | Angle (°) |
|--------------|----------------|-----------|
| V-N1         | 2.101          | N1-V-N3   | 63.38 |
| V-N3         | 2.063          | N1-V-O2   | 89.04 |
| V-O1         | 1.866          | N3-V-O2   | 83.24 |
| V-O2         | 1.953          | O1-V-O2   | 97.28 |
| V = O5       | 1.602          | O5-V-N1   | 105.2 |
| V--N2        | 3.705          | O5-V-N3   | 115.1 |
| O1--O2       | 9.838          | O5-V-O1   | 116.1 |
| N1--N3       | 2.401          | O5-V-O2   | 104.9 |
| N1--O1       | 4.920          | N1-V-O2   | 142.1 |
| N3--O1       | 2.783          | N1-V-O2   | 126.4 |
| N1-O1-O2-N3  | 12.33          | N1-O1-O2-N3 | 12.33 |
| N1-C1-N3     | 120.1          | N1-C1-N3  | 103.9 |

Figure 6. (a) Optimized structure of L (upper) and [VOL] (lower) by density function B3LYP/LANL2DZ. Optimized structure of [CoL(H2O)] complex (b) and [NiL] complex (c) by density function B3LYP/LANL2DZ.

Table 4. Important optimized bond lengths (Å) and bond angles (°) of H2L and VOL.
The computed total energy for the compounds were recorded in Table 8. The total energy of the complexes values is more negative than those of free ligands and this shows that the free ligand is less stable than that of the complexes.

### 3.10. Antimicrobial activity

The *in vitro* antipathogenic activity for H$_2$L ligand and its Cu($II$), Co($II$), Ni($II$), VO($II$), Fe($III$) complexes are examined against selected strains of bacteria Gram-positive (*S. aureus* ATCC25923 and *B. subtilis* RCMB015(1) NRRLB-543) and Gram-negative (*P. vulgaris* RCMB004(1) ATCC13315 and *E. coli* ATCC 25922) bacteria [31, 32, 35]. The fungal stains are Aspergillus fumigates (RCMB002008) and Candida Albicans (RCMB 005003). The results of antipathogenic activity are listed in (tables S2 and S3) and displayed in (figures 7 and 8).

### Table 5. Important optimized bond lengths ($\text{Å}$) and bond angles ($^\circ$) of L and [CoL(H$_2$O)].

| Type of bond | Bond length ($\text{Å}$) | Type of Angle | Angle ($^\circ$) |
|-------------|-------------------------|---------------|-----------------|
| Co-N1       | 2.013                   | N1-Co-N3      | 68.10           |
| Co-N3       | 1.897                   | N1-Co-O1      | 98.37           |
| Co-O1       | 1.939                   | N3-Co-O2      | 92.52           |
| Co-O2       | 1.899                   | O1-Co-O2      | 100.2           |
| Co-O5       | 2.265                   | O5-Co-N1      | 95.65           |
| O5-H22      | 0.996                   | O5-Co-N3      | 112.0           |
| O5-H23      | 0.976                   | O5-Co-O1      | 73.78           |
| Co-N2       | 3.774                   | N1-Co-O2      | 97.28           |
| O1-O2       | 9.838                   | N3-Co-O1      | 159.7           |
| N1-N3       | 2.401                   | N1-C1-N3      | 165.3           |
| N1-O1       | 4.920                   | N1-N3-O2-O1   | 103.9           |

### Table 6. Important optimized bond lengths ($\text{Å}$) and bond angles ($^\circ$) of L and [NiL].

| Type of bond | Bond length ($\text{Å}$) | Type of Angle | Angle ($^\circ$) |
|-------------|-------------------------|---------------|-----------------|
| Ni-N1       | 2.053                   | N1-Ni-N3      | 66.94           |
| Ni-N3       | 1.880                   | N1-Ni-O1      | 111.2           |
| Ni-O1       | 1.877                   | N3-Ni-O2      | 92.55           |
| Ni-O2       | 1.906                   | O1-Ni-O2      | 89.53           |
| Ni-N2       | 3.669                   | N1-Ni-O2      | 159.3           |
| O1-O2       | 9.838                   | N3-Ni-O1      | 171.4           |
| N1-N3       | 2.401                   | N1-C1-N3      | 120.1           |
| N1-O1       | 4.920                   | N3-O2-O1-N1   | 103.2           |

### Table 7. Calculated energies of H$_2$L Schiff base ligand and its new complexes at B3LYP/LANL2DZ.

|            | $E_a$          | HOMO$^b$      | LUMO$^c$      | $\Delta E^d$ | Dipole moment$^e$ |
|------------|----------------|---------------|---------------|--------------|-------------------|
| L          | $-1355.24$     | $-0.2186$     | $-0.0976$     | $0.1210$     | $0.2262$          |
| [VOL]      | $-1500.74$     | $-0.1914$     | $-0.1023$     | $0.0891$     | $6.953$           |
| [CoL(H$_2$O)] | $-1500.74$     | $-0.1914$     | $-0.1023$     | $0.0891$     | $6.953$           |
| [NiL]      | $-1523.36$     | $-0.1810$     | $-0.1002$     | $0.0808$     | $8.027$           |

$^a$ $E_a$ the total energy (a. u.).

$^b$ HOMO: highest occupied molecular orbital (eV).

$^c$ LUMO: lowest unoccupied molecular orbital (eV).

$^d$ $\Delta E$: $E_{\text{LUMO}} - E_{\text{HOMO}}$ (eV).

$^e$ Dipole moment (Debye).
complexes exhibit more antipathogenic activity compared to the free ligand molecules \[31, 32, 35\] within the values of the main inhibition zone. It is worth mentioning that the size of inhibition zone in case of VOL is 30 mm and is greater than the size of inhibition zone of the reference drug Gentamicin which is 25 mm in the case of P. vulgaris bacteria. The activity of all complexes could be clarified for chelation theory, as the chelation is significantly reducing the charge for the metal ions due to partial sharing of their positive charges within the donor atoms of the polydentate Schiff base as well as \(\pi\)-electrons delocalization of the three aromatic chelates of the Schiff base ligand. The presence of ethoxy group in the side chain, with its electron withdrawing ability, also enhances the lipophilic character of the metal chelates favoring the penetration through the layers of the lipid of

| Complex | \(\lambda_{\text{max}}\) free (nm) | \(\lambda_{\text{max}}\) bound (nm) | \(\Delta n\) | Chromism (%) | Type of Chromism | Binding constant \(\times 10^5\) | \(\Delta G\) kJ mol\(^{-1}\) |
|---------|-----------------|-----------------|-----------|-------------|----------------|------------------|------------------|
| CuL     | 240             | 238             | 2         | 3.27        | Hypo           | 6.25             | −33.06           |
|         | 251             | 252             | −1        | 4.21        |                |                  |                  |
|         | 489             | 482             | 7         | 6.57        |                |                  |                  |
| FeL     | 239             | 238             | 1         | 6.59        | Hypo           | 5.5              | −32.75           |
|         | 288             | 289             | −1        | 6.42        |                |                  |                  |
| NiL     | 239             | 237             | 2         | 4.52        | Hypo           | 2.3              | −30.79           |
|         | 325             | 317             | 8         | 43.58       |                |                  |                  |
| CoL     | 239             | 238             | 1         | 4.56        | Hypo           | 1.52             | −29.56           |
|         | 298             | 305             | −5        | 34.78       |                |                  |                  |
| VOL     | 239             | 237             | 2         | 1.00        | Hypo           | 3.20             | −31.40           |
|         | 253             | 252             | 1         | 1.58        |                |                  |                  |
the microorganism cell membrane. The complexes include the hydrogen bond formation through N = CH-group with active centers for the microbe cell that result from interfering with the normal cell processes. The efficiency was in the order \( P. \text{aeruginosa} > S. \text{aureus} > B. \text{subtilis} > E. \text{coli} \). Biological activity of the complexes towards organisms was in the order \( \text{VOL} > \text{CoL} > \text{CuL} > \text{NiL} > \text{H}_2\text{L} > \text{FeL} \) with regards to Gram-negative bacteria, the \( \text{VOL} \) complex had good antibacterial activity. Minimum inhibition concentration (MIC) in \( \mu \text{g ml}^{-1} \) for antimicrobial and antifungal activity of \( \text{VOL} \) and \( \text{NiL} \) pyridine Schiff base complexes is shown in table S4. Our results agree with the results obtained in [36].

3.11. DNA-binding studies

3.11.1. Absorption spectral titrations

The electronic absorption spectra of the CT-DNA interaction for the titled complexes through intercalation displayed strong red shift peak and hypochromicity with increasing CT-DNA concentration which could be as a result of the strong \( \pi \rightarrow \pi^* \) transition resulting from staking interaction between DNA and the complexes.
So, the interaction is intercalation mode through the order: CuL > CoL > VOL > FeL > NiL complexes [8, 38]. The binding constant and spectroscopy parameters for DNA reaction with the investigated complexes listed in table 8 (cf figures S5–S9).
3.11.2. Viscosity measurement

Viscosity, which is very sensitive to molecular length increase, is considered as one of the least ambiguous and most critical tests for exploring the bonding mode of complexes with DNA. Viscosity measurements were used to further elucidate the nature of the interactions between the complexes and DNA. A classical intercalator causes a significant increase in the viscosity of DNA solution due to increased separation of the base pairs resulting in an increase in overall DNA length \[37\], whereas partial or non-classical intercalation of complexes may bend the DNA helix, resulting in a decrease in its effective length and concomitant decrease in viscosity \[39\].

Figure 14. Molecular docking simulation studies of hydrophobic interactions of the Schiff base ligand H₂L and its NiL, CuL, CoL, VOL, FeL with amino acid residues of PDB ID: 1BNA are shown with dotted curves.
The effects of the Schiff base ligand and its complexes on the viscosity of DNA solutions are shown in figure 10. As seen, the relative viscosity of DNA is increased in the presence of complexes 1–5 relative to L, indicating intercalative binding modes. These viscosity results are consistent with the spectroscopic results discussed above.

3.11.3. Gel electrophoresis
It is a conclusive technique of studying DNA interaction with prepared complexes. The gel after electrophoresis showed DNA intensity loaded with the prepared complexes, has partly reduced for CoL, VOL, CuL, FeL, and NiL complexes as shown in (figure 11) [40, 41]. The DNA could bind to the investigated complexes via interactive mode as shown from experimental results.

3.12. Cytotoxicity
Overall, the outcomes of the chemical nuclease activity and binding interactions for five new complexes induced us to estimate their anticancer behavior. Accordingly, the new complexes and vinblastine drug were subjected to colorimetric (MTT) assay against Hep-G2. The results are shown in (figures 12 and 13). The results show that all titled complexes are more cytotoxic than the H2L ligand. The cytotoxic behavior may be explained in Tweedy’s chelation theory [40–45]. Most of the complexes are active against the Hep-G2 cell line while H2L has lower activity than metal complexes and its IC50 value is 21.0 μg μml−1. The IC50 values of the metal complexes range from 6.5 to 12.5 μg μml−1, and the order of IC50 for this cancer cell line can be arranged as follows: CuL > FeL > VOL > NiL > CoL > H2L.

This Cytotoxicity order agrees with the results of the CT-DNA binding constant Kb.

3.13. Molecular docking
Molecular docking study was performed to understand the drug-DNA interactions in rational drug design as well as a mechanistic study by placing a small molecule into the binding site of the target specific region of the DNA mainly in a non-covalent fashion. Molecular docking experiment was conducted using MOE version 2016.08 to get an insight into the binding mode of the H2L and its metal complexes with DNA duplex of sequence d(CGCGAATTCCGG)2 dodecamer (Code: 1BNA) to get an energetically favorable docked pose. that is shown in figures 14 and 15 and table S1 [46, 47].

The results show that the complexes interact with DNA and were deeply fitted inside the active pocket of the enzyme forming hydrogen bond interactions and hydrophobic interactions, which reflects high stability and affinity of the studied molecules to bind with the receptor. From the obtained results, CuL complex form a

Figure 15. Molecular docking simulation studies of the interaction between Schiff base ligand H2L and its NiL, CuL, CoL, VOL, FeL with the active site of the receptor of PDB ID: 1BNA. The docked conformation of the compound is shown in ball and stick representation.
strong hydrogen bonds between C = N⁺ of the imine group and pyridine ring and DAB18 residue in the DNA with short distance and relative binding energy is −6.4 k cal. Mol⁻¹. In VOL complex, the O₅₂⁺ of the vanadyl oxygen and the electron deficiency on the pyridine ring forms a moderate strength hydrogen bonds with DAB17 with binding energy value of 5.0 k cal. Mol⁻¹. In FeL and CoL complexes, hydrogen bonds were formed between coordinated water molecules of the complexes and DAB17 residue in the DNA and the binding energy was −4.6 and −4.8 k cal. Mol⁻¹ respectively. H₂L and NiL interacted with the amino acid residues of the DNA in a different way through strong stacking interaction between the conjugated aromatic system of the phenyl, pyridine, azomethine and ethoxy side chain groups and DG10, D, AB17, DAB18, DG3A12, DGB16, DCA11, the binding energy of the interaction was found to be −5.7 and 5.4 k cal. mol⁻¹. The results correlating well with the experimental results of the DNA binding results and the calculated Kᵣ values. It was concluded that the docking study showed a favorable interaction between the H₂L and complexes as shown in figures 14 and 15 and the calculated energy was listed in table S1.

4. Conclusion
In this work the preparation and characterization of the new pyridine based N₂O₂ tetradentate H₂L ligand have been reported. H₂L has been reacted with different Fe(III), Cu(II), VO(II), Co(II) and Ni(II) ions to get five new colored complexes. The analytical and thermo analytical results recommended the general molecular formula of the complexes as [M(L)] for NiL, [M(L)(H₂O)] for CoL and CuL and [M(L)(H₂O)(Cl)] for FeL complex. The study of conductance reveals non-electrolytic nature of complexes. FT-IR and 1H NMR study indicates the tetradentate nature of the ligand and its coordination through one azomethine nitrogen, two oxygen atoms and one nitrogen pyridine ring for all complexes. Magnetic moments and solid reflectance UV-vis spectral studies suggested octahedral geometry of FeL complex and square pyramidal geometry for CoL, CuL and VOL and square planar for NiL complex. The DFT calculations for the ligand show the geometry optimization for L and its complex [VOL] as a minimum energy configuration. The characterization of the five-coordinate vanadium metal is in a pyramidal structure with O5 in an axial position and atoms N1, O2 and N3 that nearly in one plane deviated by 12.33°. The complex [CoL(H₂O)] of the optimized structures reveals the lowest energy configurations. The five- coordination atoms are almost in one plane deviated by −0.604° with O5 in an axial position and occupied by atoms N1, N3, O2, and O. Finally, the optimized square structure of the complex [NiL] as being the lowest energy-configurations shows the four-coordinate nickel metal with deviations of the atoms N3, O2, O1, and N1 of almost one plane is 5.810°. Due to the chelation, the bite angle of N1-Cu-N3 (66.94°) is lower than 90°. The bond angles in the square are ranging from 66.94° to 111.2°.

In sum, from the activities of the new metal complexes as pathogenic of microorganism, the metal complexes displayed higher antimicrobial activities compared to the free ligand molecules. Finally, vanadium complex is found to be a good antimicrobial agent and the order of increasing bacterial and fungal growth inhibitory capacity is in the following order: H₂L < NiL < CuL < CoL < FeL < VOL.

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