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Synthesis, Characterization, Antimicrobial, DNA Cleavage and Fluorescent Activity of Metal ion(II) Coordinate with 2H-Chromene Azo novel ligand

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ABSTRACT

A new ligand 2H-chromene containing azo group 2-(4-nitrophenyl)-N-(4-(phenyldiazenyl)-2H-chromen-4-amine (AH), were synthesized from the condensation reaction (1:2) of 2'-hydroxychalcone and p-aminoazobenzene. Co(II), Cu(II) and Ni(II) complexes of the new ligand have been synthesized and characterized using C.H.N. analysis,^{1}H NMR spectra, FT.IR, UV/Visible, magnetic susceptible, conductance measuring, and fluorescence spectral spectroscopy; ^{13}C NMR spectroscopy of the ligand was also studied. Spectroscopic results revealed the 2H-Chromene Azo (AH) ligand behaves as monodentate chelating via the nitrogen atom of amine group at position 4 having 1:1 [M:L] ratio; suggested that the cobalt and nickel complexes have the tetrahedral structure and a distorted tetrahedral geometry for the copper complex, indicating their non-electrolyte nature. The new ligand shows a fluorescence emissioncomparing with this fluorescence quenching was noticed in its metal complexes. The antibacterial potency of the free ligand and its chelates with metal ion(II) were screened against *E.coli*, *K.pneumoniae, Staph.aureus* and *B.Subtilis*; The DNA cleavage activity of the free ligand and its 2H-chromene azo metal (II) complexes was performed by the gel electrophoresis process, which given that these compounds are effective upon DNA cleavage

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INTRODUCTION

Heterocyclic compounds represent a large family of organic compounds (Katritzky *et al.*, 2010; Brimble *et al.*, 2008), they are known for widespread applications in various fields because of their biological importance, medicinal chemistry, and chemical biology. The 2H-chromene (2H-1-benzopyran) moiety is one of the most important heterocycles detected in many biologically active natural products and in synthetic compounds (Kolokythas *et al.*, 2007; Ellis, 2009; Schweizer and Meeder, 1977) 2H-chromenes were reported to display significant antioxidant (Kwak *et al.*, 2006), antiviral (Martínez-Grau and Marco, 1997), antimicrobial (Li *et al.*, 2018) anti-inflammatory (Gebhardt *et al.*, 2007) and antifungal (Chanu *et al.*, 2017) properties, in addition to their interesting applications as highly effective fluorescent dyes (Moorthy *et al.*, 2006) Furthermore, several metal complexes of chromene derivatives possess antimicrobial (Soliman *et al.*, 2016) DNA cleavage activities (Prabhakara *et al.*, 2015) and photoluminescence properties (Aazam *et al.*, 2012) . Azo compounds and their complexes are highly important due to their applications as textile dyes, Zhigang *et al.* (2009) indicators (Abbas *et al.*, 2018), medicinal importance (Philip *et al.*, 2019) and are also inhibition of DNA, RNA and protein creation (Park *et al.*, 2007).

In the present study, we display the synthesis and characterization of Co(II), Cu(II), and Ni(II) complexes with new azo- linked chromene ligand (AH). The newly ligand and its complexes were examined for their antibacterial potency against some types of gram-positive and negative bacteria; DNA cleavage activity, and also fluorescence properties.

MATERIALS AND METHODS

All chemicals were from BDH, Merck, and Fluka products. ^{1}H and^{13}C–NMR spectra were performed using Burker 500.13 MHz. Elemental analyses were carried through a Perkin CHN Elmer analyzer. The metal content was determined by varian-AA6200 flame atomic absorption spectrophotometer. Gallenkamp M.P. apparatus were used. Molar conductance of the prepared complexes with 10^{-3}M in EtOH were measurement using SIEMENS conductivity meter. Magnetic susceptibility measurements were obtained using the MSB-MK-1 model. The UV-Vis spectra were obtained with SHIMADZU-1800 spectrophotometer using a quartz cell (200-1100 nm). The IR spectra were measured using SHIMADZU FTIR-8400S spectrophotometer using (KBr & CsI discs). The fluorescence measurements of the prepared compounds were recorded on a SHIMADZU Fluorescence instrument.

Synthesis of The Starting Material

Synthesis of chalcone compound (B) [1-(2-hydroxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one]

A mixture of p-nitrobenzaldehyde (10 mmol), 2'-hydroxyacetophenone (10 mmol) were dissolved in (25 ml) methanol, Potassium hydroxide (30 mmol) was added with stirring at room temperature for 10 h to obtain a blood red mixture, then infused into 1N HCl (20 ml), and concentrated HCl was added until the solution was acidic. The precipitate was filtrated, washed with water and recrystallized from methanol. $C_{15}H_{11}NO_4$ (269 g.mol^{-1}), Light orange powder,yield: 73%, m.p.: 210-212 ^{0}C. FTIR (KBr, cm^{-1}): 3548 (-OH group), 1639 (C=O), 1267 (C-O, phenolic). ^{1}H-NMR (500MHz, DMSO-d$_6$, δppm) δ 11.84 (s, 1H), 8.31-8.17 (m, 2H), 7.93-7.91 (m, 1H), 7.72 (d, *J*=10Hz, 2H), 7.54-7.50 (m,1H), 7.05-6.93 (m, 1H); ^{13}C-NMR (125 MHz, C_6D_6, δ ppm) δ 203.6 (C=O), 160.9 (C-O), 153.5 (C-N), 146.9 (C), 141.3 (C), 131.4 (C), 127.7 (C), 127.5 (C), 123.8 (C), 123.7 (C), 121.3 (C), 119.6 (C), 118.0 (C), 99.9, 68.8 (CH=CH).

Synthesis of 2H-Chromene Azo Ligand (AH) 2-(4-nitrophenyl)-*N*-(4-(phenyldiazenyl) -2H-Chromen-4-Amine

A mixture of the prepared chalcone compound (1.67 mmol, 0.44 g) and p-amino azobenzene (3.34 mmol, 1.49 g) was refluxed in absolute ethanol (50 ml) with concentrated hydrochloric acid for 30 h. The precipitate was filtered and washed with the hot solvent; Scheme 1. $C_{27}H_{20}N_4O_3$ (448 g.mol^{-1}), Dark purple solid yield: 85 %, m.p.: 228-230 ^{0}C, FTIR (KBr, cm^{-1}): 3485 (N-H), 3041 (aromatic C-H), 1658 (aromatic C=C), 1452 (N=N). ^{1}H-NMR (500 MHz, DMSO-d$_6$, δ ppm) δ 9.23 (s, NH), 7.91 (d, *J*=5Hz, 1H), 7.85 (d, *J*=10Hz, 1H), 7.54 (dt, *J*=15, *J*=7.5 Hz, 1H), 7.33 (d, *J*=10Hz,1H), 3.44(q, *J*=6.6Hz, 1H), 2.07 & 1.05 (s, t, *J*=7.5Hz, CH, CH); ^{13}C-NMR (125 MHz,C_6D_6, δ ppm) δ 159.8 (C-O), 151.9 (C-N), 148.9 (C-N), 148.2 (C-N), 131.4 (C), 130.0 (C), 129.9 (C), 129.7 (C), 125.1 (C), 122.7 (C), 122.5 (C), 122.3 (C), 96.5 (C), 76.9, (C) 58.6 (C).

Synthesis of 2H-Chromene Azo Metal Complexes

To a stirred solution of 2-(4-nitrophenyl)-*N*-(4-(phenyldiazenyl)-2H-chromen-4-amine (AH) (0.3 mmol, 0.134 g) in (20 ml) ethanol, (0.6 mmol) of metal chloride (M= $CoCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$ and $NiCl_2 \cdot 6H_2O$ were added at room temp. The solution mixture was reflux for two hours, then allowed to cool and left overnight at room temperature. Then filtered and washed with EtOH, ethyl ether, and dried at the desiccator on $CaCl_2$.

For $Co(AH)(H_2O)_2Cl$: pink powder; yield: 69 %, FTIR (CsI, cm^{-1}): 3419, 771 (H_2O coor.), 3047 (aromatic C-H), 545 (Co-O), 360 (Co-N), 316 (Co-Cl). For $Cu(AH)(H_2O)_2Cl$: olive green powder; yield: 74 %, FTIR (CsI, cm^{-1}): 3438, 840 (H_2O coor.), 3058 (aromatic C-H), 553 (Cu-O), 345 (Cu-N), 283 (Cu-Cl). For $Ni(AH)(H_2O)_2Cl$: Reddish pink powder; yield: 71 %, FTIR (CsI, cm^{-1}): 3315, 845 (H_2O coor.), 3047 (aromatic C-H), 441 (Ni-O), 356 (Ni-N), 310 (Ni-Cl).

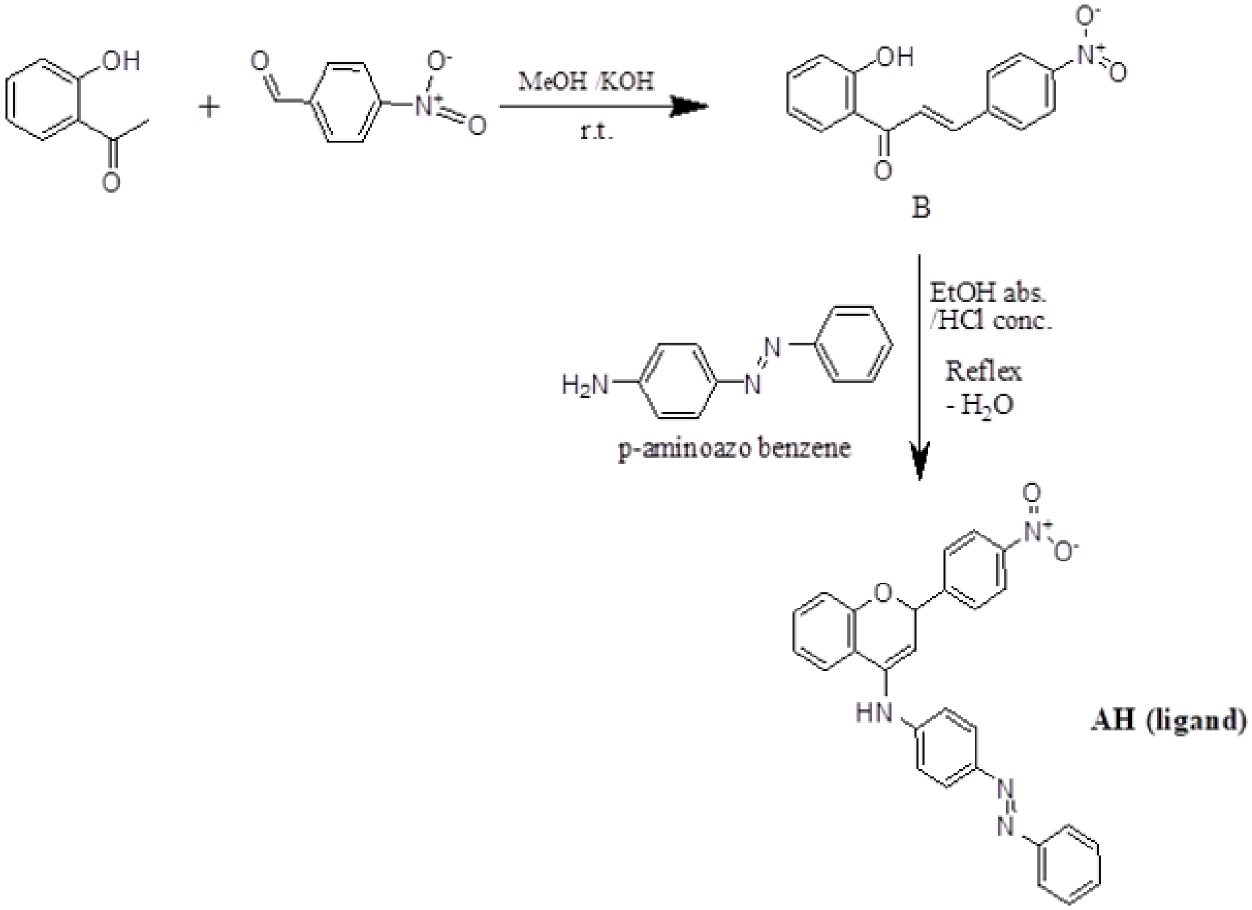
Biological Studies

Antibacterial Studies

The biological activities (*in vitro*) of the prepared ligand (AH) and its chelates were performed by disc diffusion method (Bauer *et al.*, 1966) against two gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and two gram-negative (*Escherichia coli, Klebsiella pneumonia*) using Muller Hinton agar for growing cell and the activity was measured in terms of zone of inhibition. DMSO has been applied as a negative control with ciprofloxacin (1 mg.ml^{-1}) as the standard drug.

DNA Cleavage Studies

The newly prepared ligand (AH) and its metal (II) complexes were analyzed as DNA cleaving agents using the bacterial previous studied DNA as targets by the agarose gel electrophoresis method (Sambrook *et al.*, 1989). Test samples (1 mg.ml^{-1}) were



Scheme 1: Synthesis route of the AH (ligand)

dissolved in DMSO.

RESULTS AND DISCUSSION

The analytical results of all compounds are presented in Table 1. The elemental analysis, metal determination, and the chloride content (by Mohr's method) of the prepared complexes revealed that all complexes with 1:1 stoichiometry and the calculated values are in a good acceptance with the found values.

Biological Studies

Antibacterial Studies

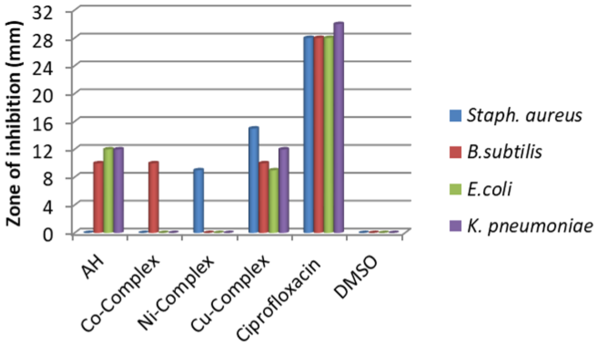


Figure 1: Graphical depicting of antibacterial activity of ligand (AH) and its chelates metal (II) against different bacterial strains in concentration (1 mg.ml^{-1})

The antimicrobial activity of the (AH) ligand and its metal (II) complexes were investigated against two Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and two Gram-negative (*Escherichia coli, Klebsiella pneumoniae).* The inhibition zones were determined and compared with ciprofloxacin (standard drug), as shown in Figure 1. The results indicate that the ligand (AH) and Co(II) complex has no effectiveness versus *Staphylococcus aureus, but*, the Cu(II) complex has more inhibition than the Ni(II) complex against this bacteria. The promoter activity of these metal complexes over the ligand can be elucidated on the principle of chelation theory (Sharma and Chandra, 2011; Chohan *et al.*, 2006). In chelating,the positive charge of the metal ion is partial participate with the donator group present in the ligand and probable π-electron delocalization upon chelated mode (El-Wahab *et al.*, 2004).

Table 1: Physical properties and analytical data for AH(ligand) and its metal complexes.

Compounc	Color	Melt. point °C	M.Wt g.mol^{-1}	Elemental Analysis Found % (Calc.)			Metal Found % (Calc.)	Chloride Found % (Calc.)
				C%	H%	N%		
AH ligand	Dark purple	228-230	448.15	72.31 (72.31)	4.53 (4.49)	12.56 (12.49)	――	――
Co(II) complex	pink	190 dec.	577.4	56.14 (56.12)	4.05 (4.01)	9.71 (9.70)	10.45 (10.20)	6.10 (6.13)
Cu(II) complex	Olive green	180	582.15	55.73 (55.67)	4.07 (3.98)	9.63 (9.62)	10.79 (10.91)	6.01 (6.09)
Ni(II) complex	Reddish pink	180 dec.	577.25	56.15 (56.14)	4.1 (4.01)	9.72 (9.70)	10.32 (10.16)	6.17 (6.14)

Table 2: Molar conductance and magnetic moment values of the prepared metal complexes

Compound	Molar Cond. S.cm^2.moL^{-1}	μeff. (B.M)
Co(II) complex	12.3	4.65
Cu(II) complex	10.4	1.89
Ni(II) complex	13.2	3.31

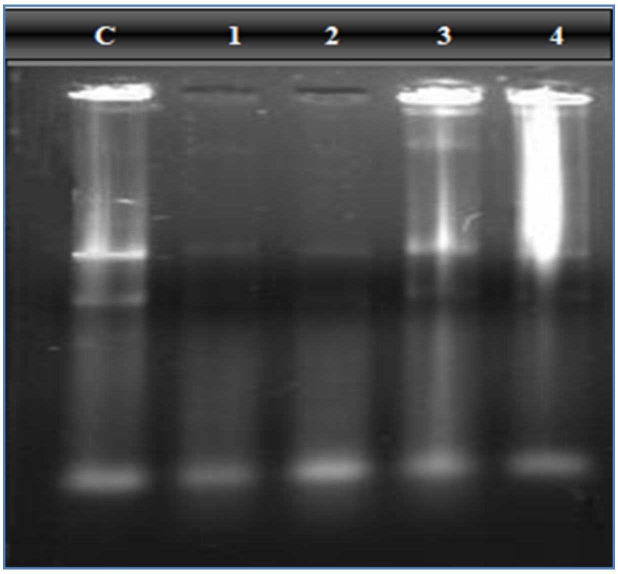


Figure 2: C, control DNA of S. aureus; S. aureus DNA remedied by: Lane 1, AH(ligand); Lane 2, Co(II) complex; Lane 3, Ni(II) complex; Lane 4, Cu(II) complex

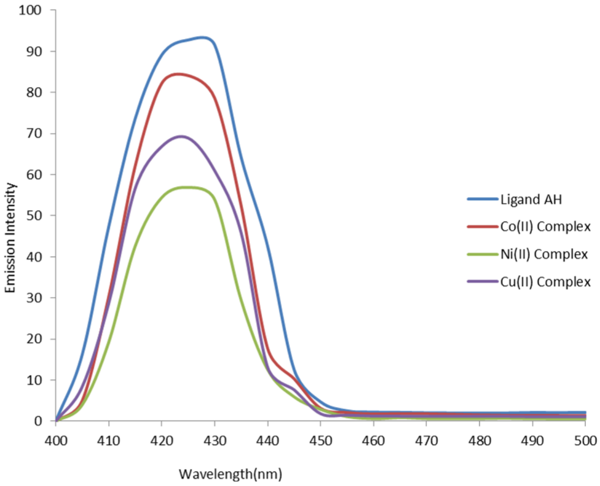


Figure 3: Emission spectra of ligand (AH) and its chelates in Ethanol at λ_{ex}= 380 nm

Ni(II) complex has no activity against *Escherichia coli, Klebsiella pneumoniae* and *Bacillus subtilis* bacteria, whereas Co(II) complex has just action against *Bacillus subtilis*, the prepared compounds showed variable effectiveness against different organisms which refers either on the impermeability into microbes cells or on variance in ribosome of microbial cells (İspir, 2009).

DNA cleavage studies

The DNA cleavage activity of the new ligand AH and its complexes have been studied against DNA of *Staphylococcus aureus*, from the results of DNA cleavage experiment as shown in Figure 2, it was noticed that the DNA control (untreated DNA) does not exhibit any cleaving and there was a difference in the migration of ligand alone and its chelates. This damage to DNA in AH ligand, Co(II), Cu(II) and Ni(II) complexes could be attributed to the cleavage of DNA. It was observed that the lane Cu(II) complex showed complete DNA cleavage of *Staphylococcus aureus*, but the free ligand, Co(II), and Ni(II) complexes showed partial cleavage of DNA. These data refer to the significant property of coordination to

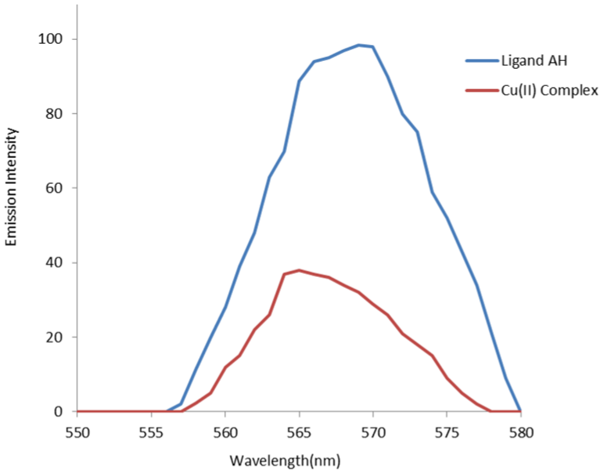


Figure 4: Emission spectra of (AH) ligand and Cu(II) complexes in DMSO at λ_{ex}= 530 nm. [Co & Ni(II) Complexes without fluorescence]

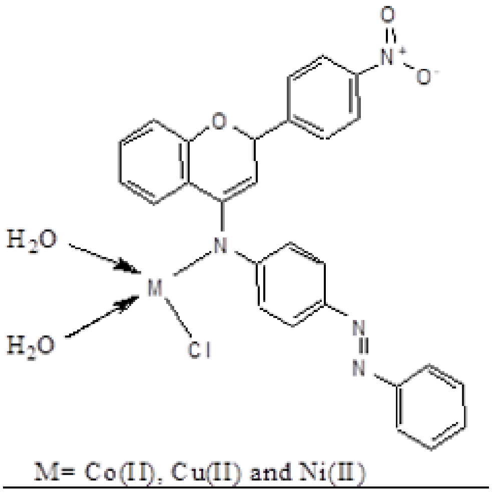


Figure 5: The proposed structure of metal complexes with2H-Chromene azo ligand AH

metal ion (Karekal *et al.*, 2013; Mahalakshmi and Rajavel, 2014).

Many previous studies infer that the compound was to split the DNA; it can prohibit the evolution of the pathogenic organism via the genome cleaving (Yousef *et al.*, 2013).

Fluorescence studies

The fluorescence properties of newly synthesized 2H-chromene azo ligand and its complexes were measured in (10^{-5} M) Ethanol and DMSO solutions. The fluorescent spectra of the free ligand and its complexes when excited at 387 nm in Ethanol, showed a maxima band at 425 nm, as in Figure 3. The low intensity of the band corresponding to complexes is due to a decrease in electron density on the ligand. The low band intensity that belongs to complexes caused the decreasing electron density of the ligand (Yamgar *et al.*, 2009).

The free ligand exhibits an emission maximum at 569 nm with excited at 530 nm in DMSO, and the Cu(II) complex noted an emission band at 565 nm as in Figure 4: but, at the same excited band (530 nm), the Co(II) and Ni(II) complexes possess no fluorescence. The chelation of the metal with ligand performing the posing of the nonfluorescent complex; consequently, the fluorescent intensity is decreased and promotes quenching (Philip *et al.*, 2019)

NMR Studies

The ^{13}C-NMR spectrum of the 2'-hydroxy chalcone (B) shows a significant signal at 203.6 ppm corresponding to carbonyl group that was absent in the spectrum of the 2H-chromene azo ligand.

The ^{1}H-NMR spectrum of the 2'-hydroxy chalcone (B), signal at 11.84 ppm was referred to the phenolic (–OH) proton, while in the spectrum of 2H-chromene azo ligand (AH), the signal at 9.23 ppm (s, 1H, NH) is due to NH proton (El-Deeb *et al.*, 2008), the disappearance of this signal in all spectra of metal complexes indicate its involvement in complexation with metal ion through nitrogen atom via deprotonation.

Infrared spectral studies

The IR spectrum of the prepared starting material 2'-hydroxy chalcone shows the characteristic band at 3548 cm^{-1} indicated that the presence of hydroxyl group and 1639 cm^{-1}remarked the α, β unsaturated carbonyl (>C=O) (Sumathi *et al.*, 2011).

In IR spectrum of 2H-chromene azo ligand (AH) exhibited significant features, the peak at 3485 cm^{-1} was assigned to the secondary (NH) vibration of the ligand which was absent in all-metal complexes, due to deprotonation of (NH) group on binding with metal ions, while the band at 3045 cm^{-1} was characteristic for the existing of aromatic (-C-H) stretching. No shift group was observed in the stretching vibration of the azo group (N=N) of the ligand. This supports the non-involved of an azo group in the metal coordination (Tunçel and Serin, 2006).

The appearance of lower frequencies due to bands of metal bonds with nitrogen, oxygen, and chloride in all metal complexes and show other bands about the region (3438-3315 cm^{-1}) and at the range (845-771 cm^{-1}) ascribed to coordinated water (Aazam *et al.*, 2012).

Electronic Spectra, Magnetic Susceptibility, and Molar Conductivity Measurements

The electronic spectra of ligand and all prepared complexes were recorded in DMSO at r.t.; in the spectra of free ligand showed two absorption bands. The first at 255 nm attributed to π–π* transition of the benzene ring (Khanmohammadi and Darvishpour, 2009; Khedr *et al.*, 2005). The second band noticed at 326 nm can be attributed to n–π* transition. An azo of free ligand (403 nm) did not note a significant shift in all the complexes, it was considered as evidence that no participation of the azo group in complex formation. The Co(II) complex showed two bands at 677 and 615 nm, which can be assigned to the d-d transitions [$^4A_2(F)\rightarrow^4T_1(F)$ &$^4A_2(F)\rightarrow$ $^4T_1(P)$]. These bands, with the magnetic moment of the complex (μ = 4.65 B.M.), usually correspond to a tetrahedral environment around Co(II) ion (Lechat *et al.*, 1993; Knittl *et al.*, 2018). The electronic spectrum of Cu(II) complex exhibited band at 989 nm and other at 891 nm that might be assigned to $^2B_2\rightarrow^2E$ and $^2B_2\rightarrow^2B_1(^2A_1)$ transitions, indicating the Cu(II) complex has distorted tetrahedral geometry (Lever, 1984; Pahonțu *et al.*, 2015) with the magnetic moment (μ = 1.89 B.M.). In the Ni(II) complex, the electronic spectrum showed bands at 646 nm and 785 nm; these bands may be attributed to $^3A_2(F)$ $\rightarrow$ $^3T_2(F)$ and $^3A_2(F)$ $\rightarrow^3T_1(F)$ transitions, respectively. It suggests a tetrahedral geometry of the Ni(II) complex (Lever *et al.*, 1984), and its the magnetic susceptibility equals 3.31 B.M., which sited at the typical field for a tetrahedron geometry.Figure 5 showed the proposed structure of all metal complexes. Molar conductance of the complexes was recorded in ethanol (10^{-3} M). The lower value of molar conductance for all prepared complexes indicates their non-electrolytic character (Geary, 1971), as shown in Table 2.

CONCLUSION

In this study, the 2H-Chromene azo ligand and its chelates with Cobalt(II), Copper(II) and Nickel(II) ions have been synthesized and characterized using various analytical and spectral data. The results demonstrate that the ligand is monodentate; all metal chelates exhibited non-electrolyte and tetrahedral geometry. Antibacterial activities of the free ligand and its metal complexes were investigated against gram (+) and Gram (-) bacteria strains and revealed that Cu(II) and Ni(II) complexes were effective comparables with free ligand against *S. aureus* bacteria. Cu(II) complex activity upon the DNA cleavage studies showed more effective against the DNA isolated from *S. aureus* bacteria than the free ligand and other its metal chelates.

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