



Synthesis, Structural Characterization, Thermal properties and Biological Activity of New Complexes Derived from Ligands with N, O Donor Atoms

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Abstract

The study includes preparation and characterisation of a new tridentate Mannich - β -amino carbonyl ligand and its metal complexes are reported. The formation of the ligand (R)-2-((4-(dimethylamino)phenyl) ((2-nitrophenyl)amino)methyl)-5,5-dimethylcyclohexane-1,3-dione (HL) was achieved from the reaction of 4-dimethylaminobenzaldehyde, 2-nitroaniline and dimedone in a 1:1:1 mole ratio, respectively. The reaction of the ligand with Co(II), Ni(II) and Cu(II) metal ions in a 2:1 (L:M) mole ratio resulted in the isolation of monomeric complexes. The ligand and its metal complexes were characterised using a range of analytical and spectroscopic techniques including; elemental microanalysis, magnetic susceptibility, conductance, TGA, FT-IR, electronic spectra and ^1H , ^{13}C -NMR spectra. The characterisation data indicated the isolation of six coordinate monomeric complexes with the general formula; $[\text{M}(\text{HL})_2]$ (where M= Co(II), Ni(II) and Cu(II)). The ligand and its complexes were screened against several bacterial strains and fungi species. The obtained data indicated that the complexes are more active against several kind of organism, under survey, compared with the free ligand.

Keywords: Dimedone; Mannich - β -amino carbonyl ligand; Monomeric metal complexes; Thermal properties; Structural study.

Introduction

Mannich reaction are one of the most important reactions in organic synthesis C-C bond forming and medicinal chemistry [1]. In these reactions, three or more compounds are used as starting materials. Organic species with N and carbonyl oxygen atoms and their complexes have shown a range of applications in several fields. These include their role as reagents in catalysis and analytical chemistry, biomedical [2, 4], industry [5], agriculture [6] and environmental chemistry [7].

The Mannich reaction is an important synthetic path that played a key role in the development of organic and biochemistry. The synthesis of various drugs including antibacterial, antiprotozoal [8], and other natural products [9, 10] are derived via Mannich approach. Further, Mannich reaction has used in the synthetic of intermediates that required in the pharmaceuticals industry. Organic ligands that based on Mannich approach have used

as chelating agents in the field of inorganic and coordination chemistry [11]. Mannichbased ligands with N and carbonyl O atoms and their metal complexes have an important role in the coordination chemistry and have used as mimics for biomolecules [12]. Mannich-based metal complexes have shown anti-anginals, anti-cancer, anti-tuberculosis, anthelmintic action, hypolipidemic and flavouring activity [13]. More, Mannich compounds have considered as potential scavengers in the removal of heavy elements from water and waste treatment [14].

Experimental

Materials and Methods

All reagents used in this work were commercially available and used as received. The NMR spectra (^1H - and ^{13}C -NMR) for the ligand and complexes were recorded in DMSO- d_6 using a Bruker 300 MHz instruments (300 MHz for ^1H and 75 MHz for

^{13}C) with a tetramethylsilane (TMS) as an internal reference. The FT-IR spectra were recorded as KBr discs in the range 4000-400 cm^{-1} using FTIR-600 FT-IR spectrometer. Melting points, were determined on an electro thermal Stuart apparatus, model SMP40. The electronic spectra were recorded using a (UV-Vis) spectrophotometer type Shimadzu UV-160 in the range 1000-200nm using quartz cell of (1.0) cm length with concentration 10^{-3} mol L^{-1} of samples in DMSO at 25 °C.

The molar conductivity measurements of the complexes were recorded at 25 °C for 10^{-3} - 10^{-5} M solutions of the samples in DMSO using an Eutech Instruments Cyber scan con 510 digital conductivity meter. Elements analysis (C, H and N) and metal content were carried out on a Heraeus instrument (Vario EL) and Shimadzu (A A- 7000) atomic absorption spectrophotometer, respectively. The chloride content for complexes was determined using potentiometric titration method on 686-Titro Processor-665 Dosim A-Metrohm/Swiss. Thermo gravimetric analysis was carried out using Differential Scanning Calorimetry (DSC) on STA PT-1000 Linseis Company/Germany. Magnetic moments at 33°C were measured with a magnetic susceptibility balance on Johnson Matthey.

The evaluation of ligand and its metal complexes against four bacterial species (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*) and two types of fungi (*Candida* and *Trichomoniasis*). Were performed using agar-well diffusion. In this method, the wells were dug in the media with the help of a sterile metallic borer with centres at least 6 mm. Recommended concentration (100 μL) of the test sample 1 mg/mL in DMSO was introduced in the respective wells. The plates incubated immediately at 37°C for 24 h. The activity evaluated by measuring the diameter of inhibition zones (mm).

Synthesis

Synthesis of Mannich-base Ligand (HL)

The ligand was prepared according to approach reported in [15, 17], which based on Mannich condensing and as follows; To a solution of anhydrous calcium chloride 0.5g, 5mmol in ethanol (10ml) was added successively; 4-dimethylaminobezaldehyde (0.74g, 5 mmol), 2-nitroaniline (0.69 g,

5mmol) and dimedone (0.7g, 5 mmol) and three drop of concentration HCl. The reaction mixture allowed stirring about 12h at RT and a yellow solid that formed was filtered off, washed with EtOH (30ml) and water (30ml), and then dried in air. Yield: 0.9g, (44%); m.p = 219°C. Mwt = 409 amu. FT-IR (KBr), 3433 ν (N-H), 1647 ν (C=O), 1601 δ (N-H), 1510 ν (C=C) aromatic and ν (NO₂) 1460 cm^{-1} .

Synthesis of Mannich-base Complexes

The monomeric complexes were isolated adopting an analogue approach. Therefore, the synthesis of [Co(HL)₂]. Is included as an example for the synthesis of the complexes and as follows; To a solution of HL (0.4g, 0.97mmol) in ethanol (5ml) was added a solution of CoCl₂.6H₂O (0. 11g, 0.46mmol). The mixture was stirred about overnight at RT. The reaction mixture allowed to slow evaporation at RT and the obtained solid product that formed was filtered off, washed with 20ml of ethanol and 10ml of diethylether. Yield: 0.21g (50%), mp 255°C ,M.Wt= 877.91amu. [Ni (HL)₂]. (2) Yield: 0.2g (50%), mp > 360°C, M.Wt = 877.67amu. [Cu(HL)₂]. (3) Yield: 0.25g (57%), mp 287°C, M.Wt = 882.52amu. (Table (1)). The FT-IR and electronic data are listed in Tables (2) and (3), respectively.

Determination of Biological Activity

The sensitivity of bacteria and fungi against the prepared compounds were explored by Kirby-Bauer method. In this work, the colony of organism was mixed with a solution of 85% NaCl, until the suspension becomes (0.5 Mcf). This suspension was applied on the surface of a Petri dish filled with Mueller Hinton agar. The holes were made with the same distance and exact concentration. Recommended concentration (100 μL) of the test sample 1mg/mL in DMSO was introduced in the wells. The dishes were incubated for 24 h at 37°C and the inhibition zones were measured and compared with standard values [18]. The role of DMSO solutions in the biological evaluation were examined separately that indicated no activity against any bacterial strains or fungi species.

Results and Discussion

The ligand (R)-2-((4-(dimethylamino) phenyl) ((2-nitrophenyl)amino) methyl)-5,5

dimethylcyclohexane-1,3-dione (HL) was obtained adopting the Mannich approach using CaCl_2 as a catalyst. The reaction of dimethylaminobenzaldehyde, 2-nitroaniline and dimedone in a 1:1:1 molar ratio, respectively resulted in the formation of the ligand, Scheme (1). The ligand behaves as a tridentate species providing carbonyl oxygen and nitrogen atom of the secondary amine as donor atoms. The reaction of the ligand with the metal chloride of Co(II), Ni(II) and Cu(II), in a 2:1 (L:M) mole ratio resulted in the isolation of six-coordinate neutral monomeric complexes of the general formula $[\text{M}(\text{HL})_2]$ (were $\text{M} = \text{Co}(\text{II}), \text{Ni}(\text{II})$ and $\text{Cu}(\text{II})$)

respectively (Scheme (2)). The isolated monomeric complexes are air-stable solids and dissolve in DMSO and DMF, bar from other common organic solvents. The complexation behaviour and coordination geometries of complexes were assumed from their physico-chemical data respectively, These data (Table 1) agree well with the suggested formula. Conductance measurements of the complexes in DMSO solutions indicated complexes are neutral. The entity of Mannich-base was confirmed by elemental analysis (Table 1), FT-IR (Table 2), UV-Vis spectroscopy (Table 3) and ^1H , ^{13}C -NMR spectra.

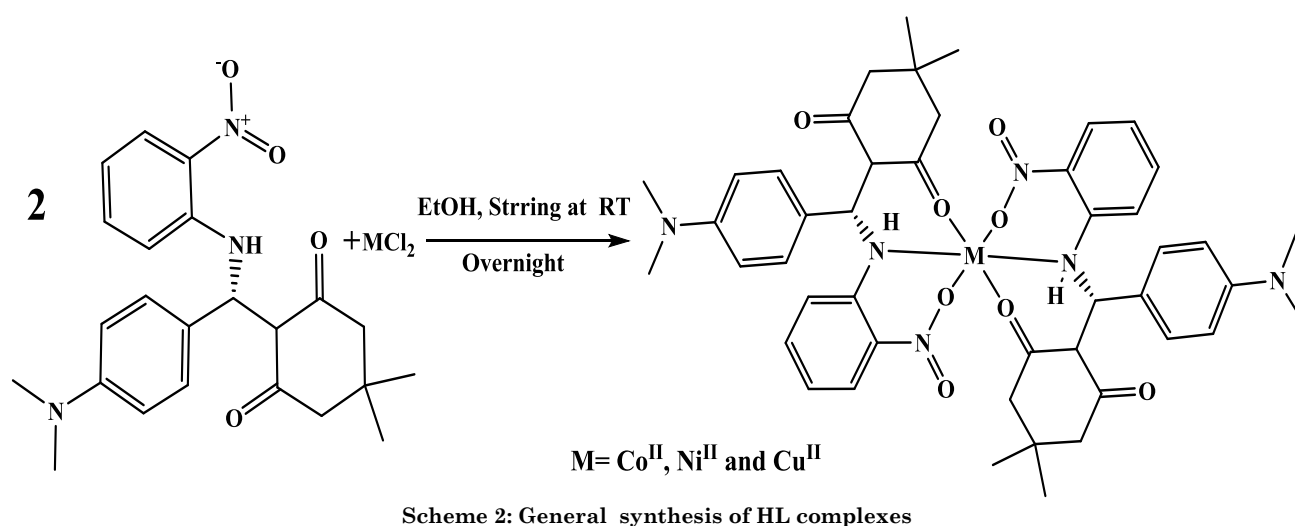
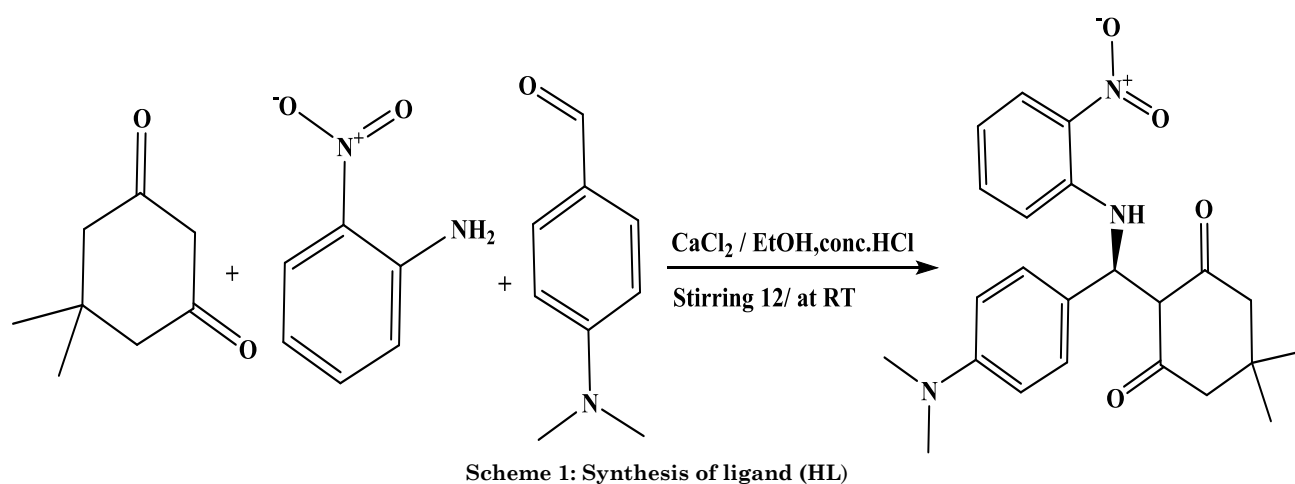


Table 1: Microanalysis and physical properties of HL its complexes

Complex	Molecular formula	M.Wt	Colour	m.p. °C	Yield (%)	Micro analysis calculated found (%)			
						C	H	N	M
HL	$\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_4$	409	Yellow	219	44	(67.02) 67.45	(6.23) 6.60	(10.00) 10.26	-
$[\text{Co}(\text{HL})_2]$	$\text{C}_{46}\text{H}_{54}\text{CoN}_6\text{O}_8$	877.91	Pale green	255	50	(62.34) 62.93	(6.00) 6.21	(9.22) 9.56	(6.45) 6.67
$[\text{Ni}(\text{HL})_2]$	$\text{C}_{46}\text{H}_{54}\text{NiN}_6\text{O}_8$	877.67	Greenish-yellow	360*	50	(62.24) 62.94	(6.96) 6.21	(9.24) 9.57	(6.34) 6.68
$[\text{Cu}(\text{HL})_2]$	$\text{C}_{46}\text{H}_{54}\text{CuN}_6\text{O}_8$	882.52	Pale brown	287	57	(62.18) 62.60	(5.97) 6.17	(9.11) 9.51	(7.00) 7.19

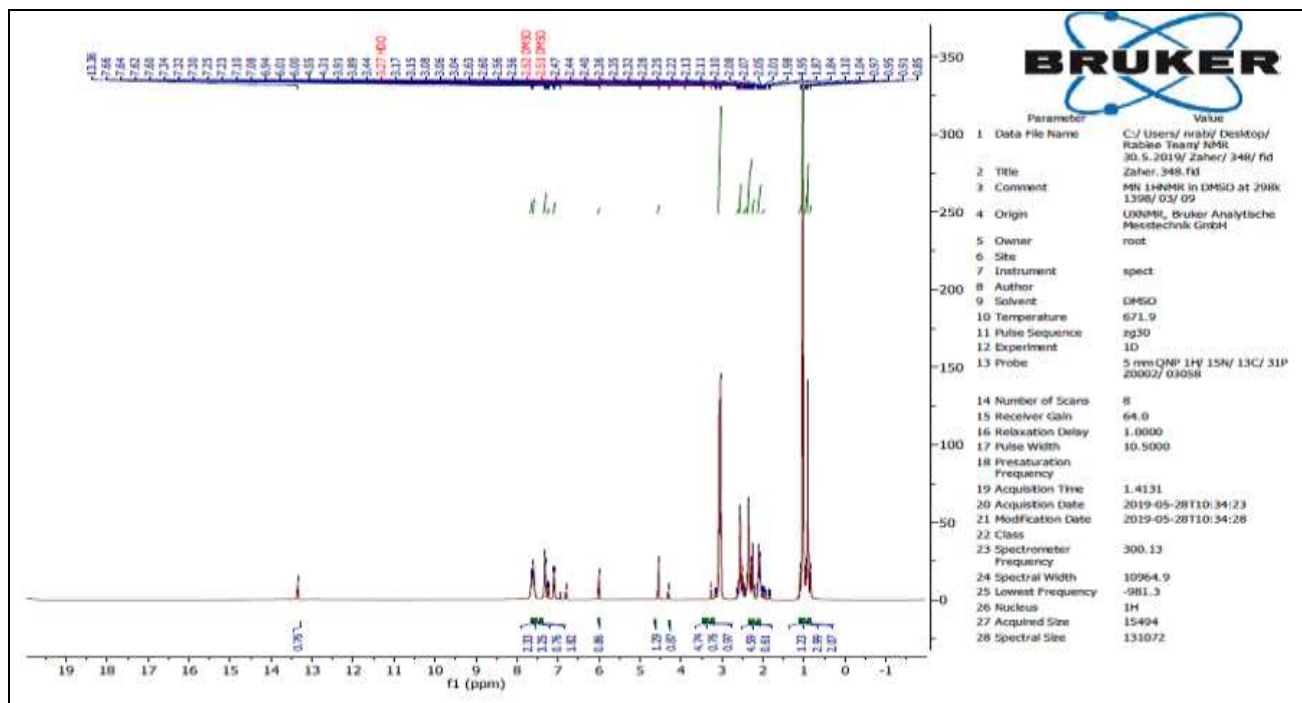


Figure 1: ¹H NMR spectrum of HL in DMSO-d₆ solutions

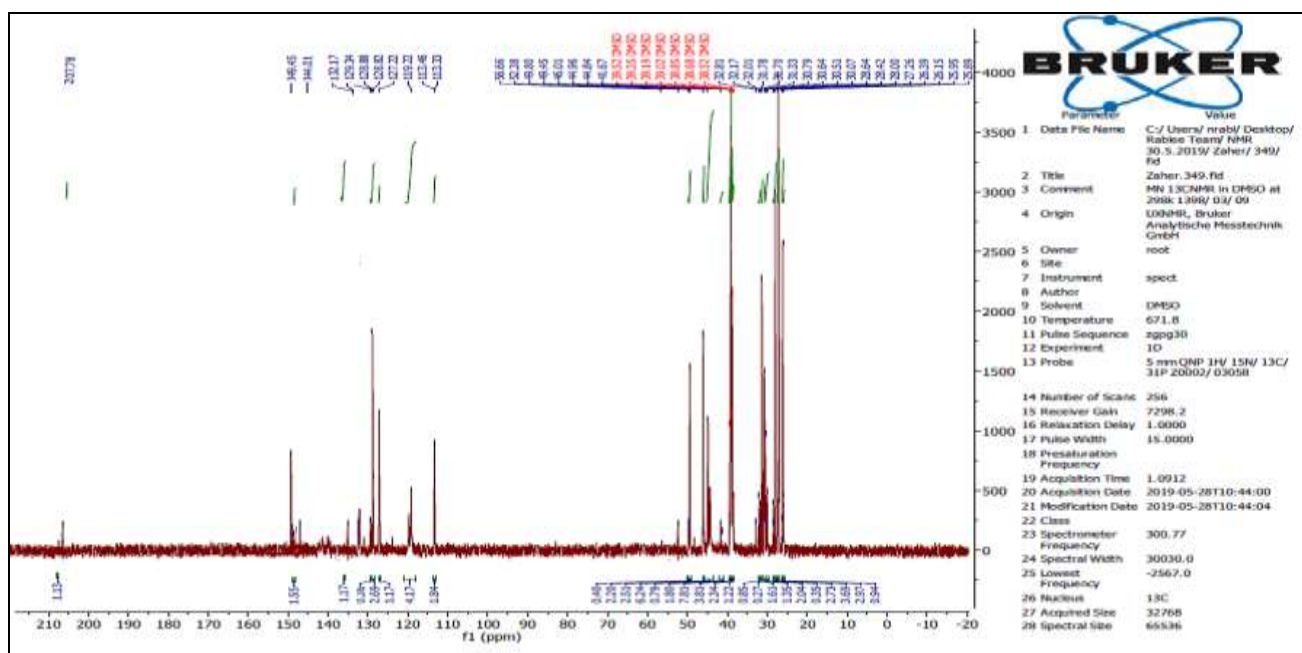


Figure 2: ¹³C NMR spectrum of HL in DMSO-d₆ solutions

FT-IR and NMR

The important infrared bands of the ligand and its monomeric complexes together with their assignments are listed in (Table 2). The HL spectrum revealed a peak brood at 3433 cm⁻¹ assigned to tutomerism between (NH) of the secondary amine and OH enol of the carbonyl group [19], and the spectrum exhibited a band at 1647cm⁻¹ assigned to v(C=O) of the carbonyl group [20].

The band at 1647cm⁻¹ that related to v(C=O)_{keto} in free ligand was shifted to higher frequency at 1653,1662 and 1666 cm⁻¹ in complexes, respectively. The shifting of the carbonyl moiety may attributed to the

coordination of the oxygen atom of the carbonyl to the metal centre in a similar fashion to that reported in literature [21]. Further, the spectra of the metal complexes showed new bands, which are not observed in the spectrum of the ligand, around 598-550cm⁻¹ assigned to v(M-O) [21, 20]. Bands recorded around 480-471cm⁻¹ attributed to v(M-N) [21, 22], respectively. Finally, The spectra of complexes revealed additional peaks, at 3425, 3440 and 3433cm⁻¹ in the complexes of Co^(II), Ni^(II) and Cu^(II), respectively. The presence of these peaks may be related to intramolecular hydrogen bonding between NH and OH [19], The ¹H-

NMR spectra of the ligand (HL) in DMSO-d₆ solvents are depicted in Figure (1). The spectrum of the free Mannich-base (HL) in DMSO-d₆ solvent shows peak at 6.01 ppm equivalent to one proton that assigned to (N-H) the secondary amine and The signal at 13.35 ppm that belongs to O-H which

equivalent to one proton. The ¹³C-NMR spectra of the free Mannich-base (HL) in DMSO-d₆ solvents is depicted in Figure (2). The free Mannich-base (HL) shows peak at 207.87ppm assigned to C=O of the carbonyl group of the cyclohexyl segment.

Table 2: The FT-IR spectral data of HL and its complexes (cm⁻¹)

Compounds	v(NH) v(OH)	v (C-H) _{ar}	v (C-H) _{al}	v(C=O) _{ket.}	δ(N-H)	v(C=C) _{aro.}	v(NO ₂)	v(M-O)	v(M-N)
HL	3433	3043,3022	2958,2912, 2887,2873	1647	1601	1510	1460	-	-
[Co(HL) ₂]	4325	3037,3020	2958, 2875	1653	1603	1514	1465	598.575	480
[Ni(HL) ₂]	3440	3070,3016	2960, 2875	1662	1620	1516	1456	584.550	472
[Cu(HL) ₂]	3433	3070,3014	2960, 2877	1666	1615	1510	1466	579.563	471

Electronic Spectra and Magnetic Moment Measurements

The electronic spectra and magnetic moments data of the Mannich-base complexes are collected in (Table 3). The electronic spectra of the complexes display various peaks around 297-291nm attributed to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$, respectively. Additional peaks at 378-445nm assigned to charge transfer (C.T) [23,24]. The electronic spectrum of Co (II) reveals bands in the d-d region at 613 and 656nm that assigned to ${}^4T_{1g}^{(F)} \rightarrow {}^4A_{2g}^{(F)}$, respectively indicating a distorted octahedral structure about the cobalt centre. The obtained magnetic moment

value is in accordance with Co(II)-complexes in which the arrangement about the Co atom is a distorted octahedral [25,26]. Band observed in the Ni(II)-complex at 612nm attributed to ${}^1A_{1g}^{(F)} \rightarrow {}^1A_{2g}^{(F)}$ confirming a distorted octahedral arrangement about the metal centre. The magnetic value of the complex is consistent with its octahedral arrangement. The magnetic moment value of Cu (II) is agreed with the distorted octahedral geometry. The spectrum of Cu(II) complex revealed peaks at 597and 645nm, which attributed ${}^2B_{1g} \rightarrow {}^2B_{2g}$, respectively. These transitions are characteristic for Cu-complex with distorted octahedral structure [25, 27].

Table 3: Electronic spectra data in DMSO solutions and magnetic moment of HL complexes

Complex	λ_{nm}	ϵ_{max} (dm ³ mo ⁻¹ cm ⁻¹)	Assignment	Suggested geometry	μ_B BM
[Co(HL) ¹] ₂	291	1935	L.F	Distorted octahedral	2.6
	399	1955	C.T		
	445	120	C.T		
	613	92	${}^4T_{1g}^{(F)} \rightarrow {}^4A_{2g}^{(F)}$		
	665	117	${}^4T_{1g}^{(F)} \rightarrow {}^4A_{2g}^{(F)}$		
[Ni(HL) ¹] ₂	297	2333	L.F	Distorted octahedral	2.9
	388	1697	C.T		
	612	45	${}^1A_{1g}^{(F)} \rightarrow {}^1A_{2g}^{(F)}$		
[Cu(HL) ¹] ₂	295	2322	L.F	Distorted octahedral	1.9
	378	2380	C.T		
	597	108	${}^2B_{1g} \rightarrow {}^2B_{2g}$		
	645	80	${}^2B_{1g} \rightarrow {}^2B_{2g}$		

Thermal Analysis

Thermal analysis results of the ligand (HL) and $[\text{Co}(\text{HL})_2]$ complex were determined from ambient temperature to 1200°C in the atmosphere of nitrogen. The first exothermic peak detected at 219°C may attribute to the loss of a molecule of the $(\text{NH}_3 + \text{NH}_2 + \text{CO}_2 + \text{H}_2\text{CO} + (\text{CH}_3))$ segment; (obs.= 0.648 mg, 30.26%; calc.=0.639mg, 29.28%).

The second step occurred at 625°C indicated the loss of $(\text{CO} + \text{NH}_2 + \text{C}_2\text{H}_2 + \text{C}_6\text{H}_2 + \text{C}_7\text{H}_4)$ fragment; (obs.=1.22 mg, 56.92%; calc.=1.21mg, 56.47%). The third step recorded at 1005°C indicated the loss of (4H_2) fragment, (obs.= 0.044mg, 2.055%; calc.=0.042mg, 1.97%). The final residue of the (4C) calc.= 48.06mg, 11.73%.The differences between the observed and the calculated values may due to sublimation. The DTGA indicated several peaks at 219, 404 and 457°C . The second peak may indicate the melting point of the ligand. In the DSC analysis, peaks pointed at 404 and 457°C correlated to endothermic decompositions process.

However, the peak at 219°C refer to exothermic decompositions process. The exothermic and endothermic peaks may indicate combustion of the organic ligand in nitrogen atmosphere. The thermal analysis of $[\text{Co}(\text{HL})_2]$, The first exothermic peak detected at 310°C may attribute to the loss of a molecule of the $(\text{N}_2 + \text{NH}_3 + \text{CO} + \text{CO}_2 + \text{N}_2\text{H}_4 + \text{C}_2\text{H}_2 + \text{CH} + \text{C}_3\text{H}_6)$ segment; (obs. =1.272mg, 28.82%; calc.=1.257mg, 28.49%). The second step occurred at 550°C indicated the loss of $(\text{CO}_2 + 2\text{CO} + \text{CH}_4 + \text{HCN} + \text{C}_2\text{H}_4 + \text{C}_2\text{H}_6 + \text{C}_4\text{H}_{10} + \text{C}_6\text{H}_5)$ fragment; (obs. = 1.691 mg, 38.32%;calc.=1.6mg, 38.30%).

The third step recorded at 1000°C indicated the loss of $(\text{CO} + \text{CH} + \text{C}_3\text{H}_2 + \text{C}_6\text{H}_2)$ fragment,(obs.=0.7855mg, 17.80%; calc.= 0.7694mg, 17.43%). The final residue of the $(\text{C}_8\text{H}_3 + \text{Co})$ calc.= 157.9, 17.98%. The DTGA indicated several peaks at 197, 214, 400 and 950°C . A series of peaks pointed at 197 and 400°C correlated to exothermic decompositions process. However, the detected peaks at 214 and 950°C refer to endothermic decompositions process.The exothermic and endothermic peaks may indicate combustion of the organic ligand in nitrogen atmosphere.

Biological Activity

The synthesised ligand and its metal complexes were tested for their microbiological activity against two strains of bacterial species: G-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and G-negative (*Escherichia coli* and *Pseudomonas aeruginosa*). Additional to two types of fungi were explored (*Candida* and *Trichomoniasis*). The selected bacteria considered to be as the mostly harmful and deadly kind of bacteria. These bacteria are widely detected in the surgery operation rooms of hospitals. Further, two types of fungi were explored that have impact on human being, animals and plants.

Table (4), displays the evolution of diameter zone (mm) of inhibition of HL-complexes against the growth of various bacteria and fungi strains. The strains of bacteria, under this study, revealed high resistant against HL, which mean that the ligand showed no activity against all type of bacteria.

However, the metal complexes have shown antimicrobial activity against several bacteria species, compared with the free ligand, The highly activity of the complexes, especially Co (II) complex, could be discussed on the basis of chelation theory and Overtone's model [28]. According to the chelation hypothesis, the complex has the ability to move and across the cell membrane of organism. This may relate to the decrease of the polarity of the metal ion, by the partial sharing of metal positive charge with donor groups.

Subsequently, this will enhance the lipophilic property of the chelation system of metal allowing the complex to cross the lipid layer of the cell tissue [29]. This may attribute to the size of the metal ion and/or the stability of the complex in the tested medium, the complex suffers decomposition in the medium,From the obtained data, the ligand indicated no activity against the two types of fungi. Otherwise, the complexes revealed activation toward fungi organism. Generally, the increase of the anti-fungal activity of complexes could describe by the chelation concept [30], which related to the delocalization of π -electrons across the entire chelate segment. This mean that the polarity of the ligand and the central metal atom decreases and then results in the incursion of

the complex through the lipid tissue of the cell membrane.

Table 4: The biological evaluation of ligand and its complexes

Compounds	<i>Escherichia coli</i> (G-)	<i>Pseudomonas aeruginosa</i> (G-)	<i>Bacillus stubtilis</i> (G+)	<i>Staphylococcus aureus</i> (G+)	<i>Candida</i>	<i>Trichomoniasis</i>
HL	-	-	-	-	-	-
[Co(HL) ₂]	12	7	17	20	19	11
[Ni(HL) ₂]	7	11	2	9	8	5
[Cu(HL) ₂]	3	16	4	12	7	3

Conclusion

The preparation and characterisation of a new tridentate Mannich β -amino carbonyl ligand and its metal complexes are reported. The formation of the ligand (R)-2-((4-(dimethylamino) phenyl) ((2-nitrophenyl) amino) methyl)-5,5-dimethylcyclohexane-1,3-dione (HL) was achieved from the reaction of 4-dimethylaminobenzaldehyde, 2-nitroaniline and dimedone in a 1:1:1 mole ratio, respectively. The reaction of the ligand with Co(II), Ni(II) and Cu(II) metal ions in a 2:1

(L:M) mole ratio resulted in the isolation of monomeric complexes. Physico-chemical and spectroscopic methods were implemented to confirm mode of bonding and over all structure of the complexes. These results lead to the preparation of six coordinate complexes.

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