



Preparation, Characterization and Biological Activity Studies of Some Metal Complexes with New Schiff Base for Trimethoprim Ligand

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Abstract

A new ligand complexes have been synthesis from reaction of metal ions of Mn^{II} , Co^{II} , Ni^{II} , Cu^{II} , Zn^{II} , Cd^{II} and Pd^{II} with schiff base [(E)-1-((2-amino-5-(3, 4, 5-trimethoxybenzyl) pyrimidin-4-ylimino) methyl) naphthalen-2-ol [HL)]. The prepared [HL] was characterized by FT-IR, UV-Vis spectroscopy, 1H - ^{13}C -NMR spectra Mass spectra and melting point. The compounds were characterized by techniques UV-Vis and FT-IR spectral studies, micro analysis (C.H.N), determination of atomic absorption, chloride content, molar conductivity measurements, magnetic susceptibility and melting point. The ligand acts as a monobasic tridentate, coordinating through deprotonated phenolic O and azomethine N atoms. The compounds are neutral electrolytic in dimethylsulfoxide. The analytical and spectral data for these compounds indicated to (1:2) (metal: ligand) mole ratio. The metal content and magnetic moment suggest the square planar geometry for the palladium ion, but the other metal complexes show six-coordinate. Compounds were having been screened for their antimicrobial activities against various bacteria and fungi organisms using inhibition method.

Keywords: Preparation, Characterization, Azomethine, Inhibition method.

Introduction

Antibiotic is a a compound or substance which inhibit the growth of bacteria [1]. Trimethoprim (TMP) and its derivatives are broad spectrum antimicrobial agents with anti-parasitic activity [2] and were first described by Roth and coworker [3]. Schiff bases, also known as azomethine due to presence of ($-C=N-$) group have been extensively used as ligands in coordination chemistry in order to their stellart donor capacities as chelating agent [4].

A number of azomethine molecules show biological activities such as anticancer, antifungal, antibacterial, anti-corrosion, antitumor, anti-inflammatory activities and antiproliferative [5-8]. Particularly, range of transition metal complexes of azomethine ligands derived from the reaction of 2-hydroxyl-1-naphthaldehyde and various primary amines became the topic of contemporary research [9]. In the present work was to prepare a novel ligand derived from trimethoprim drug of azomethine with number metal complexes and study of their biological activity as antimicrobial agents.

The antimicrobial activity was evaluated against two bacterial strains and two fungal species.

Experimental

All chemicals reagents were used without further purification and purchased from the Merck Chemical Company in very high purity. The amine and 2-hydroxy-1-naphthaldehyde were purified by standard procedures. Their purity was determined by thin layer chromatography (TLC).

Instrumentation

Melting points were recorded by using Stuart SMP40 apparatus. U.V-Vis. Spectra were obtained in the range (200-1100) nm in DMSO using a (Shimadzu UV-visible-160 A Ultra Violet-Visible Spectrophotometer). FT-IR-Spectra were determined by (Shimadzu FTIR- 8400s) between (400-4000) cm^{-1} as potassium bromide disk.

The 1H , ^{13}C -NMR spectra were performed in Bruker Ultrashield 300 MHz for 1H -NMR and 75 MHz for ^{13}C -NMR respectively, and using

DMSO as solvent, Turkey / Gazi University. Mass spectra was obtained by positive (lc/ms-ms) using agilent q_trap3200 mass spectroscopy Mashhad University of Medical Sciences, Iran. Elemental micro analysis (Carbon, Hydrogen and Nitrogen) recorded by (Vario EL chns, Elementar) at Iran.

Atomic absorption spectroscopy was gained on a (Varian-AA 775 Atomic Absorption Spectrophotometer). Chloride was determined for compound by potentiometric titration method on (686-Titro Processor-665 Dosimat Metrohm Swiss).

Molar conductivity measurements ($10^{-3}M$) in Dimethylsulfoxide of compound were obtained by using (Philips PW 9526 Digital Conductivity meter). Magnetic moment balance (Sherwood Scientific) was used to record magnetic of compounds. The purity determination for the ligand by using TLC silica-gel Polygram SILG/UV 254 plates.

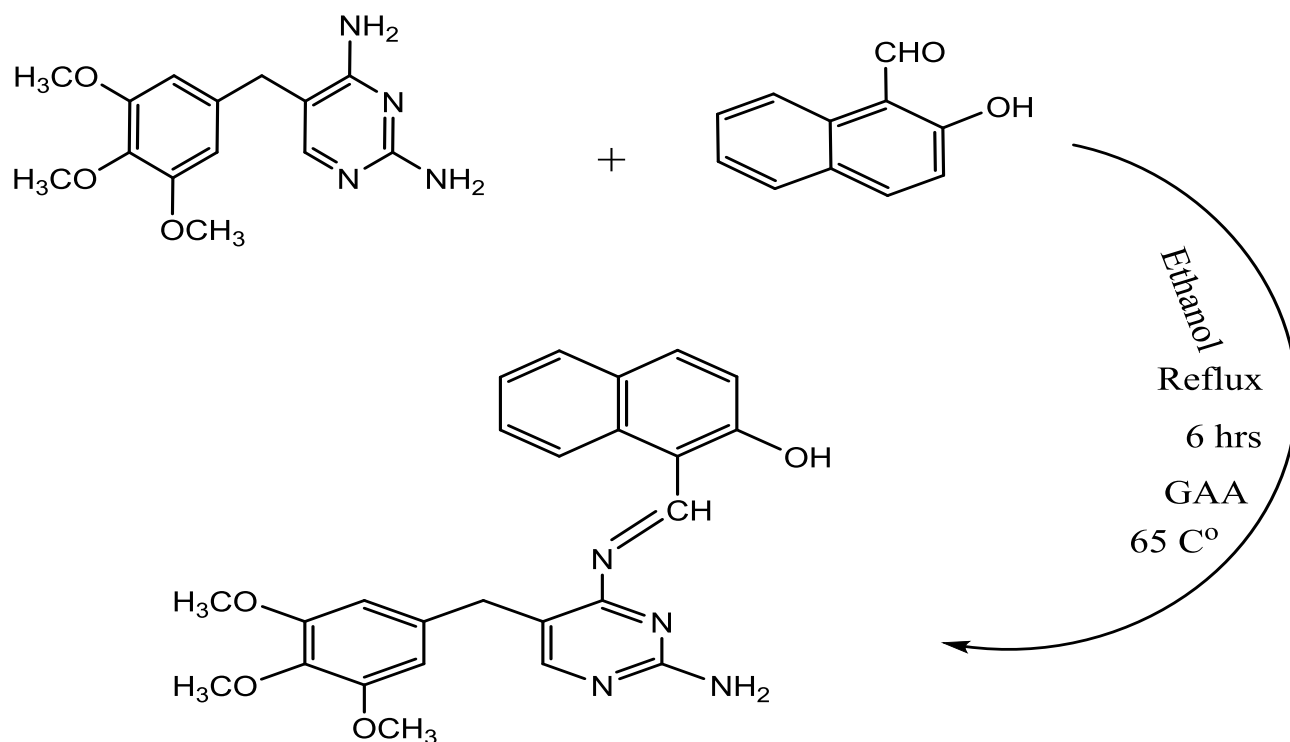
Preparation of the Compounds

Preparation of the ligand [HL] [10]

ligand [HL] was prepared with a weight (0,059g,0.344 mmol) of 2 hydroxynaphthaldehyde, dissolved in (10 ml) from Et-OH with continuous stirring until the solubility was completed, the weight of (0.1g, 0.344mmol) trimethoprim and dissolved in (10 ml) of Et-OH with stirring until solubility was complete.

After the dissolving, the second solution was added to the first solution and drops of glacial acetic acid were added with stirring. And the process of reflux for 6 hrs ($65^{\circ}C$).

The of the reaction (monitored by TLC), the yellow coloured compound is left for overnight and the deposit and washing of ethanol and air dried. The yield was obtained at a weight of (0.1419g, 0.3192mmol), m.p ($152^{\circ}C$), yield (92%), Scheme (1).



Scheme 1: Synthesis route of the ligand [HL]

Preparation of the Complexes [11]

The compounds were prepared from reaction the [HL] with the metal salts in a 2:1 mole ratio. The metal solution of $MnCl_2 \cdot 4H_2O$ (0.022g, 0.1124mmol) in (10 ml) Et-OH is stirred for (10 minutes). The [HL] solution (0.1g, 0.2249mmol) in (10 ml) ethanol after being adjusted to (pH= 9) by added potassium hydroxide to the ligand solution.

The mixture under reflux for 2 hrs. After the mixture was filtered, the precipitate is washed with an excess of Et-OH, then dried at room temperature for (24hrs). A mustard solid is obtained. A similar method to that mentioned in the preparation of Mn^{II} compound is applied for the preparation of metal ions compounds Figure (1). The physical properties and micro analysis of [HL] and its compounds is given in Table (1).

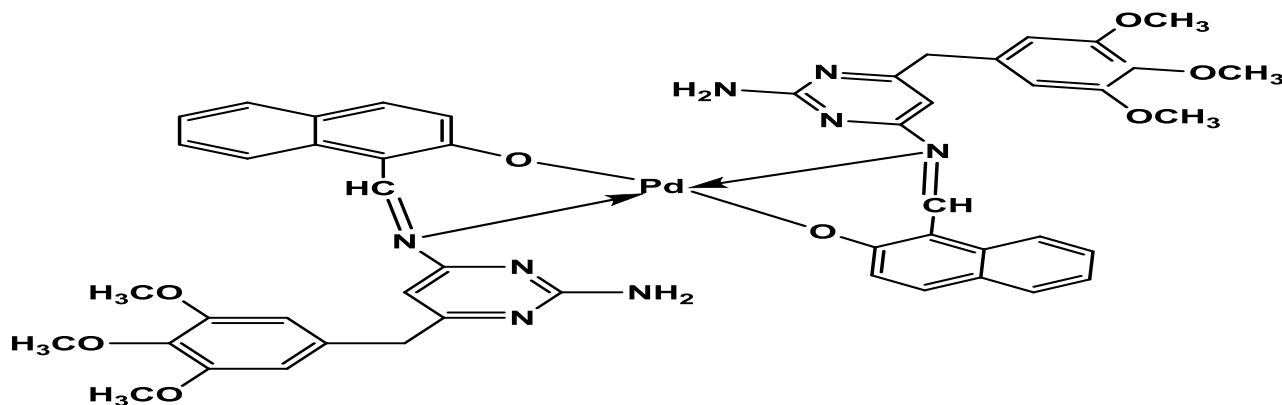
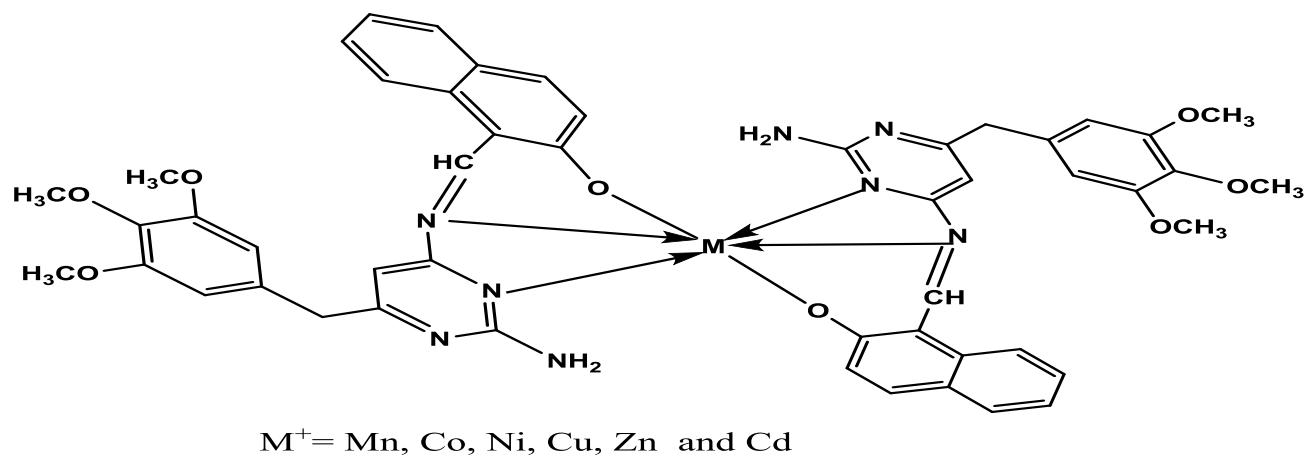


Figure 1: The proposed structural formula of the complexes

Table 1: Physical properties and micro analysis of [HL] and Compounds

Compound	Empirical Formula (formula wt.)	M.P Dec .	Yield %	Color	Elemental Analyses Found (Calc.) %(calculated) (% Found/(Calcd.))					Molar conductivity ($\text{Ms}^{-1} \text{cm}^2 \text{mol}^{-1}$)
					C	H	N	M	Cl	
[HL]	$\text{C}_{25}\text{H}_{24}\text{N}_4\text{O}_4$ (444.48)	152	92	Yellow	67.38 (67.55)	4.88 (5.44)	12.19 (12.60)	-	-	-
[Mn(L) ₂]	$\text{C}_{50}\text{H}_{46}\text{MnN}_8\text{O}_8$ (941.89)	175	71	Masturd	63.24 (63.76)	3.78 (4.92)	11.81 (11.90)	5.28 (5.83)	Ni 1	18.3
[Co(L) ₂]	$\text{C}_{50}\text{H}_{46}\text{CoN}_8\text{O}_8$ (945.88)	186	60	Brown	63.19 (63.49)	3.99 (4.90)	11.77 (11.85)	6.03 (6.23)	Ni 1	11.4
[Ni(L) ₂]	$\text{C}_{50}\text{H}_{46}\text{NiN}_8\text{O}_8$ (945.64)	239*	60	Mastrud	63.73 (63.51)	3.78 (4.90)	11.12 (11.85)	6.43 (6.21)	Ni 1	16.8
[Cu(L) ₂]	$\text{C}_{50}\text{H}_{46}\text{CuN}_8\text{O}_8$ (950.50)	251*	62	Deep Green	63.03 (63.18)	4.38 (4.88)	11.34 (11.79)	7.01 (6.69)	Ni 1	9.5
[Zn (L) ₂]	$\text{C}_{50}\text{H}_{46}\text{ZnN}_8\text{O}_8$ (952.36)	119	78	Deep brown	63.12 (63.06)	3.46 (4.87)	10.96 (11.77)	6.59 (6.87)	Ni 1	9.8
[Cd(L) ₂]	$\text{C}_{50}\text{H}_{46}\text{CdN}_8\text{O}_8$ (999.36)	215	66	Brown	60.31 (60.09)	3.95 (4.64)	11.19 (11.25)	11.16 (11.25)	Ni 1	11.5
[Pd(L) ₂]	$\text{C}_{50}\text{H}_{46}\text{PdN}_8\text{O}_8$ (993.37)	245*	62	Deep brown	60.22 (60.45)	4.11 (4.67)	11.20 (11.27)	10.68 (10.71)	Ni 1	13.5

M.P=melting point Decompose = * Calc. =calculated

Biological Activity

The standard disc agar diffusion method [12] was resulted to determine the antibacterial and antifungal activity of the prepared compounds.

The compounds were dissolved in DMSO (which have no inhibition activity), conc. of 100 $\mu\text{g/ml}$. Uniform size filter paper disks were impregnated by equal volume (0.1 ml) from the particular concentration of dissolved

compounds and closely topic on incubated agar surface. After incubation for 48 h at 37°C, inhibition of the organisms which it is clear by clear zone surround each disk was measured and used to calculate mean of inhibition zones.

Results and Discussion

The azomethine ligand [HL] was synthesis by the condensation reaction. The element analysis data of the synthesis azomethine and its complexes with the metal salts are in good convention with the calculated results from expected formula of each prepared compounds in Table (1). All these complexes are colored solid, stable in air, insoluble in water and common solvents, except DMF and DMSO. The conductivity results for the compounds in DMSO, ranging in the (18.3-9.5) region, indicate that the complexes are non electrolytes [13]. Chloride ion (Cl) with silver nitrate solution was (+) [14, 15]. The calculated and experimental values of (M %) in each complex are in fair agreement as shown in Table (1).

Infrared Spectra

Important spectral bands for the [HL] and its compounds are presented in Table (2), Figures (2, 3). The spectra of free azomethine ligand showed the band at 3448 cm^{-1} was due to stretching vibration of

phenolic u (O-H) group [16]. This band was absent in all the complexes, indicating deprotonation on coordination of the ligand to metals [16]. Infrared spectra of the compounds and [HL] were compared, it was observed that absorption bands $\nu_{\text{asy}}(\text{NH}_2)$ and $\nu_{\text{sy}}(\text{NH}_2)$ modes of pyrimidine (NH_2) group in the free ligand due to (3425, 3332 cm^{-1}) are not significantly shifted with respect to those of the complexes [17]. The imine (C=N) out of ring functional group of the free ligand was observed as strong band (1658) cm^{-1} was blue-shifted to (1643-1627) cm^{-1} , indicating coordination of azomethine nitrogen of the azomethine ligand to metal ion [18]. In addition, the band at (1284) cm^{-1} assigned the phenolic u (C-O) stretching vibrations of the free ligand was blue-shifted to (1242-1238) cm^{-1} upon complexation suggesting the involvement of the phenolic oxygen atom in the coordination [19].

At lower frequency the compounds appearance new bands at (551-439) cm^{-1} and (451-405) cm^{-1} which are due to $\nu(\text{Metal-N})$ and $\nu(\text{Metal-O})$, respectively [20, 21]. It can be concluded that (HL) binds to the metal ions through Schiff base nitrogen and phenolic oxygen and the [HL] behaves as neutral tridentate ligand with all complexes except Pd^{II} the [HL] behaves as neutral bidentate ligand.

Table 2: FT-IR spectral data (wave numbers) cm^{-1} of the ligand and its complexes with some metal ions

Compound	$\nu(\text{O-H})$	$\nu_{\text{asym}}(\text{NH}_2)$ $\nu_{\text{sym}}(\text{NH}_2)$	$\nu(\text{C=N})$ imine out ring	$\nu(\text{C=N})$ pyrimidine	$\nu(\text{C-O})$ phenolic	$\nu(\text{C-H})$ arom.	$\nu(\text{C-H})$ alph.	$\nu(\text{Metal-N})$	$\nu(\text{Metal-O})$
[HL]	3448	3425 3332	1658	1593	1284	3005	2904 2835	-	-
[Mn(L) ₂]	-	3406 3321	1643	1585	1238	-	2889 2835	478 459	435
[Co(L) ₂]	-	3406 3325	1639	1585	1242	-	2927 2835	551 513	451
[Ni(L) ₂]	-	3406 3321	1643	1583	1242	-	2993 2835	493 459	420
[Cu(L) ₂]	-	3483 3417	1639	1585	1242	3066	2927 2835	486 455	432
[Zn(L) ₂]	-	3494 3334	1631	1585	1242	3097	2931 2835	528 474	447
[Cd(L) ₂]	-	3479 3417	1639	1585	1238	3074	2997 2835	466 439	405
[Pd(L) ₂]	-	3332 3194	1627	1593	1242	-	2927 2839	455	432

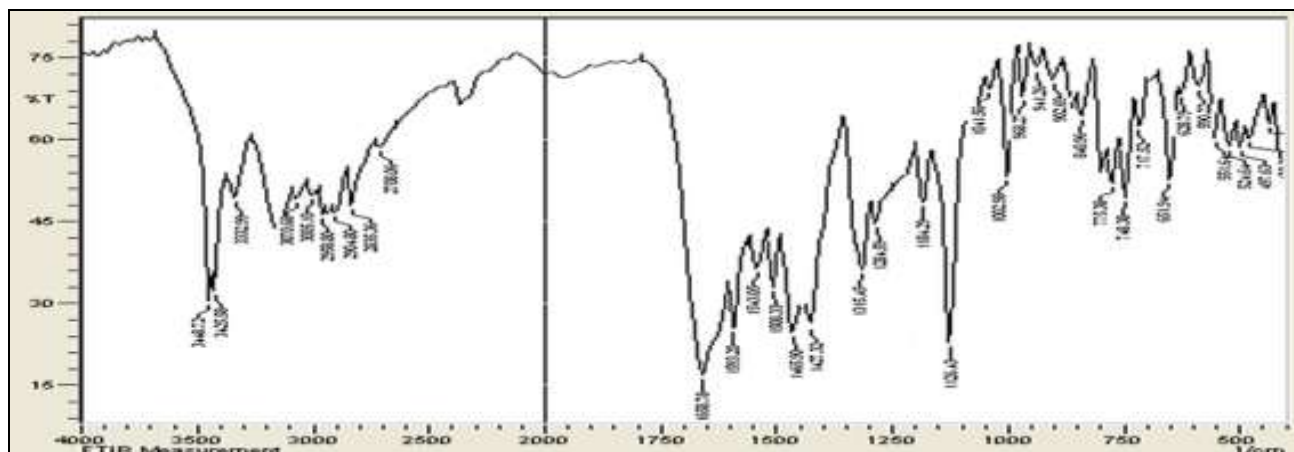


Figure 2: The FT-IR spectrum of ligand [HL]

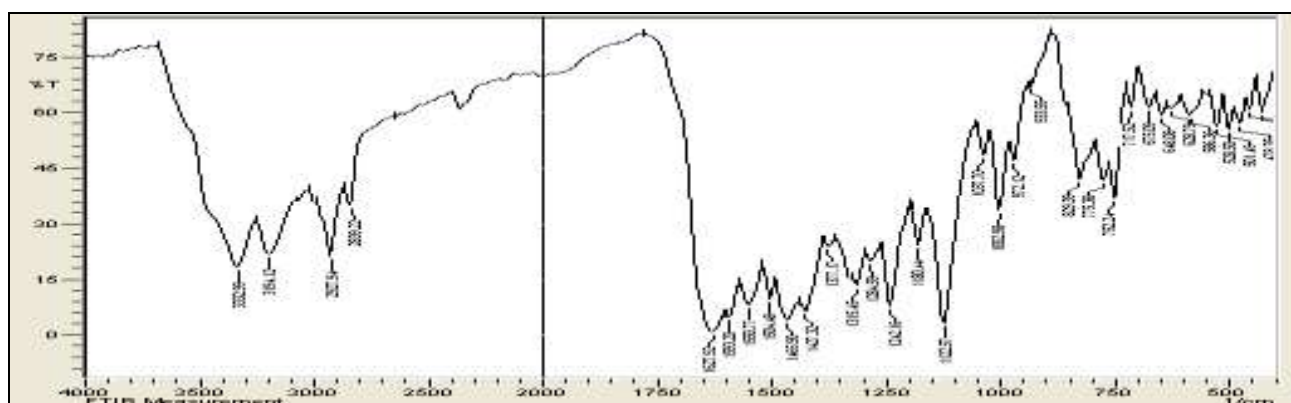


Figure 3: The FT-IR for the [Pd (L)₂] complex

¹H-NMR Spectrum of ligand and Complex [Pd (L)₂]

The ¹H-NMR Spectra for ligand and its [Pd (L)₂] complex were registered in Dimethylsulfoxide as solvent. The [HL] showed peak appearance of a signal at δ (10.78 ppm) due to (OH) phenolic for the [HL] that disappeared on complexation indicating

the involvement of phenolic (O) in the link *via* deprotonation in the complex[22]. The peak appeared at δ (9.49ppm) referred to the proton of imine group (CH=N) in the ligand that was shifted to δ (9.53ppm) in the complex [23, 24]. The ¹H NMR spectrum of [HL] and of the [Pd (L₂)] complex are shown in the Figures (4 and 5) and in the Table (3).

Table 3: ¹H-NMR data for [HL] and its complex measured in DMSO-d₆ and chemical shift in ppm (δ)

Compounds	Functional groups	δ (ppm)
<p>Schiff base</p>	(OH) For phenolic group	(10.78)(1H,s)
	(CH=N imine) (C ₁₁)	(9.49) (1H,s)
	Ar-CH(C ₁₄)	(8.95) (1H,s)
	Ar-CH(C ₄)	(8.10) (1H,s)
	Ar-CH(C ₅ ,C ₈)	(8.07) (2H,m)
	Ar-CH(C ₃ ,C ₆ ,C ₇)	(7.21,7.39,7.51)(3H,m)
	(NH ₂) For amine group	(6.56)(1H,s)
	Ar-CH (C ₁₈ , C ₂₂)	(6.64)(2H,m)
	for OCH ₃ groups	(3.72)(9H,m)
	DMSO-d ₆ solvent	2.51
<p>[Pd(L)₂]</p>	(CH=N imine) (C ₁₁)	(9.53)(1H,s)
	Ar-CH(C ₁₄)	8.95(1H,s)
	Ar-CH(C ₄)	8.07(1H,s)
	Ar-CH(C ₅ ,C ₈)	8.03(2H,m)
	Ar-CH(C ₃ ,C ₆ ,C ₇)	(7.81,7.54,7.52)(3H,m)
	(NH ₂) For amine group	(6.55)(1H,s)
	Ar-CH (C ₁₈ , C ₂₂)	(6.64)(2H,m)
	for CH ₂ group C ₁₆	(3.77)(2H,s)
	for OCH ₃ groups	(3.72)(9H,m)
DMSO-d ₆ solvent	2.50	

s=sing late , m=multi plate

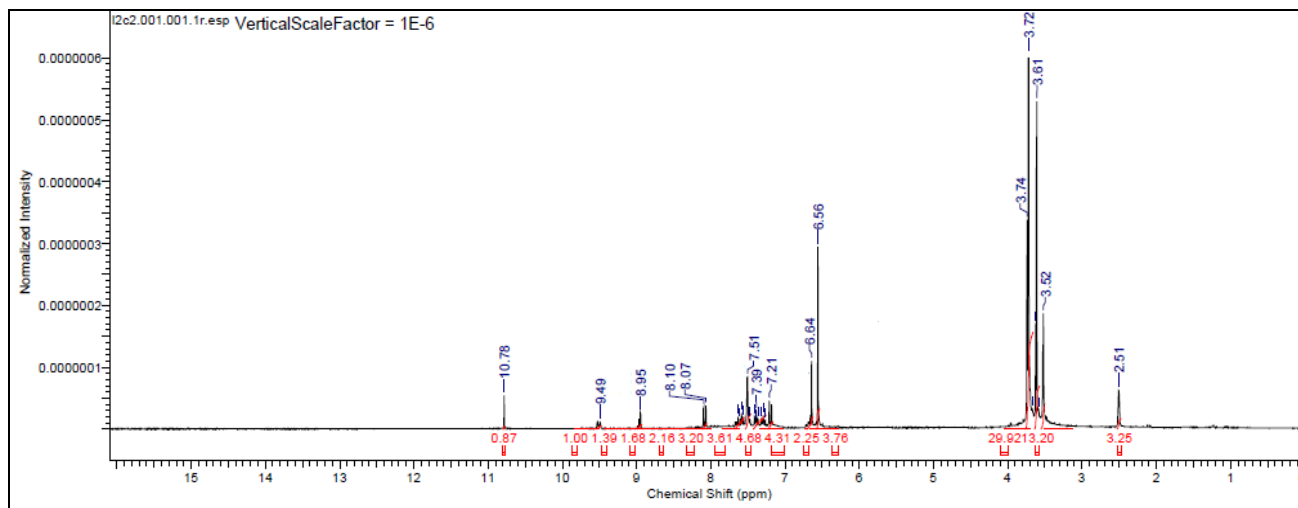


Figure 4: The ¹H-NMR for the ligand [HL]

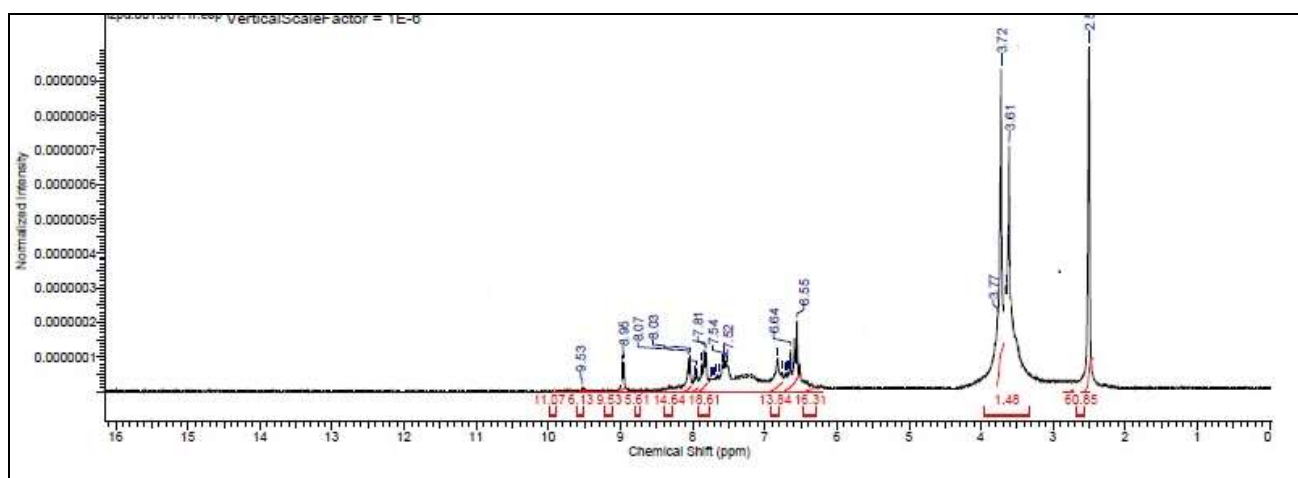


Figure 5: The ¹H-NMR for the complex [Pd(L)₂]

¹³C-NMR Spectrum of ligand [HL]

¹³C-NMR spectrum and the assignments for

ligand [HL] are shown in Table (4) and Figure (6).

Table 4: ¹³C-NMR data for ligand measured in DMSO-d₆ and chemical shift in ppm (δ)

Compound	Functional groups	δ (ppm)
<p>[HL]</p>	for (C-OH) group C ₁	172.93
	for (C-NH ₂) group C ₁₃	163.25
	C ₁₂ for Aromatic ring	162.90
	for (C=N)imine group C ₁₁	161.78
	C ₁₄ for Aromatic ring	154.08
	C ₁₂ , C ₁₉ for Aromatic ring	153.17
	C ₂₀ for Aromatic ring	141.24
	C ₁₀ for Aromatic ring	138.69
	C ₄ for Aromatic ring	135.99
	C ₁₇ for Aromatic ring	129.61
	C ₉ for Aromatic ring	129.17
	C ₅ for Aromatic ring	127.75
	C ₇ for Aromatic ring	124.36
	C ₆ for Aromatic ring	122.68
	C ₃ for Aromatic ring	119.84
	C ₈ for Aromatic ring	114.11
	C ₁₅ for Aromatic ring	106.51
	C ₂ , C ₁₈ , C ₂₂ for Aromatic ring	106.30
for (OCH ₃) group C ₂₆	60.40	
for (OCH ₃) groups C ₂₅ , C ₂₇	56.23	
DMSO-d ₆ solvent	40.21	
for (CH ₂) group C ₁₆	39.93	

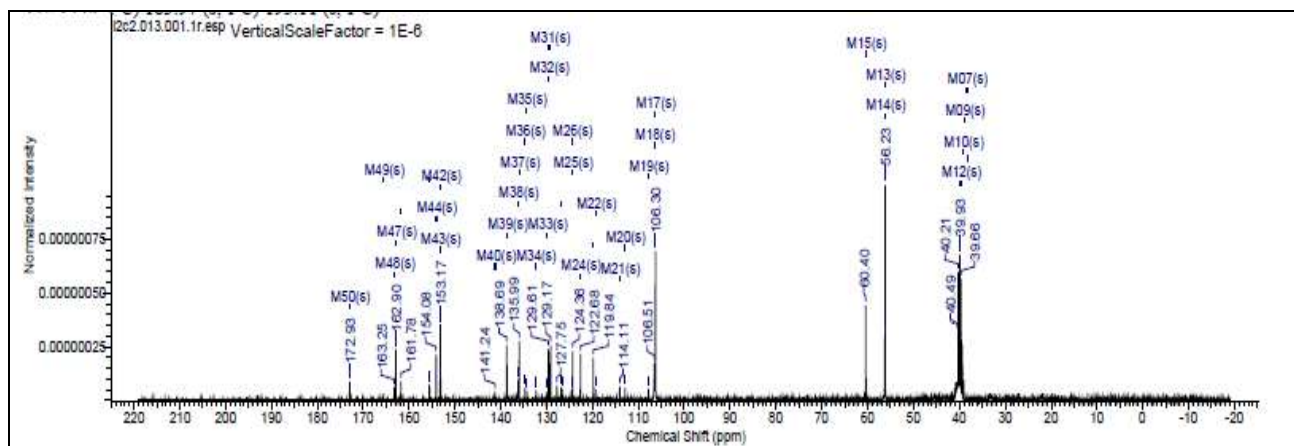


Figure 6: The ¹³C-NMR for the ligand [HL]

EI-Mass Spectrum of ligand [HL]

The electro spray (+) mass spectrum of [HL], shows the parent ion peak at (M/Z= 445.2),

which agree with [M⁺], other fragments their relative abundance and fragmentation pattern are shown in Figure (7).

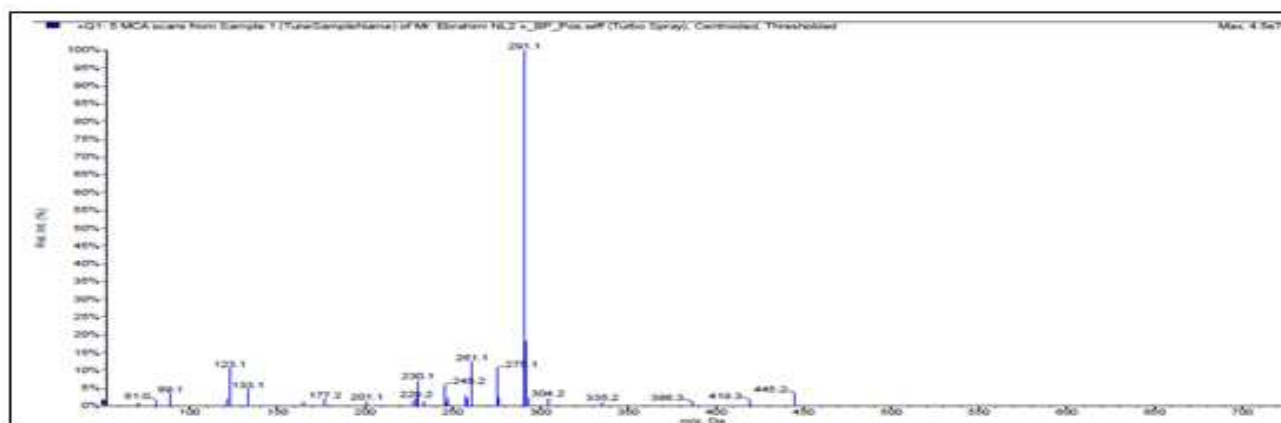


Figure 7: EI-mass spectrum for the ligand [HL]

UV-Vis, Magnetic Moments and Molar Conductance

According to the magnetic moment values, all prepared complexes are paramagnetic except complexes Zn-complex, Cd-complex and Pd-complex are diamagnetic because the Zn, Cd and Pd ions have filled d-orbital. The data of magnetic susceptibilities were in the Table

(5). Conductivity indicates that all compounds behave as neutral [13]. The data of molar conductance were listed in the Table (1). The UV-Vis spectra for [HL] and its complexes are shown in Table (5), Figure (8 and 9), together with the magnetic moment indicate octahedral geometry for complexes expect the Pd complex was square planer geometry around the metal atoms studied.

Table 5: Electronic spectral data of ligand [HL] and its metal complexes

Compound	Wave number		ϵ_{max} molar ⁻¹ cm ⁻¹	Assignment	μ_{eff} (BM)	Suggested structure
	Nm	cm ⁻¹				
(HL)	275	36363	2138	$\pi \rightarrow \pi^*$	-	-
	341	29325	630	$n \rightarrow \pi^*$		
[Mn(L) ₂]	269	37174	1648	L.F	5.6	Octahedral
	295	33898	1962	L.F		
	365	27397	758	L, F		
	409	24449	537	C.T		
	811	12330	2	${}^6A_{1g} \rightarrow {}^4T_{1g}(g)$		
[Co (L) ₂]	268	37313	1517	L.F	4.7	Octahedral
	301	33222	1478	L.F		
	397	25188	445	C.T		
	822	12165	9	${}^4T_{1g} \rightarrow {}^4T_{2g}(F)$		
	269	37174	1645	L.F		Octahedral

[Ni (L) ₂]	299	33444	1838	L.F	3.6	
	316	31645	790	L.F		
	412	24271	417	C.T		
	811	12330	27	³ A _{2g} → ³ T _{1g} (F)		
[Cu(L) ₂]	271	36900	1967	L.F	1.7	Octahedral
	301	33222	2262	L.F		
	433	23094	1062	C.T		
	856	11682	22	² E _g → ² T _{2g}		
[Zn (L) ₂]	276	36231	2324	L.F	-	Octahedral
	347	28818	680	L.F		
	387	25839	490	C.T		
[Cd(L) ₂]	276	36363	1758	L.F	-	Octahedral
	352	28409	730	L.F		
	422	23696	390	C.T		
[Pd(L) ₂]	288	34722	2144	L.F	-	Square planner
	346	28901	1482	L.F		
	432	23148	717	C.T		
	810	12345	16	¹ A _{1g} → ¹ E _g		

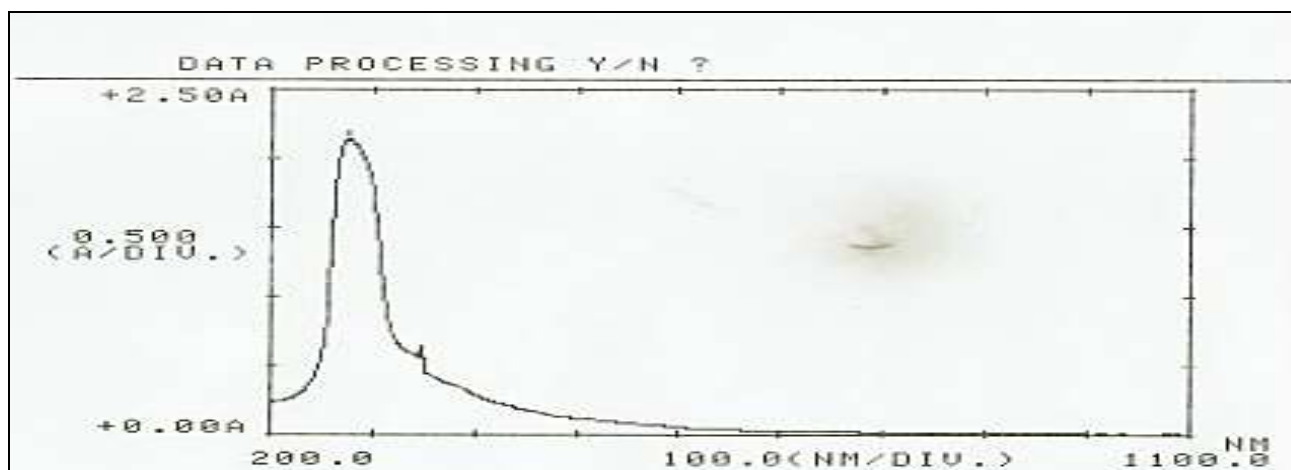
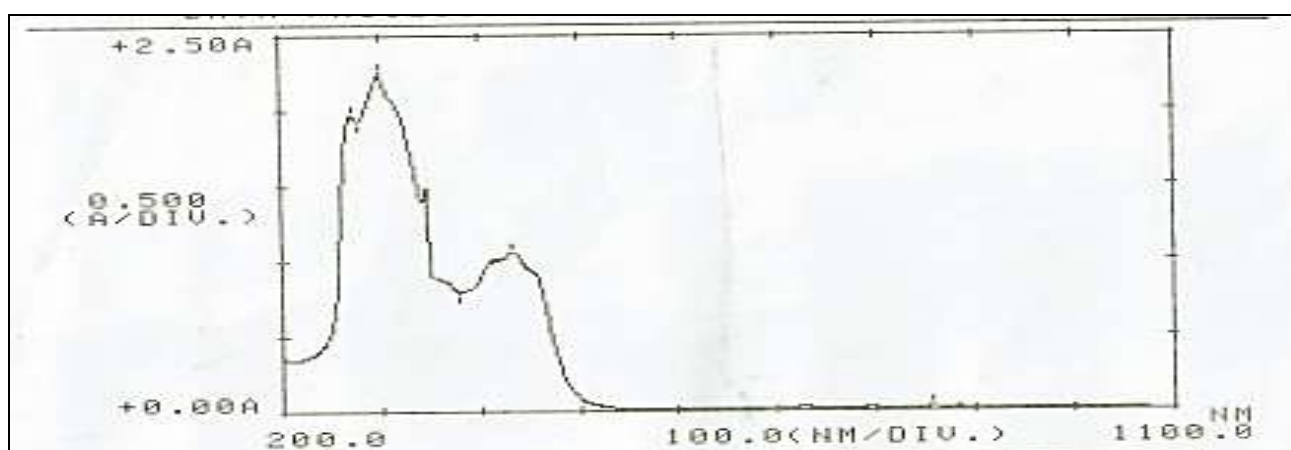


Figure 8: Electronic spectra of the ligand [HL]

Figure 9: Electronic spectra of the ligand complex [Cu (L)₂]

Biological Activity

The antibacterial activity of the parent Schiff base [HL] and its metal complexes against bacteria such as *Bacillus subtilis* (G+) and *Escherichia Coli* (G-) and fungi such as *Canidia albicans* and *Candida tropicalis* were tested in order to evaluation their potential antimicrobial agents. The data are in Table

(6), Figures (10, 11). According to the data can be observed that the [HL] and its complexes did show very good biological activity. This means that the activity of the latterly synthesized azomethine against different microorganisms is enhanced with chelation with different biological active metals [25].

Table 6: Inhibition circle diameter in millimeter for the ligand [HL] and its complexes

Compounds	Inhibition Zone Diameter (mm)			
	<i>Escherichia Coli (G⁻)</i>	<i>Bacillus subtilis(G⁺)</i>	<i>Conidia albicans</i>	<i>Candida tropicalis</i>
[HL]	33	31	20	22
[Mn(L) ₂]	31	23	15	16
[Co(L) ₂]	32	24	24	25
[Ni(L) ₂]	28	22	28	26
[Cu(L) ₂]	29	27	20	16
[Zn(L) ₂]	29	24	27	22
[Cd(L) ₂]	28	27	23	19
[Pd(L) ₂]	28	25	19	17
DMSO	-	-	-	-



Figure 10: The biological activity of the ligand and its complexes

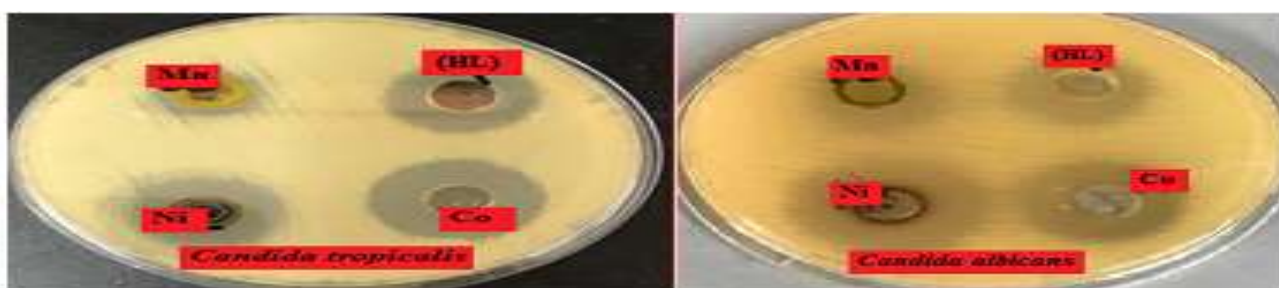


Figure 11: The biological activity the ligand and its complexes

Conclusion

The prepared new [HL] and their complexes were characterized by Electronic spectra, FT-IR spectroscopic technique, ¹H, ¹³C-NMR, mass spectra, magnetic moment, (C.H.N) and their biological activity. Complexes were prepared by reaction of the synthesized [HL]

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