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Bioactive Study and Theoretical Treatment for Metal Ion Complexes.

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ABSTRACT

The complexes 4-((2-Hydroxy-1-naphthyl) methylene amino)-1,5-dimethyl -2-phenyl -1H-pyrazol-3(2H)-one (HL) with Co(II), Zn(II), Cd(II) and Hg(II) were prepared. The ligand and complexes have been characterized by $^1\text{H-NMR}$, Mass spectra, elemental microanalyses (C.H.N.), FT-IR, UV-Vis, magnetic moment and molar conductance. The ligand and its metal complexes were screened for their bioactivity against bacterial species, 2 Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and 2 Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) as well fungi like *Penicillium expansum* and *Candida albicans*. The work also include a theoretical treatment of the formed complexes in the gas phase, this was done using the (hyperchem-8) program for the molecular mechanics and semi-empirical calculations. The heat of formation (ΔH_f°) and binding energy (ΔE_b) at 298K for the compounds were calculated by using PM3 method.

Keywords: Bioactivity studies, Theoretical treatment, Metal ion complexes

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INTRODUCTION

Schiff bases and their metal ion complexes are considered as prosperous category of organic component sowing to their electrochemical analysis, biochemical synthesis, extraction of metal ions, antifungal, antimicrobial as well as catalytic activities [1-4]. Various types of Schiff bases forming metal complexes have been widely investigated, exhibiting extensive range of different applications, especially in biological systems [5-7]. The investigation of mono, di, tridentate metal ion complexes discover their outstanding properties which are cooperative in understanding the numerous features of coordination chemistry of metals [8]. The presence of N and O atoms in such structure make them structurally similar to the neutral biological systems and are used in explaining the mechanism of racemization reactions. Several biological activities (antibacterial, antitumor, herbicidal and antifungal) are reviewed due to the presence of imines groups ($-C=N$) present in living systems [9].

EXPERIMENTAL

Materials and Instrumentation

The following chemicals were commercially available and were used without further purification: 2-hydroxy-1-naphthaldehyde, 4-amiophenazone, CH_2Cl_2 , $CHCl_3$, CH_3OH , and C_2H_5OH from Merck DMSO, acetic acid glacier and Petroleum ether from Riedel, $CoCl_2 \cdot 6H_2O$, $CaCl_2$, $ZnCl_2$, $CdCl_2 \cdot 2H_2O$, $HgCl_2$ and diethyl ether Aldrich.

Mass spectra were obtained by using Mass 100P Shimadzu. ^{13}C - 1H -NMR spectra were acquired in DMSO- d_6 solution using Bruker AMX 300 MHz spectrometer. Elemental (C.H.N) analyses were carried out on a Perkin-Elmer automatic equipment model 240.B. FT-IR spectra were recorded in the range (4000-400) cm^{-1} on a Shimadzu 3800, spectrometer. Electronic absorption spectra were recorded in the range (200-900) nm for solution in DMSO (1×10^{-3}) on a Shimadzu 160 Spectrophotometer. Magnetic properties were measured using (Magnetic susceptibility balance model MSR-MKi). Metal ratios were identified using a Shimadzu (A.A) 680G atomic absorption Spectrometer. Conductivity measurements were measured for solution in DMSO (1×10^{-3}) using a Jenway 4071 digital conductivity meter at room temperature. Melting points were obtained on a Buchi SMP-20 capillary melting point apparatus and are uncorrected, Chloride ion content is specified by using potentiometric titration method at a 686-Titro processor – 665 Dosimat Metrohm Swiss

Preparation of Ligand (HL) [10]

The ligand was prepared by condensing 2-hydroxy-1-naphthaldehyde in hot ethanolic solution of 4-aminophenazone in (1:1) molar ratio, and then, the reaction mixture was heated to reflux for 3 hours. The 1H -NMR spectra of the ligand provide compelling evidence of the presence of azomethine group. The product was dried and crystallized from a mixture (1:1) (water: ethanol) at room temperature as shown in Scheme (1). Yield: 94%, mp = 212°C. 1H -NMR (Figure 1) (DMSO, ppm): 9.08 (s, $-CH=N$, 1H), 7.08 to 7.98 (m, aromatic), 10.99 (s, $-OH$, 1H), 3.1 (s, CH_3 , 3H), 3.39 (s, CH_3 , 3H), (2.5, DMSO). ^{13}C NMR (Figure 2) (DMSO, ppm): 161.3 ($-HC=N$), 111 to 136 (arom), 27.04 (C- CH_3), 59.33(N- CH_3).

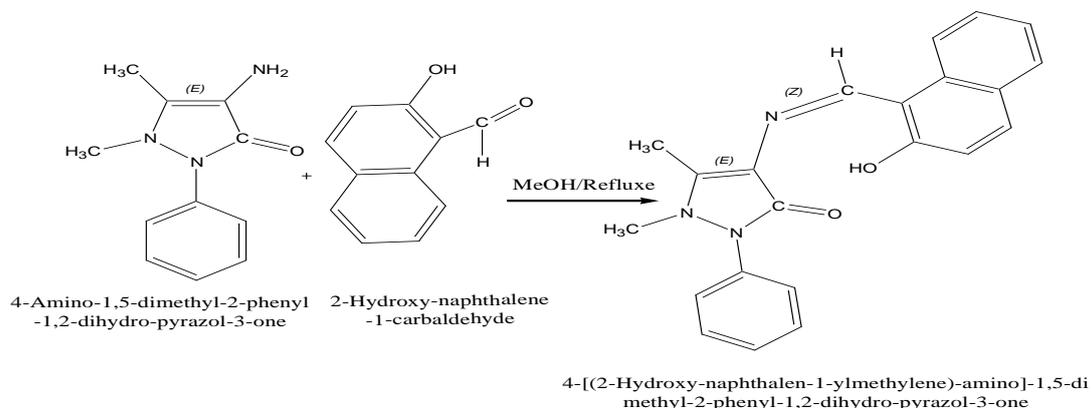
Preparation of Complexes

$Co(II)$, $Zn(II)$, $Cd(II)$ and $Hg(II)$ complexes (Scheme 2) were prepared in a similar manner using the method by Al Zoubi and Al-Hamdani. Thus, a solution of 1 mmol of metal salts in 10 mL of ethanol was added to an ethanol solution containing 1 mmol Ligand (HL) and was refluxed for 5 hours. The resulting solution was concentrated to a small volume (3 mL) on a rotary evaporator, and the product was separated by the addition of small amount of pet-ether (Petroleum ether) (60-80°C) mixture. Elemental microanalysis, colors, and yields for the complexes are given in Table 1.

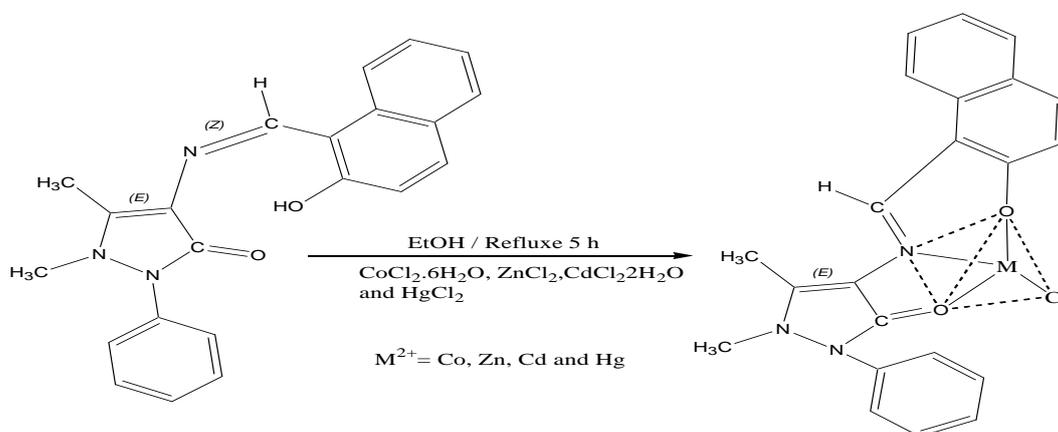
Study of Bioactivity

All the metal complexes, ligand and metal salts were screened against (*Bacillus subtilis* and *Staphylococcus aureus*) (gram positive) and (*Escherichia coli* and *Pseudomonas aeruginosa*) (gram negative) bacteria as well fungi like *Penicillium expansum* and *Candida albicans*, by using the wall agar diffusion method.

Using solvent (DMSO), the concentration of the compounds by this exposure was (10^{-3} M) by using disc sensitivity inspection. This method involves the exposure of the zone inhibition toward the diffusion from micro-organism on agar plate. The plates were incubated for 24 and 48 hours of bacteria and fungi respectively at 37 °C.



Scheme (1): Preparation of the Ligand (HL)



Scheme 2: Preparation of the Complexes

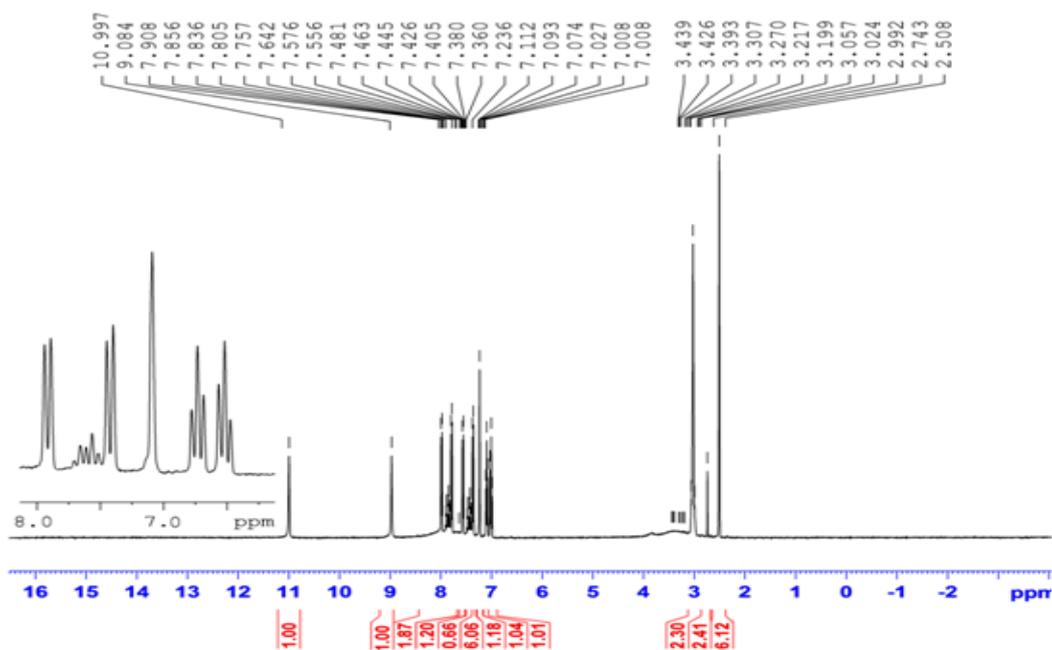
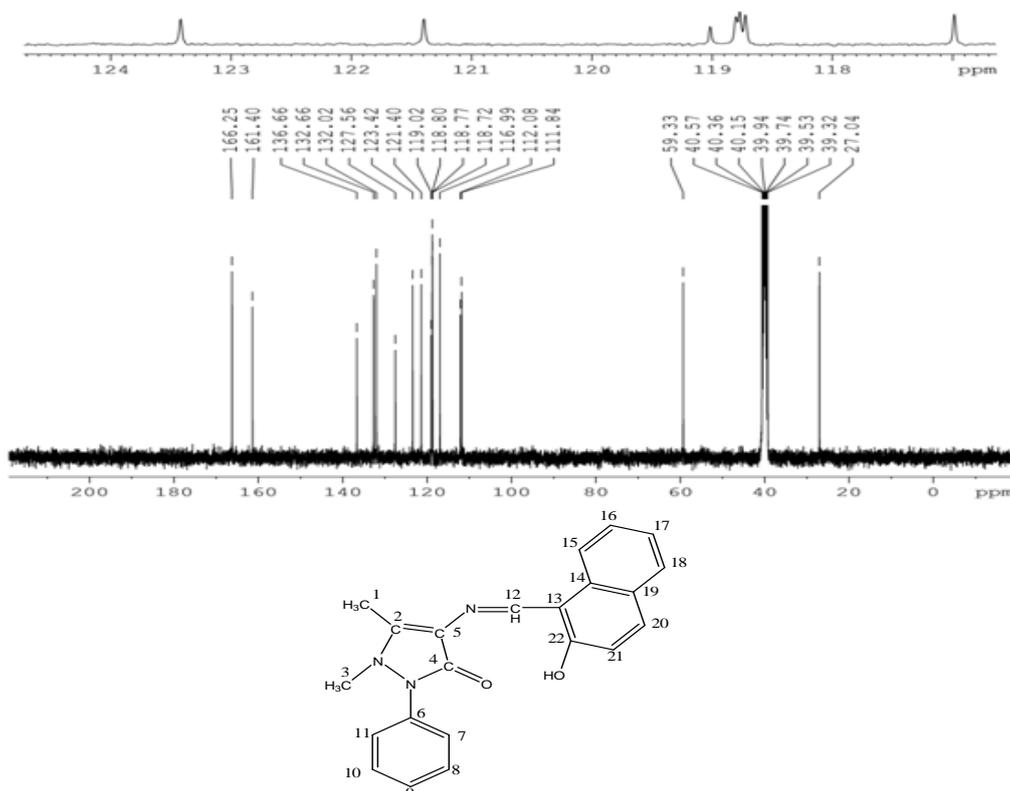


Figure 1: $^1\text{H-NMR}$ spectrum of ligand


 Figure 2: ^{13}C -NMR spectrum of ligand

RESULTS AND DISCUSSION

Complexes were obtained upon reaction between metal ions and tridentate ligand 4-[(2-Hydroxy-naphthalen-1-ylmethylene)-amino]-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one with molar ratio (1:1) (M:L). Nonnumeric complexes of the ligand with Co(II), Zn(II), Cd(II), and Hg(II) were prepared by heating 1 mmol of the ligand with 1 mmol of metal salt using ethanolic solution. However, deprotonation of the ligand occur facilitating the formation of the complexes $[\text{M}(\text{II})(\text{L})\text{Cl}]$ (M with Co(II), Zn(II), Cd(II) and Hg(II)) (Scheme 2).[11] The complexes are air-stable solids, solution in DMF and DMSO, sparingly soluble in MeOH, CHCl_3 , and CH_2Cl_2 . The analytical data (Table 1) agree well with the suggested formulae. The most important infrared bands of the ligand and its complexes together with their assignments are collected in Table 2.

Table 1: Microanalysis results and some physical properties of the Ligand and its complexes

Molecular Formula = M_{wt}	Color	m.p. $^{\circ}\text{C}$	Element microanalysis % Calcu. (Found)					Molar Conductivity ($\text{cm}^2 \cdot \Omega^{-1} \cdot \text{mol}^{-1}$)
			M	C	H	N	Cl	
HL =357 $\text{C}_{22}\text{H}_{19}\text{N}_3\text{O}_2$	Yellow crystals	212	-	73.92 (73.94)	4.88 (5.36)	4.88 (5.36)	-	-
CoL=450.78 $\text{C}_{22}\text{H}_{18}\text{N}_3\text{O}_2\text{CoCl}$	Greenish yellow	288-290d	13.07 (14.55)	58.62 (57.87)	4.02 (3.78)	9.32 (10.11)	7.86 (7.00)	16
ZnL=457.24 $\text{C}_{22}\text{H}_{18}\text{N}_3\text{O}_2\text{ZnCl}$	Light yellow	271-272d	14.30 (15.45)	57.79 (58.09)	3.97 (3.04)	9.19 (10.45)	7.75 (8.29)	20
CdL=504.26 $\text{C}_{22}\text{H}_{18}\text{N}_3\text{O}_2\text{CdCl}$	Light yellow	309-311d	22.29 (23.54)	52.40 (53.09)	3.60 (2.88)	8.33 (9.56)	7.03 (6.46)	17
HgL=592.44 $\text{C}_{22}\text{H}_{18}\text{N}_3\text{O}_2\text{HgCl}$	Light yellow	321-322d	33.86 (32.45)	44.60 (54.69)	3.06 (4.23)	7.09 (8.99)	5.98 (6.08)	9

Abbreviation: d, decomposition

Mass spectra for complexes

The Mass spectra of complexes [HL, LCo and LCd] showed the parent ion peaks at ($m/z=357$), ($m/z=450.78$), ($m/z=504.24$) and ($m/z=592.44$) correspond to ($M= C_{22}H_{19}N_3O_2$), ($M= C_{22}H_{18}N_3O_2CoCl$) ($M= C_{22}H_{18}N_3O_2CdCl$) and ($M= C_{22}H_{18}N_3O_2HgCl$) respectively. The fragmentation pattern is shown in Table (2), Figure (3) spectrum of Hg(II) complex..

Table 2: The Fragmentation Pattern data for ligand and its metal Complexes

Compounds	Peaks
HL = $C_{22}H_{19}N_3O_2 = 357$	257, 242, 99, 67
LCo = $C_{22}H_{18}N_3O_2CoCl = 450.78$	451, 414, 398, 177, 126, 67, 59
LCd = $C_{22}H_{18}N_3O_2CdCl = 504.24$	504.5, 459, 444, 398, 177, 126, 67, 59
LHg = $C_{22}H_{18}N_3O_2HgCl = 592.44$	593, 558, 482, 156, 126, 72, 59

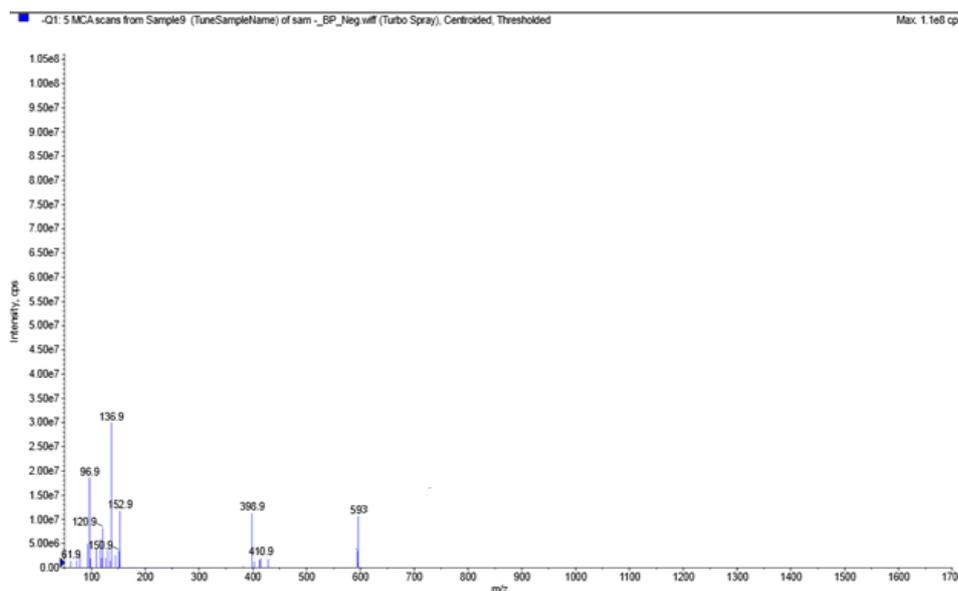


Figure 3: Mass spectrum for Hg(II) complex

FT-IR spectra

All peculiar features of the Infrared spectra are consistent with the structural characteristics of the Schiff base and its metal complexes. A characteristic stretching vibration (Table 3) was observed in the region 1641 cm^{-1} attributed to $\nu(C=N)$. The broad bands around 3420 cm^{-1} are assigned to H-bonded $-OH$ stretching vibrations; a strong band at 1744 cm^{-1} and 1284 cm^{-1} in the IR spectra of the Schiff bases are assigned to $\nu(C=O)$ and phenolic $\nu(C-O)$ vibrations, respectively. In comparison with the vibrational spectra [11,12] (Table 3) of the metal complexes with the free ligand, the $\nu(C=N)$ and $\nu(C=O)$ are shifted to the lower wave number ($(C=N)$ ($21-6\text{ cm}^{-1}$) ($C=O$) ($39-11\text{ cm}^{-1}$)), ie, in the range 1585 to 1588 cm^{-1} shows the coordination of nitrogen of azomethine group to the metal ion. This indicates that the ligand was coordinated with the metal ions through the imine and carbonyl group. The metal complexes (as shown in Table 3) showing broad band in the region 3378 to 3388 cm^{-1} and 2 weak bands in the region 695 to 708 cm^{-1} and 766 to 821 cm^{-1} are attributed to the presence of coordinated water molecules because of $\nu(OH)$ rocking and wagging modes of vibrations, respectively [13-15]. The reduction in bond order, upon complexation, can be attributed to delocalization of metal electron density (t_{2g}) to the π -system of the ligand. These shifts confirm the coordination of the ligand via the nitrogen of the azomethine group and oxygen of carbonyl and the phenol groups to metal ions. At lower frequency the complexes exhibited bands around 520 to 459 cm^{-1} assigned to the $\nu(M-N)$ and exhibited bands around 455 to 418 cm^{-1} assigned to the $\nu(M-O)$ for complexes [12,13].

Table 3: FT-IR spectral data (wave number) cm^{-1} for the ligand and its complexes

Compounds	ν (O-H) phenol	ν (C-H) aromatic	ν (C-H) aliphatic	ν (C=O)	ν (C=N)	ν (M-N)	ν (M-O)
HL	3420	3041	2870	1744	1641	-	-
LCo	-	3035	2824	1705	1623	459 497	418
LZn	-	3051	2879	1727	1627	479 520	448 420
LCd	-	3043	2845	1720	1635	470	428
LHg	-	3044	2852	1733	1620	498	424 455

Electronic spectra

The electronic spectrum of the free ligand HL, electronic transitions $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ at 265 and 317 344 nm respectively. Finally, the diamagnetic of Zn(II), Cd(II) and Hg(II) complexes exhibited absorption bands at 276, 277 and 280 nm due to $\pi \rightarrow \pi^*$. Appearance of these bands are due to $n \rightarrow \pi^*$ transition associated with azomethine linkage at 345, 356 and 352nm. Moreover, the spectra of the complexes also shows bands at 410, 421 and 424 nm due to the charge transfer (M \rightarrow L CT) as the electronic configuration of these complexes Zn (II), Cd (II) and Hg (II) respectively [16,17]. The electronic spectrum of Co(II) complex showed four broad peaks at 289 nm, 378 nm, 396nm and 668 nm assigned to $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ and CT, ${}^4A_2 \rightarrow {}^4T_1$ respectively, suggesting a tetrahedral geometry [16,18] (Figure 4) spectrum of gH(II) complex.

In this case the magnetic moment for Zn(II), Cd(II) and Hg (II) complexes are diamagnetic, but Co(II) complex 4.10 B.M which confirmed the tetrahedral geometry for complexes [16]. All the absorption bands were fully assigned in Table (4).

Table 4: Electronic data magnetic moment and molar conductivity for ligand and its complexes

compounds	λ_{max} nm	Wave number cm^{-1}	Assignment	μ_{eff} M.B.
HL	269 325 337	27100 30769 29673	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ $n \rightarrow \pi^*$	-
LCo	289 378 396 668	34602 36455 25252 14970	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ C.T ${}^4A_2 \rightarrow {}^4T_1$	4.10
LZn	276 345 410	36231 28985 24390	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ C.T	Diamagnetic
LCd	277 356 421	36101 28089 23752	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ C.T	Diamagnetic
LHg	280 352 424	35714 28409 23584	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ C.T	Diamagnetic

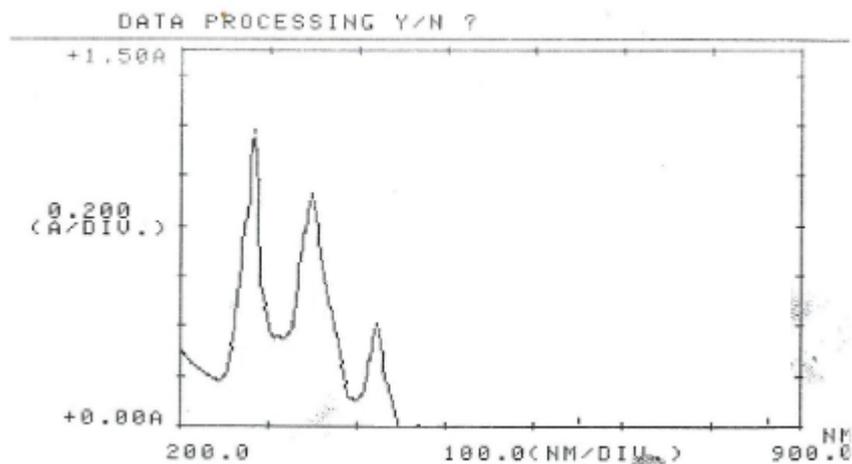


Figure 4: UV-Vis Spectrum of gH(II) complex

Electrostatic potentials

Electron distribution governs the electrostatic potential of the molecules. The electrostatic potential (E.P) describes the interaction of energy of the molecular system with a positive point charge. The E.P is useful for finding sites of reaction in a molecule; positively charged species tend to attack a molecule where the electrostatic potential is strongly negative (electrophilic attack).[19, 20] The E.P of the free ligand was calculated and plotted as 2D contour to investigate the reactive sites of the molecules, Figure 5. Therefore, one can interpret the stereochemistry and rates of many reactions involving “soft” electrophiles and nucleophiles in terms of the properties of frontier orbital HOMO and LUMO. The results of calculations show that the LUMO of transition metal ions prefer to react with the HOMO of 3-donor atoms of 2 oxygen of carbonyl, phenol and nitrogen of imine group for free ligand [20].

All theoretically probable structures of free ligand and their complexes have been calculated by (PM3) and (ZINDO/1) methods in the gas phase to search for the most probable model building stable structure, the heat of formation (ΔH_f°), binding energy (ΔE_b) total energy and dipole moment for free ligand and their metal complexes were calculated Table 5. The theoretically calculated wave numbers for this ligand showed that some deviations from the experimental values; these deviations are generally acceptable in theoretical calculations. Calculation of parameters has been optimized bond lengths of the free ligand and its metal complexes which to give excellent agreement with the experimental data as shown in Figure 6.

Table 5: Conformation energetic (in KJ. mol⁻¹) for naringin and its metal complexes

Compounds	ΔE_{tot}	ΔH_f°	ΔE_b	Dipole (Debyes)
HL	-91542.629	73.9510	-5133.684	6.296
LCo	-156578.4393	-208.83966	-5970.20766	3.01
LZn		1575.83 (AMBER)		3.44
LCd		301.789 (AMBER)		2.88
LHg		230.987 (AMBER)		4.01

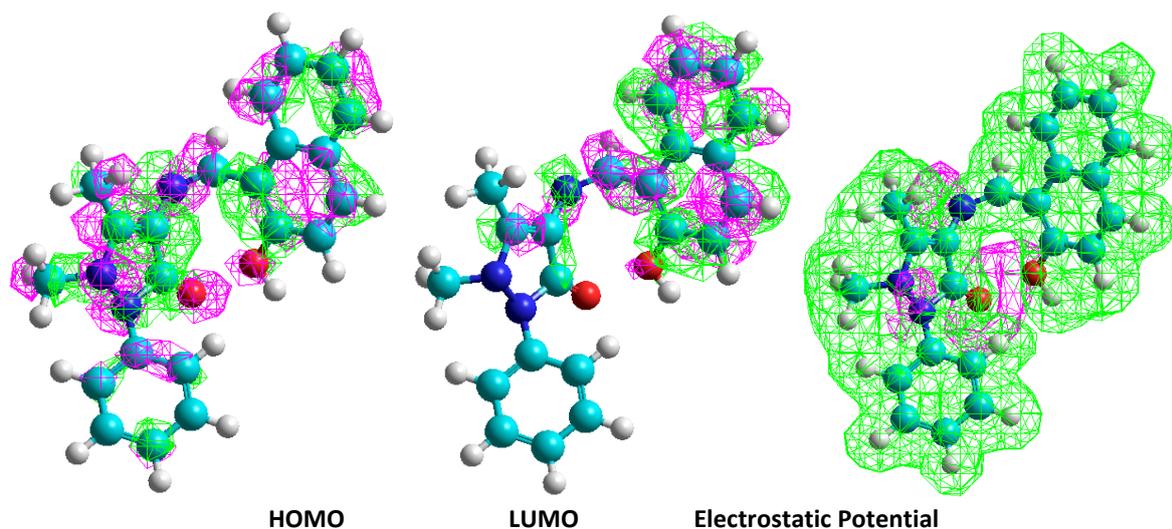
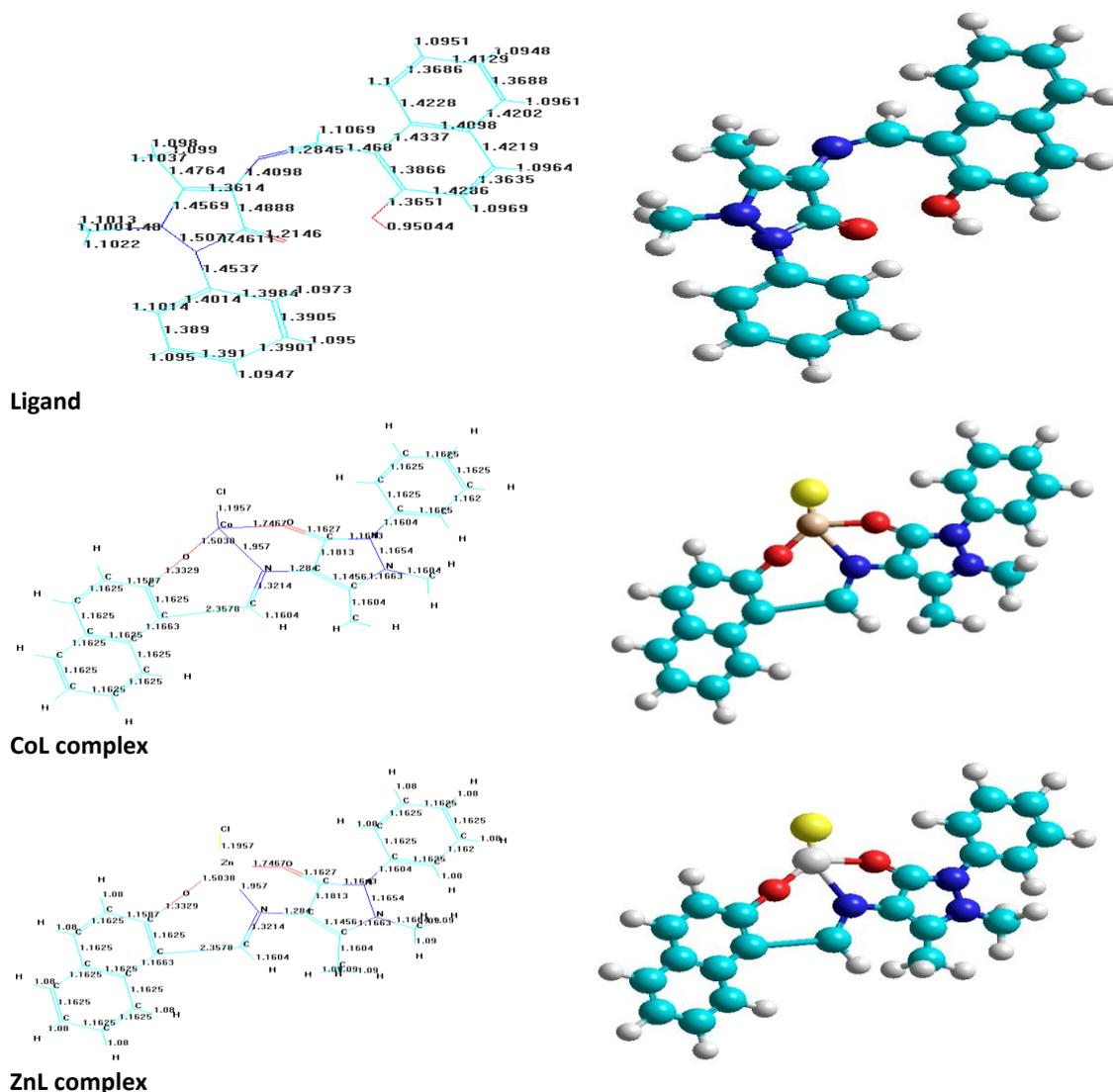


Figure 5: Electrostatic potential (HOMO and LUMO) contours for ligand



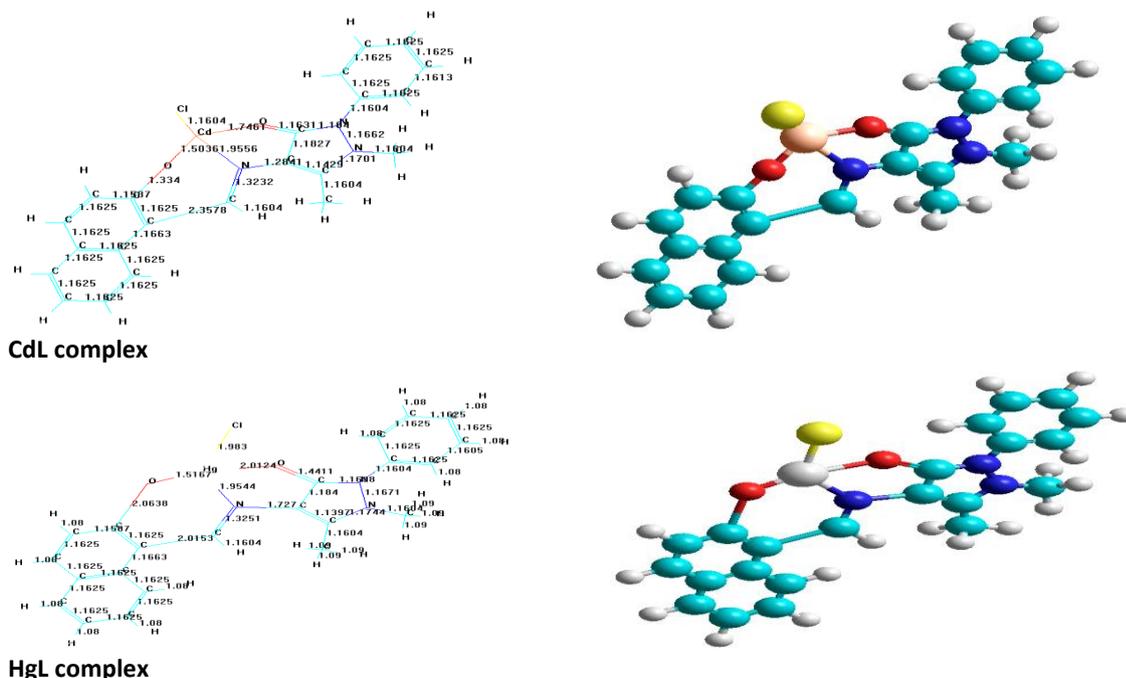


Figure 6: Conformational structure of (HL) and their metal complexes

Antimicrobial activity of ligand and complexes

Ligand and their complexes of metal ions were screened for antibacterial and antifungal activity (*Penicillium expansum* and *Candida albicans*). The entire tested compound exhibited variable antibacterial and antifungal activity as shown in Figures 7 *Escherichia coli* and *Staphylococcus aureus* for compounds.

Ligand which did not exhibit have antifungal activity, but activity normal exhibit have *Staphylococcus aureus*, *Bacillus subtilis* *Pseudomonas aeruginosa* and *Escherichia coli*

Mercury, Cobalt and Cadmium Complexes also exhibited activity high antibacterial against *Staphylococcus aureus*, *Bacillus subtilis* *Pseudomonas aeruginosa* and *Escherichia coli* as well as fungi *Penicillium expansum* and *Candida albicans* activity high (a, b, c) concentration Tabs (6- 11), but activity normal Zn complex (a, b) concentration and activity high in (c) concentration.

Table 6: Diameters (mm) at suppression for bacteria to the compounds *Escherichia coli*

Compounds/ Samp	HL	a= mg/ ml	b= mg/ ml	c= mg/ ml
CoL /3	11	25	22	18
ZnL / 1	10.5	14	12	10.8
CdL / 5	10.5	18	26	19
HgL /6	11	26	22	20

Table 7: Diameters (mm) at suppression for bacteria to the compounds *Staphylococcus aureus*

Compounds/ Samp	HL	a= mg/ ml	b= mg/ ml	c= mg/ ml
CoL /3	11	28	25.5	22.5
ZnL / 1	11	16	14.5	12
CdL / 5	11	22	19	22
HgL /6	14	30	27	22

Table 8: Diameters (mm) at suppression for bacteria to the compounds *Pseudomonas aeruginosa*.

Compounds/ Samp	HL	a= mg/ ml	b= mg/ ml	c= mg/ ml
CoL /3	11.5	25	22	20
ZnL / 1	11	16	14	12
CdL / 5	10.5	18	16	13
HgL /6	11	20	17	14

Table 9: Diameters (mm) at suppression for bacteria to the compounds *Bacillus subtilis*

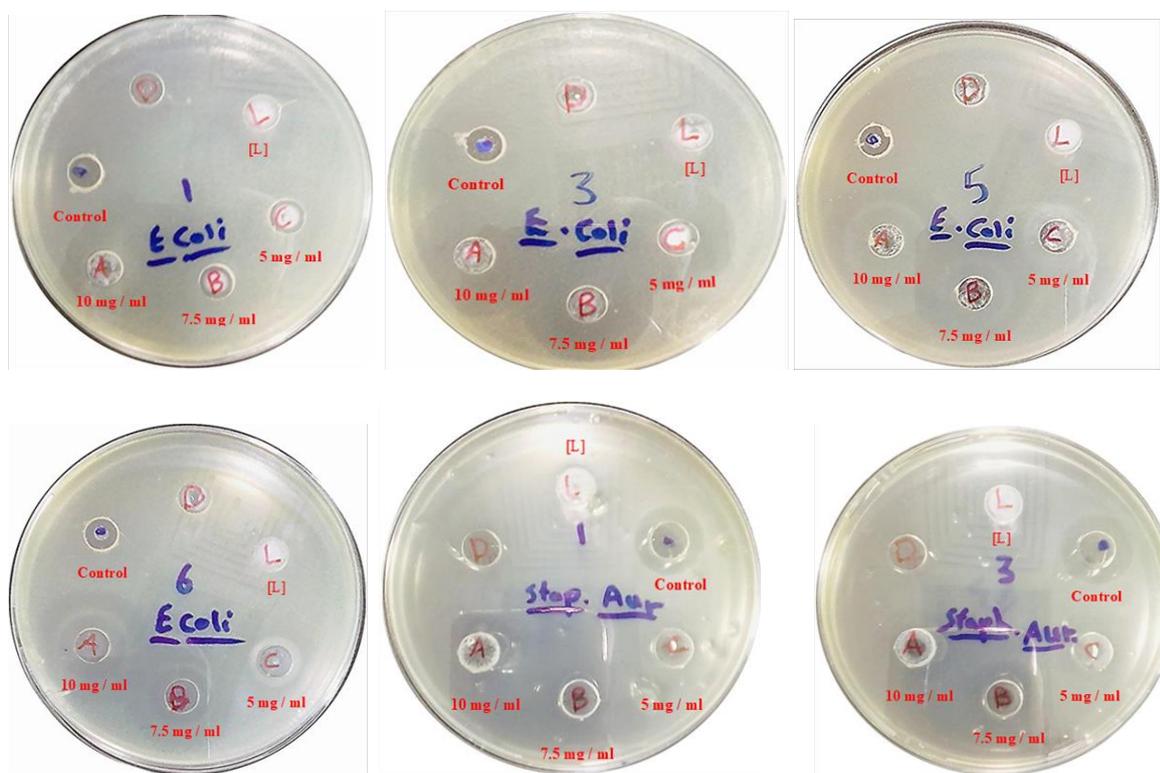
Compounds/ Samp	HL	a= mg/ ml	b= mg/ ml	c= mg/ ml
CoL /3	11	18	16	17
ZnL / 1	10	13	12	16
CdL / 5	11	22	20	18
HgL /6	11	28	25	22

Table 10: Diameters (mm) at suppression for fungi to the compounds *Candida albicans*

Compounds/ Samp	HL	a= mg/ ml	b= mg/ ml	c= mg/ ml
CoL /3	-	10	17	18
ZnL / 1	-	10	11	17
CdL / 5	-	10	16	22
HgL /6	-	15	18	21

Table 11: Diameters (mm) at suppression for fungi to the compounds *Penicillium expansum*

Compounds/ Samp	HL	a= mg/ ml	b= mg/ ml	c= mg/ ml
CoL /3	-	5	7	5
ZnL / 1	-	12	12	10
CdL / 5	-	12	22	23
HgL /6	-	18	26	28



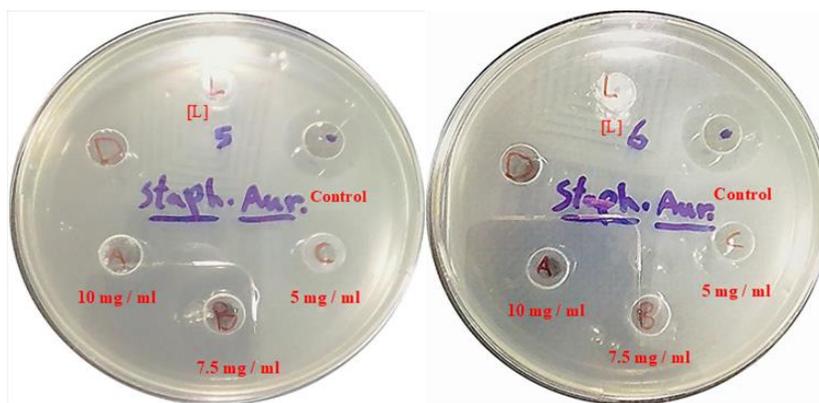


Figure 7: *Escherichia coli* and *Staphylococcus aureus* for compounds

CONCLUSION

In this paper ligand (HL) complexes with the Co(II), Zn(II), Cd(II) and Hg(II) were prepared. The configurations were performed to coordinate the ligand through the nitrogen and two oxygen atoms. Therefore, from the presented results the complexes have tetrahedral geometry.

The prepared metal complexes were screened for their biological activity against bacterial species, 2 Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and 2 Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) as well as fungi *Penicillium expansum* and *Candida albicans*.

Hyper Chem-8 program was used to predict structural geometries of compounds in the gas phase. The heat of formation (ΔH_f) and binding energy (ΔE_b) at 298 K for the A, free ligand, and its complexes were calculated by PM3 method.

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