Synthesis, Characterization of New Metal Complexes of Ligand [3-(4-hydroxyphenyl)-2-(3-(4-methoxybenzoyl) thio ureido)propanoicacid] (HMP) and Study of the Biological Activity

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Abstract

A new ligand [3-(4-hydroxyphenyl)-2-(3-(4-methoxybenzoyl)thioureido)propanoic acid] (HMP) was synthesized by reaction of Tyrosine and, 4- methoxy benzoylisothiocyanate , and used for the synthesis of a series of new Metal complexes Cobalt(II) Nickel (II), Copper (II), Zinc(II) , Palladium(II), Cadmium (II), mercury (II). The mineral complexes were characterized by molar conductivity, elemental analyzes (C H N), and spectroscopic techniques Such as : electronic spectral UV-Vis , Infrared FT-IR , ^{1}H- NMR, ^{13}C-NMR and magnetic properties . The results confirmed that the ligand (HMP) behaves as a Bidentate and coordinates with the metal ion via the oxygen atom of the carboxyl group and a nitrogen atom of the amine group. The antimicrobial properties of all newly synthesized compounds were also demonstrated against the bacterial pathogenic organisms Escherichia coli (G-) and Staphylococcus aureus (G+) by the agar-well diffusion technique. These complexes are more effective against bacteria compared to the Amoxicillin antibiotic. The aim of this study describes the synthesis and characterization of the new ligand (HMP) derived from Tyrosine and its metal complexes with knowing their effect on selected bacteria.

Key words : Tyrosine , 4- methoxy benzoyl chloride , biological activity , metal complexes.

Introduction

The major role in structure blocks of proteins and intermediates in metabolism is played by amino acids. One of the 20 non-essential amino acids that are used by cells to synthesized proteins is tyrosine [(1)] , Chelating ligands based on N, O and S donor atoms show the immense extent organic performance [(2)] . The body

produces it naturally from another amino acid called phenylalanine, which is found in meat, dairy products, eggs, oats, wheat and beans, and is used continuously within the body[3].It is worth noting that the body's inability to manufacture tyrosine from phenylalanine is a condition called phenylketonuria . The brain uses it to make neurotransmitters such as dopamine and adrenaline [4]. Some compounds of metal ions with amino acid derivatives are very important in the biological framework and in the study of the effects of pharmaceutical drugs used as therapeutics or to increase functional similarity and reduce the effect of some toxic metals [5]. In previous studies, new ligand compounds (HNP) were synthesized from tyrosine with (4-Nitrobenzoyl isothiocyanate) and metal ions (manganese, cobalt, nickel, zinc, cadmium and mercury) as a result of electronic spectra studies [6], and also has been prepared ligand derived of Vanillaldehyde and (L-tyrosine and L-phenylalanine) and its complexes with iron(III) were and determined using various physicochemical such as FT-IR and UV-Vis [7]. The aim of this work to prepare new ligand [3-(4-hydroxyphenyl)-2-(3-(4-methoxybenzoyl)thioureido)propanoic acid] (HMP) in addition to its metal complexes with Cobalt (II), Nickel (II) Cupper(II) Zinc(II), Palladium(II), Cadmium(II) and Mercury(II).

Experimental

Material and reagent : The chemicals used in this work were all reagent grade by from known sources BDH, Fluka and Sigma-Aldrich

Instruments: Nuclear magnetic resonance was recorded using Bruker 500 MHz USA at Tahran university / Central Laboratory of the College of Science and using DMSO-d_6 AndTMS as aggregates to determine the zero point. Electronic spectra were obtained using (Spectraphotometer Shimadzu UV-Vis -1700) at 25 ºC in ($1x10^{-3}$) M DMSO. FT-IR Spectra were recorded with a Shimadzu 8000Sspectrophotometer using KBr discs in the (400–4000) cm^{-1} range. Elemental analysis (C H N) have been determined for prepared ligand, also a few of their complexes at Tehran University / Iran utilizing Elementar Analysensysteme GMBH،device. Metal contents of the complexes were determined by atomic absorption technique by using Shimadzu(A620). Magnetic susceptibility measurements were obtained by balance magnetic susceptibility by model MSB .

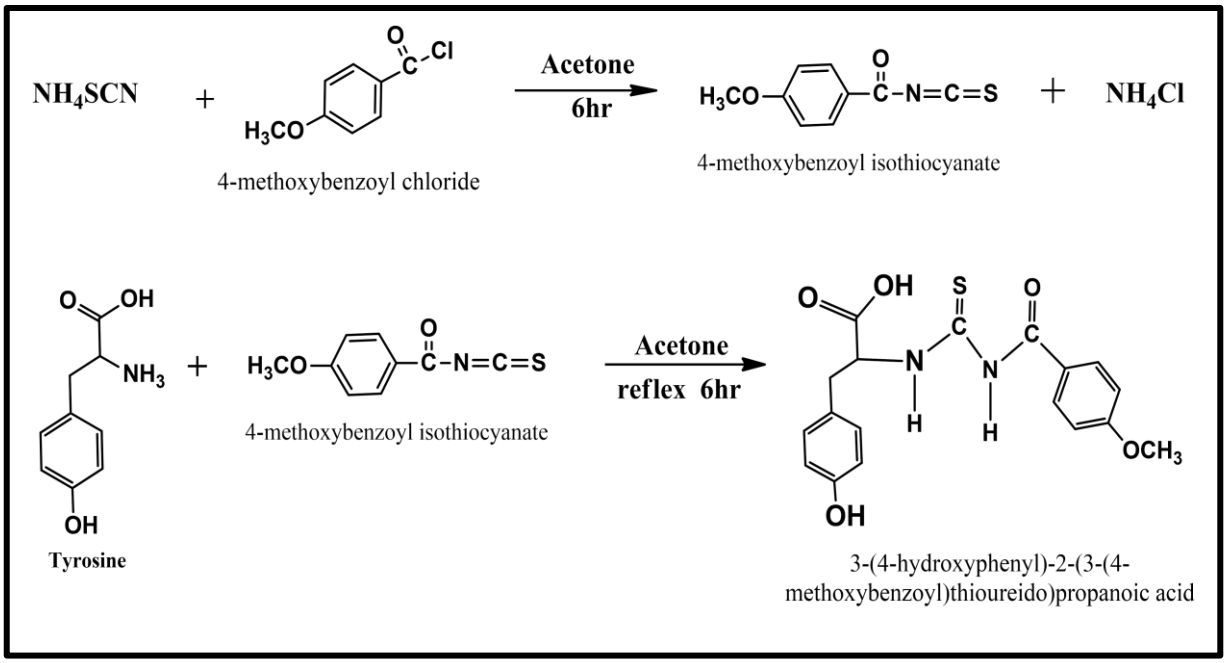
Synthesis

Synthesis of ligand (HMP)

The ligand was prepared by two steps :

1- Preparation of the(4- methoxy benzoyl isothiocyanate) Mixture of 4- methoxy benzoyl chloride (4.446g ،26mmol) and Ammonium thiocyanate (1.979g, 26mmol) in 25mL of acetone was stirred under refluxed for 3 hrs , and then filtered, the filtrate was used for further reaction.

2- Preparation of [3-(4-hydroxyphenyl)-2-(3-(4-methoxybenzoyl)thioureido)propanoic acid] (HMP) From (4.706g ،26mmol) of the amino acid tyrosine in 15mL Acetone was rapidly added to 4- methoxy benzoylisothiocyanate and maintaining reflux. After refluxing for 6hrs, there resulting solid was collected, washed with acetone. And recrystallization from ethanol, yield (75%) m.p = (166-168) C° ,Scheme (1) .



Scheme 1: Preparation of the ligand (HMP)

General method for synthesis metal complexes

1- Dissolved(0.748 g، 2mmol) of the ligand (HMP) in a base alcohol solution (0.112 g, 2mmol) of KOH in 8mL of absolute ethanol and the pH is set at 8

2- The solution of following metal salts, $CoCl_2.6H_2O$ (0.237g, 1mmol) $NiCl_2.6H_2O$(0.237g,1mmol),$CuCl_2.2H_2O$ (0.170 g ,1mmol) , $ZnCl_2$(0.136 g ,1mmol) , $CdCl_2$ .H_2O (0.201g ,1mmol),and $HgCl_2$(0.271 g ,1mmol) , $PdCl_2$ (0.177 g ,1mmol) in10mL ethanol , were added drop wise to the solution of the

ligand (HMP^-K^+) .The precipitate formed immediately after stirring the mixture at room temperature for 3hr. The resulting precipitates were filtered off, washed with ethanol, and water recrystallized from ethanol and dried. Physical properties were given in Table (1).

Antibacterial assay (in vitro) :

The biological activity of the ligand (HMP) and their complexes has been tested against two types of Gram-negative bacteria (E- coli) and Gram-positive bacteria (Staphylococcus aureus) were studied Each of these bacteria is one of the most common types of pathogenic bacteria. biological effectiveness solutions were prepared at a concentration of 10^{-3} mg/L each of the metal complexes and its ligand dissolved in dimethyl sulfoxide (DMSO). The medium was prepared according to the instructions provided by Muller-Hinton Agar Company by dissolving (37 g) of agar in 1liter of distilled water . Bacteria is spread in plates and on the surface of the food medium (Muller-Hinton agar) using (Loop full), likewise formation several holes with a diameter of 6 mm in these plates using a sterile alcohol drill. The prepared solutions were added to these bores in an amount of 0.1mL using (Micropipette) and placed in the incubator for 24 hours at a temperature of 37°C, after which the diameter of the inhibition zone was measured in millimeters [8] .In addition, the antibiotic Amoxicillin was tested for comparison with prepared compounds . During the experiments, bacteria and Amoxicillin were placed in nutrient agar medium respectively. Different test microorganisms were inoculated into agar medium . The Gram method was also used to diagnose bacteria. Microbial growth was determined by measuring the area of the inhibition diameter with the help of a scale . The diameter of the inhibitory region was measured after 24h in solvent (DMSO) for bacteria and Amoxicillin respectively.

Characterization of ligand (HMP) and its metal complexes

The prepared compounds were stable in air and moisture at room temperature. It was insoluble in water, diethyl ether and carbon tetrachloride , but soluble in dimethylformamide (DMF), dimethyl sulfide (DMSO) , absolute ethanol (ETOH) also acetone.

Elemental analysis data for the Ligand and Complexes

Synthesized ligand (HMP) and its metal complexes are shown in Table (1). They are consistent with the suggested stoichiometries. The melting points and

color of the synthesized ligand and its metal complexes are as well as shown in Table (1).

Molar conductivities of all synthesized

The molar conductivity of the metal compounds was studied in a solution of dimethyl sulfoxide (10^{-3}M) and at ambient temperature using Cond 3110 SET1 model, and the test showed that all the complexes are non- electrolytes

Magnetic moment

The measured magnetic susceptibility and effective magnetic moment (μeff) values for Co(II), Ni(II), Cu(II), the complexes show μeff (3.94 , 2.96 , 1.75) B.M respectively which can be normal values for high-spin tetrahedral complexes [9] .

Table (1): Physical properties, elemental analysis and conductivity data for the synthesized (HMP) and metal complexes.

Compound	Color	M.p °C or dec.	% Yield	μeff (B.M)	Molar condu. $Ohm^{-1}.Cm^2.mol^{-1}$	M% Calculation (Found
(HMP)Ligand	orange	**(166-168)**	%75	-	17	-
[Co(HMP)$_2$]	Green	**(178-180)**	%68	3.94	13	7.31 (7.43)
[Ni(HMP)$_2$]	yellowish green	**230 Dec**	%77	2.96	18	7.29 (7.01)
[Cu(HMP)$_2$]	orange	**(218-220)**	%80	1.75	5	7.84 (8.03)
[Zn(HMP)2]	yellow	**231Dec**	%66	0	16	8.05
[Pd(HMP)2]	brown	**(188-190)**	%78	0	14	12.47
[Cd (HMP)$_2$]	light yellow	**(204-206)**	%64	0	9	13.08
[Hg (HMP)$_2$]	dark yellow	**(208-210)**	%70	0	11	21.17 (21.20)

Infrared spectra studies of Ligand (HMP) and complexes

The FTIR spectrum infrared data (KBr disk) of [3-(4-hydroxyphenyl)-2-(3-(4-methoxybenzoyl)thioureido)propanoic acid] (HMP) (Table 2) and Fig. (1) , Showed bands due to υ (NH) amide, υ (OH), υ(C=O) (amidic) and (C=S) which were observed at, (3311) cm^{-1}, (3469) cm^{-1}, (1602) cm^{-1}, (1234) cm^{-1}, respectively . While another absorption band appeared were the υ (COO) sym was noticed (1417) cm^{-1} . and these spectra showed exceptional difference between the bands belonging to the stretching vibration of the υ (NH) band for the amine group in the range between (3417 -3468) cm^{-1} shifted to lower frequencies by (106-157) cm^{-1} suggesting the possibility of coordination bonding through the nitrogen atom in the amine group[10,11] . while the resulting band shifted frequencies less by (39-60) cm^{-1}, as well the absorption specific to υ(COO)sym was observed in the range (1452-1483) cm^{-1} shifted to higher frequencies by (35-66) cm^{-1} , which indicates the coordination of the central carboxylic group [12] , the stretching vibration band $\upsilon(C=O)_{amid}$ and υ(C=S) either show no change or very little in their frequencies (1589-1606)cm^{-1} and (1232-1244)cm^{-1} respectively therefor, indicating do not coordinate to the metal ion. Metal-oxygen and metal-nitrogen were confirmed by the presence of the stretching tremor of υ (M-O) and υ (M-N) around (491-516)cm^{-1} and (418-462)cm^{-1} respectively[13,14]. Table (2) describe the important bands and assignment for free ligand (HMP) and its complexes .

Table (2): FT-IR frequencies in (cm^{-1}) for ligand (HMP) and its metal complexes

Compound	υ(N-H) υ(O─H)	υ(COO) Sym	υ(COO) assym	υ(C=O)	υ (C=S)	υ(M-O)	υ (M─N)
Ligand	3311 (m) 3469	1417 (m)	1728 (s)	1602(w)	1234(s)	—	—
$[Co(HMP)_2]$	3417 (b)	1463(s)	1668(s)	1589 (m)	1244(s)	516 (m)	425 (m)
$[Ni(HMP)_2]$	3417 (b)	1467(m)	1668(m)	1600 (w)	1238 (s)	491 (m)	420 (w)
$[Cu(HMP)_2]$	3468 (b)	1475(m)	1680(s)	1604 (m)	1232 (s)	511 (m)	460 (m)
[Zn(HMP)2]	3417 (b)	1479 (s)	1668(s)	1597(m)	1234 (s)	505 (m)	418 (w)
$[Cd(HMP)_2]$	3417 (b)	1479 (s)	1674(s)	1602 (m)	1232 (s)	513 (m)	439 (w)

[Hg(HMP)$_2$]	3417(b)	1452(s)	1669(s)	1606 (w)	1232 (m)	505 (m)	462 (m)
[Pd(HMP)2]	3421 (b)	1483 (s)	1689(s)	1606 (w)	1236 (s)	514 (m)	462 (m)

b=broad , S= strong , M=medium , w=weak

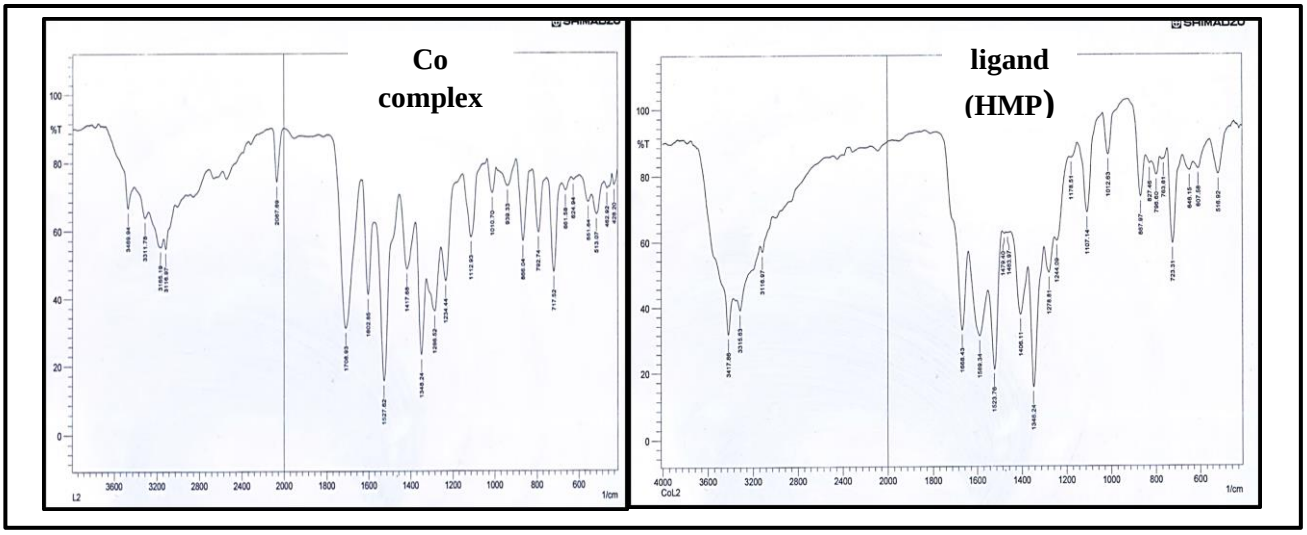


Fig (1): FTIR spectra of ligand(HMP) and Co complex

^{1}H -NMR studies of ligand (HMP)

The proton nuclear magnetic resonance spectrum for the ligand (HMP) was carried out using (DMSO) as a solvent and the following peaks were detected, Figure (2)The binary signal at δ (2.17) ppm belonging to (1H,NH $_{amine}$) , And the appearance of a single signal at δ (2.50) ppm which is attributed to the solvent(d^6- DMSO) , And the emergence of multiple signals at δ (2.73- 3.39) ppm due to (2H,CH_2 and (1H,CH) , And the appearance of a single signal at δ (3.93) ppm belonging to (3H, OCH_3) as well as the emergence of a single signal at δ (9.71) ppm belonging to (1H, OH phenolic). It was also observed that multiple signals appeared at δ (6.69- 8.41) ppm, which belong to aromatic protons, and a single signal appeared at δ ((7.79) ppm , due to (1H,NH $_{sec.CO}$) and a mono signal at δ (11.68) ppm which is attributed to (1H,COOH)[15] .

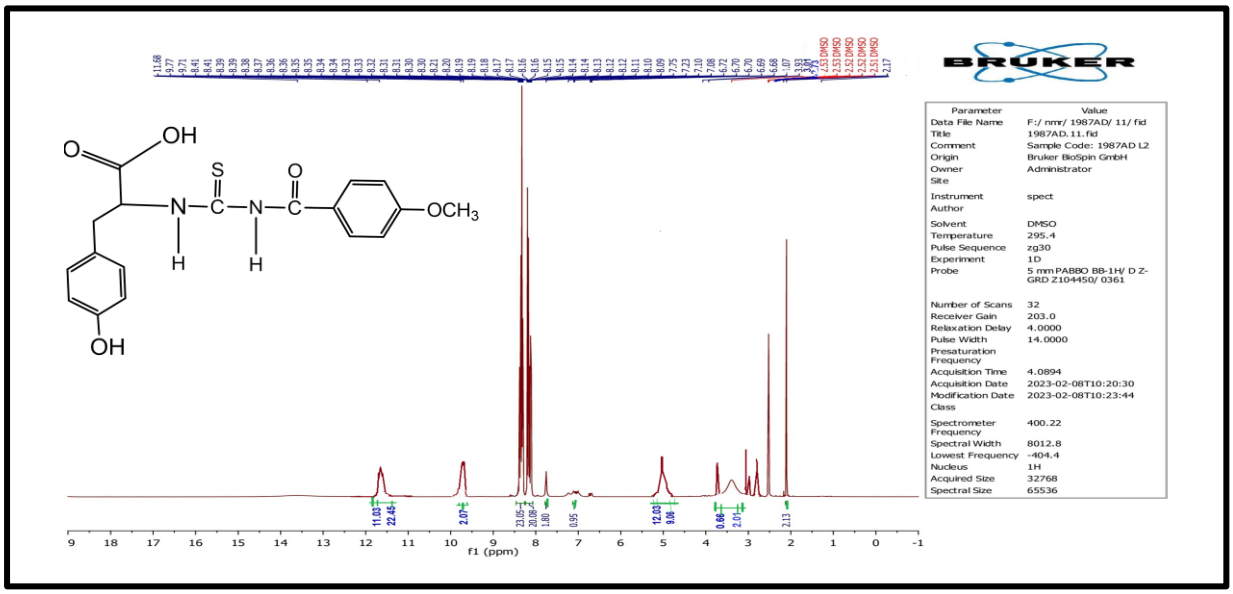


Figure (2): ^{1}H -NMR Spectrum of ligand(HMP)

^{13}C-NMR studies of ligand (HMP)

^{13}C-NMR spectrum of the free ligand(HMP) in d^{6}-DMSO , Figure (3) showed for following signals: Signals at δ (50.02) ppm for (CH_2) , and also δ (61.13) ppm for CH-COOH , and multiple signals at δ (40.48 - 39.22) ppm that are due to the d^{6}-DMSO solvent , Multiple signals were also observed that appeared at δ (149.44- 123.70) ppm , which are due to aromatic carbons , and the appearance of one signal at δ (167) ppm belonging to (C = O), and another signal appeared at δ (172.57) ppm due to COOH , and another signal appeared at δ (182.26) ppm belonging to the (C = S) [16] .

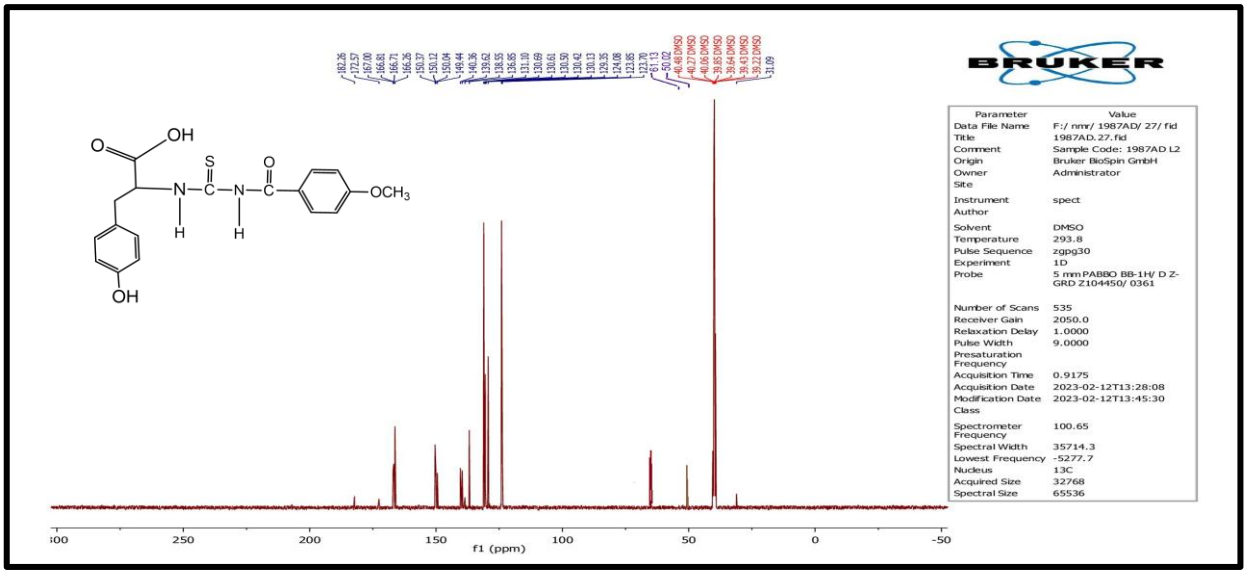


Figure (3): ^{13}C-NMR Spectrum of ligand(HMP)

Electronic spectra studies of ligand (HMP) and complexes

The ligand (HMP) spectrum showed absorption band at (265) nm (37735) cm $^{-1}$ and with a molar absorbance of ϵ_{max} L.mol^{-1}.cm^{-1} (1762) and returns to the bundle π-π*, and Weak package at (315) nm , (31746)) cm $^{-1}$ and with a molar absorbance of ϵ_{max} L.mol^{-1}.cm^{-1} (289) and returns to the bundle n-π* . And the data obtained from Uv–vis spectra of the synthesized of the ligand (HMP) given in (Table3) and Fig (4)

-The spectrum of cobalt compound [Co (HMP)$_2$] d^7 (green -colored) Shows Four bands. At (39370) cm^{-1}, (33670) Cm^{-1}, (21097) cm^{-1} and (15479) cm^{-1} retunes to intra-ligand (C.T), $^4A_2$ (F) → $^4T_1$ (P), $^4A_2$ (F) → $^4T_1$ (F), and $^4A_2$ (F) → $^4T_2$ (F) respectively as Show in table (3). and the repulsion parameter(B$^-$) was calculated and found to be (555) Cm^{-1} and(β) was found to be equal (0. 571) cm $^{-1}$ from the relation β = B$^-$/ B$_0$ .these parameters are accepted to Co^{+2} Tetrahedral complex [17] .

-The spectrum of the Nickel compound [Ni (HMP)$_2$] d^8 (greenish yellow color) showed an absorption band at (39062) cm^{-1}, (31746) cm^{-1}, (15220) cm^{-1}and(11148) Cm^{-1} retunes to reveals the following electronic transfer transition (C.T), $^3T_1$ (F) → $^3T_1$ (P), $^3T_1$ (F) → $^3A_2$ (F) , and $^3T_1$ (F) → $^3T_2$ (F) . respectively,the repulsion parameter (B$^-$) value establish to be (901)cm $^{-1}$ while β it was found (0.865) these parameters are accepted to Tetrahedral complex for Ni^{+2}.

-The spectrum of the copper compound [Cu (HMP) $_2$](d^9) (orange color) showed three absorption band at (37453) Cm^{-1}, (14814) cm^{-1} and (11682) cm^{-1} which due to the Electronic transfers C.T, $^2B_1$(g) → $^2A_1$(g), and $^2B_1$(g) → $^2B_2$(g). respectively which was a good agreement for square Planer complexes for Cu (II) [18] .

-The spectrum of the Palladium compound [Pd (HMP) $_2$](d^8) (dark brown color) showed three absorption band at (37313)cm^{-1} , (11273)cm^{-1} and (10121)cm^{-1} attributed to (C.T) , $^1A_1$(g)→ $^1A_2$(g) , $^1A_1$(g)→ $^1B_1$(g) transition respectively which was a good agreement for square Planer complexes for Pd (II) [19] .

-The spectrum of the complexes of [Zn(HMP)$_2$] , [Cd(HMP)$_2$] and [Hg(HMP)$_2$] shows bonds are returning to electronic transition C.T respectively. (37037) cm^{-1}, (37312)cm^{-1}and (37453) cm^{-1} [20] respectively All transition with their assignments are summarized in Table (3).

Table (3) Electronic spectral data of ligand (HMP) and its complexes in DMSO Solvent.

Compounds	*λ(nm)*	*$\bar{v}$ (cm^{-1})*	*ABC*	*€ max*	*Transitions*	*Geometric shape*
Ligand (HMP)	265 315	37735 31746	1.762 0.289	1762 289	π-π* n-π*	----------
$[Co(HMP)_2]$	254 297 474 646	39370 33670 21097 15479	1.200 0.598 0.080 0.240	1200 598 80 240	C.T $^4A_2(F)\rightarrow{}^4T_1(P)$ $^4A_2(F)\rightarrow{}^4T_1(F)$ $^4A_2(F)\rightarrow{}^4T_2(F)$	Tetra herald
$[Ni(HMP)_2]$	256 315 657 897	39062 31746 15220 11148	1.900 0.974 0.035 0.032	1900 974 35 32	C.T $^3T_1(F)\rightarrow{}^3T_1(P)$ $^3T_1(F)\rightarrow{}^3A_2(F)$ $^3T_1(F)\rightarrow{}^3T_2(F)$	Tetra herald
$[Cu(HMP)_2]$	267 675 856	37453 14814 11682	1.271 0.134 0.047	1271 134 47	C.T $^2B_1g\rightarrow{}^2A_1g$ $^2B_1g\rightarrow{}^2B_2g$	Square planar
$[Pd(HMP)_2]$	268 887 988	37313 11273 10121	1.400 0.050 0.070	1400 50 70	C.T $^1A_1g\rightarrow{}^1A_2g$ $^1A_1g\rightarrow{}^1B_1g$	Square planar
$[Zn(HMP)_2]$	270 340	37037 29411	0.874 0.213	874 213	C.T C.T	Tetra herald
$[Cd(HMP)_2]$	268 366	37313 27322	1.732 0.221	1732 221	C.T C.T	Tetra herald
$[Hg(HMP)_2]$	267 361	37453 27700	1.673 0.233	1673 233	C.T C.T	Tetra herald

C.T = Charge transfer

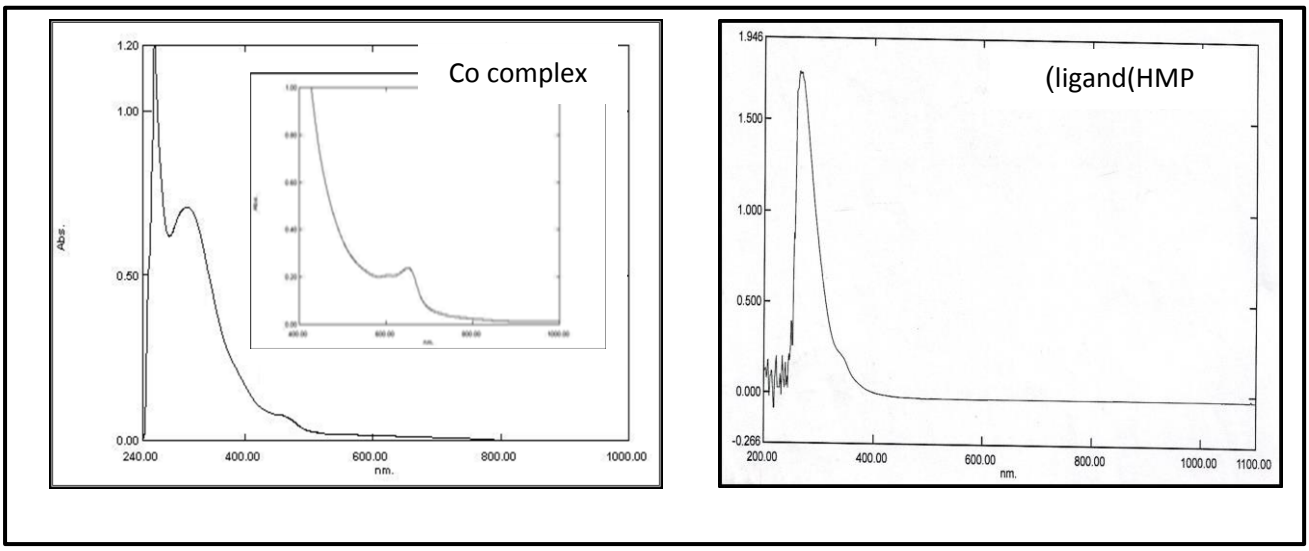


Fig. (4): Electronic spectrum of ligand(HMP) and Co complex

Biological activity study

The biological activity of the ligand and their complexes has been tested against two types of bacteria by using the inhibition Zone method .

Escherichia Coli: when we comparing the results with the percentage of Amoxicillin we find the Ligand (HMP) ,Cd ,Co and Hg complexes Showed a greater inhibition than Amoxicillin while the Ni , Cu ,Zn and Pd Complex showed the lower inhibition than Amoxicillin antibiotic.

Staphylococcuse aurous: when we comparing the results from the results in table (4) and Figures (5,6) ,with the percentage of Amoxicillin antibiotic inhibition we find the Hg complex showed a greater inhibition than Amoxicillin , As for the ligand and the Cd complex they showed an inhibition rate similar to Amoxicillin, while the Ni, Co ,Cu , Zn and Pd complexes showed smaller than Amoxicillin.

Table(4) Effect of ligands (HMP) and metal complexes dissolved in DMSO solvent on two types of bacteria compared to Amoxicillin

Comp.no	Anti-bacterial Activity	
	Staphylococcus(+)	E.coli(-)
DMSO	-	-
Ligand (HMP)	15	16
Amoxicillin	15	14
[Co(HMP)$_2$]	13	15
[Ni(HMP)$_2$]	10	10
[Cu(HMP)2]	14	13
[Zn(HMP)$_2$]	10	12
[Hg (HMP)$_2$]	20	15
[Cd (HMP)$_2$]	15	20
[Pd(HMP)$_2$]	14	13

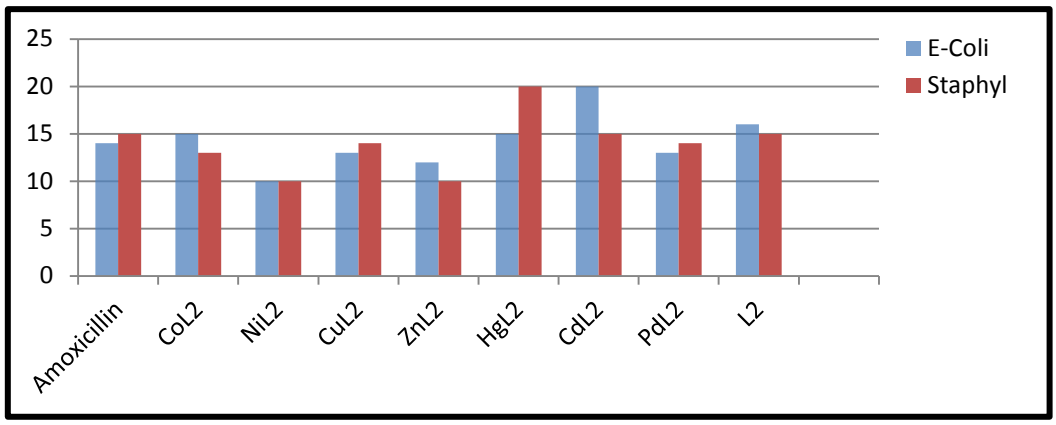


Fig. (5):Effect of ligand (HMP) and its metal complexes in inhibiting bacterial

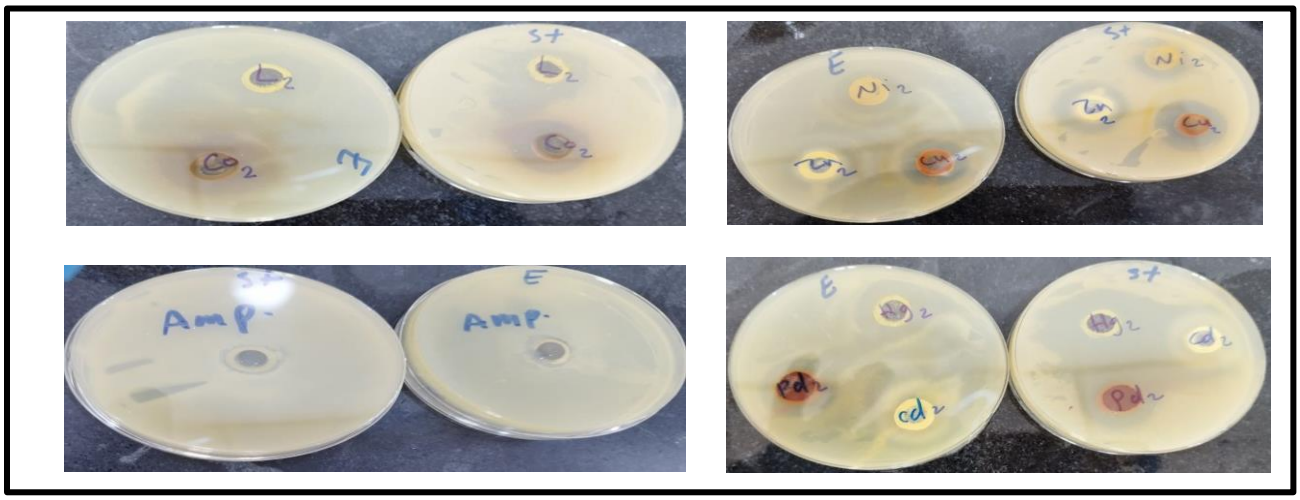
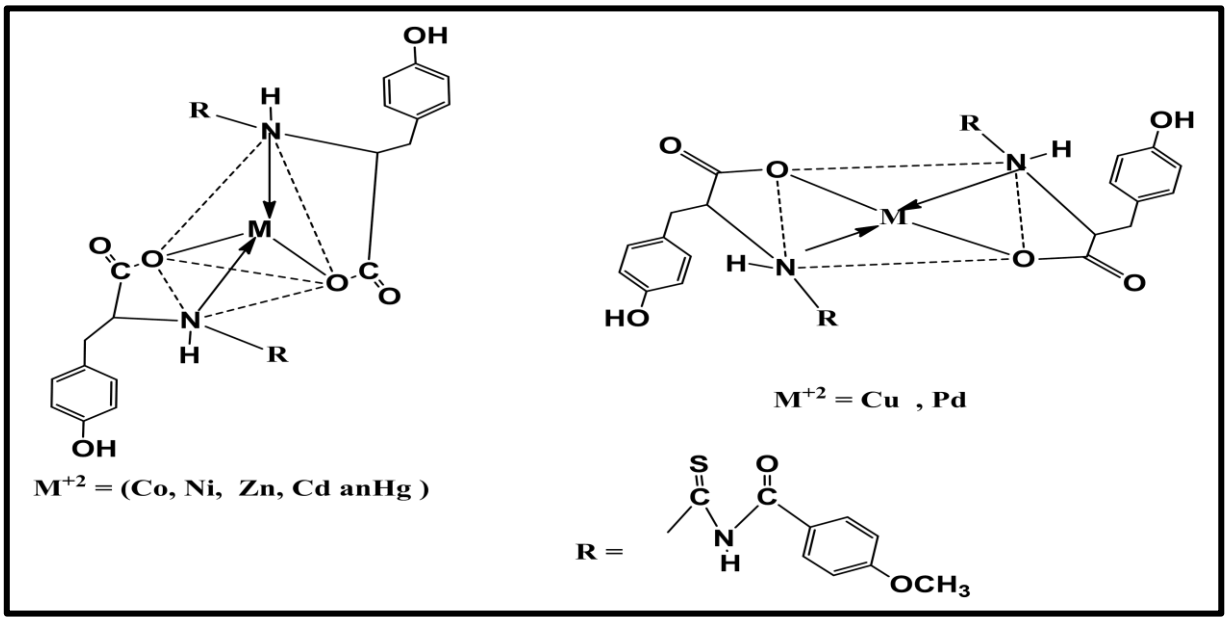


Fig- (6) The effect of ligands (HMP) and metal complexes on Staphylococcus and Escherichia- Coli

Conclusions

The new ligand [3-(4-hydroxyphenyl)-2-(3-(4-methoxybenzoyl)thioureido)propanoicacid (HMP) and its complexes with metal ions were prepared and characterized by analytical and spectroscopic data . The (HMP) ligand acts binary, in coordination with the Oxygen and nitrogen atoms of the (COO-) and (NH) groups respectively as the illustration of Figure (7). Electronic and magnetic measurements suggested tetrahedral Structure for all complexes except Cu (II) and Pd (II) complex has a square planar geometry . The biological efficiency of the ligand(HMP) and its complexes were performed and compare it the antibiotic Amoxicillin and the experimentation showed that the ligand and all the complexes have good activity against bacteria.



Figure(7):The proposed chemical structure formula of the complexes

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