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STRUCTURE AND ANTIMICROBIAL STUDIES OF NEW HYDRAZONE LIGAND, 3-[2-(1,5-DIMETHYL-3-OXO-2-PHENYL-2,3-DIHYDRO-1*H*-PYRAZOL-4-YL)HYDRAZINYLIDENE]-1-PHENYLBUTANEDIONE, AND ITS Co(II), Fe(III), Ni(II) and Cu(II) COMPLEXES

[*1,2]Ndidiamaka Justina Agbo, [1]Pius Oziri Ukoha

[1]Co-ordination Chemistry and Inorganic Pharmaceuticals Unit, Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, Enugu State, Nigeria

[2]Chemistry Advanced Research Centre,

Sheda and Science and Technology Complex (SHESTCO), Garki-Abuja, Nigeria

[*]Corresponding Author: agbo.ndj@gmail.com

ABSTRACT

A new ligand, 3-[2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)hydrazinylidene]-1-phenylbutanedione, (hereafter designated as HL**),** and its Co(II), Fe(III), Cu(II) and Ni(II) complexes were synthesized. $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, $[Cu(HL)_2]Cl_2$ and $[Ni(HL)_2]Cl_2$ represent the Fe(III), Co(II), Cu(II) and Ni(II) complexes of ligand HL respectively. The compounds were characterized by UV, IR, elemental analysis, mass spectrometry, and NMR spectroscopy. The molar conductivity values indicated that $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, and $[Ni(HL)_2]Cl_2$ were electrolytes and $[Cu(HL)_2]Cl_2$ was non-electrolyte when compared with $CuSO_4$ and KCl salts. The chloride analysis revealed the presence of chloride ions outside the coordination sphere of all the complexes. Bonding of the ligand to the central metal atom in the complexes may have occurred by δ-bonding through the participation of carbonyl oxygen of pyrazolone and diketones and hydrazo nitrogen group. Based on these data, an octahedral geometry was assigned to all the complexes. NMR result showed that HL existed in hydrazo and azo forms. The antimicrobial tests revealed that HL, $[Cu(HL)_2]Cl_2$ and $[Ni(HL)_2]Cl_2$ complexes had activities against *B. subtilis*, *S. typhil*, *S. pneumeniae*, *E.coli* and *Proteus moratis*. HL and $[Ni(HL)_2]Cl_2$ had good activities on *B. subtilis* and Eco 6 respectively up to 0.3125 **μg/**cm^3. This implies that HL, $[Cu(HL)_2]Cl_2$ and $[Ni(HL)_2]Cl_2$ compounds may be used as antibacterial agents against drug-resistant strains.

KEYWORDS: Antipyrines, Diketone, Butanedione, Hydrazone, Metal complexes, Antimicrobial studies.

INTRODUCTION

Antipyrine and their derivatives have attracted considerable interest because of their pharmaceutical and therapeutic properties [1-3]. Since 1884, when antipyrine was marked as a drug used in management of pains, inflammation and fever, great attention was focused on pyeazolone derivatives as potent anti-inflammatory [2], analgesic and antipyretic agents [2, 3]. The coordinating property of 4-aminoantipyrine ligand has been modified to give a flexible ligand system, formed by condensation with variety reagents like aldehydes, ketones and carbazides [4]. As a result, a large number of hydrazones derived from 4-aminoantipyrines and diketones have been synthesized. The literature survey reveals that more attention has been given to Schiff's base derived from 4-aminoantipyrine with several aldehydes. But less work has been reported on the condensation process in 4-aminoantipyrine with diketone like: 3-[2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)hydrazinylidene]-1-Phenylbutandione. There are no known reports on hydrazones derived from butandione and 4-aminoantipyrine.

The aim of the study is to synthesize a novel ligand derived from butandione and 4-aminoantipyrine and characterize the ligand and its complexes with spectroscopic techniques. Also, to carry out antimicrobial studies on this ligand and its Fe(II), Co(II), Cu(II) and Ni(II) complexes. In this research, synthesis and physical properties of the compounds were carried out at Post Graduate Laboratory, Department of Pure and Industrial Chemistry University of Nigeria, Nsukka. Elemental analysis was done at Micro-analysis Laboratory, University of Strathclyde, Scotland. NMR and Mass spectroscopy were done at Campbell Micro-Analytical Lab, University of Otago, New Zealand, while UV and IR were done at National Research Institute for Chemical Technology, Zaria. Antimicrobial activity was done at the Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

MATERIALS AND METHODS

All chemicals used were of analytical grade and were products of Sigma Aldrich. They were used as purchased without further purification unless otherwise stated.

Heating was done on Gallenkamp Magnetic Stirrer/Thermostat hot plate. John-Fisher melting point apparatus was used in determining melting points of compounds. UV Visible spectra were obtained on Varian Carry-100 Uv-visible spectrophotometer (Made in California, U.S.A) whereas Perkin-Elmer FTIR spectrometer and Bruker DPX 400 NMR spectrophotometer were used to run ^{I}H and ^{13}CNMR spectra of compounds respectively.

Carbon, hydrogen and nitrogen were determined on a Heraeus Carlo Erba 1108-CHN analyser. Conductivity of 1.0 x 10^{-3}mol/dm^{-3} methanol solution of compounds was determined using WTW-LF90 conductivity meter (Made in London, England).

The microorganisms used for the study, *P. aeruginosa, S. aureus, E. coli, B. sabtilis. S. pneumeniae, Proteusspp, S. intermedius and K. pneumoniae* were clinical isolates obtained from pig, poultry and human. The *in vitro* antibacterial studies were carried out using agar well diffusion method [5, 6].

Synthesis of 3-[2-(1, 5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)hydrazinylidene]-1-phenylbutandione (HL)

The ligand was prepared by adopting the method of Heinosuke Yasuda [7]. 4-Aminoantipyrine (0.0006 mole) was dissolved in diluted hydrochloric acid (1 cm^3 in 5 cm^3 water) and diazotized with (0.0009 mole) of sodium nitrite solution below 5 ºC with stirring. The resulting diazotized 4- aminoantipyrine was poured into a solution of 0.0006 mole of 1-phenyl-1, 3-butanedione and 0.0305 mole of sodium acetate respectively using mechanical stirring at room temperature. An orange powdery product was collected for HL and characterized (yield: 51.37%, M.pt: 66 ºC). The orange compound was recrystallized and dried in a desiccator.

Synthesis of metal complexes

The metal complexes were prepared by adopting the method of El-Saied *et al* [8]. The metal solution of 2 moles of a metal salt with I mole of 3-[(E)-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dhydro-1H-Pyrazole-4-yl)diazenyl]1-Phenylbutandione in about 50 cm^3 EtOH was stirred for a periods of 6 h at 60 ºC. The resulting solids were filtered off, recrystallized with EtOH and dried in desiccator over $CaCl_2$.

Antimicrobial Activity of ligand (HL) and its complexes

Preliminary antibacterial screening of the ligand and complexes in DMSO were examined by using agar-well diffusion method [9, 10]. The compounds were dissolved at concentration of 10 mg/cm^3 in order to obtain a final concentration of 0.156 mg/cm^3. A single colony of each test bacteria was suspended in 2 cm^3 of sterile nutrient broth. Cork borers of 6 mm in diameter, wells were bored in the inoculated agar plates. A micropipette of 50 cm^3 of each test compound was delivered into the well. The plates were left on the bench for 30 min to allow the substance to diffuse into the agar. The plates were incubated at 37 ºC for 24 h. After incubation, the plates

were observed for inhibition zones around the wells and the diameters of the zones were measured with a meter rule. On the basis of preliminary test, the compounds affecting significant zones of inhibition were then selected and used for the minimum inhibition concentration (MIC) determination by double serial dilution of test compound.

Ampicillin was used as the reference standard. The bacteria which include *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Streptococcus pneumeniae, Proteus molatis, Streptococcus Intermedius (G106) and Klebsiella pneumeniae* were obtained from stock culture (clinical isolate) and were maintained separately on solid medium containing agar. All the materials used were sterilized.

RESULTS AND DISCUSSION

Physicochemical properties of the compounds

The yield, melting point, colour, texture, conductivity and chloride content of synthesized compounds are presented in Table 1.

Table 1: Physical properties of HL and its complexes.

Compound	Colour	Texture	Melting point (^{o}C)	Yield (%)	Molar Cond. (S /cm)	Cl^-
HL	Orange	Powder	64- 66	51.37	0.48 X10^{-6}	Present O.S
[Fe(HL)$_2$]Cl$_3$	Brown	Powder	123-124	47.89	1.30 x 10^{-3}	Present O.S
[Co(HL)$_2$] Cl$_2$	Black	Crystalline	57- 58	43.47	1.8 x 10^{-3}	Present O.S
[Cu(HL)$_2$] Cl$_2$	Black	Granular	70 71	65.45	30.2 x 10^{-4}	Present O.S
[Ni(HL)$_2$]Cl$_2$	Black	Granular	69-70	62.31	3.4 x 10^{-4}	Present O.S

Legend: O.S = outer-sphere, I.S = inner-sphere

The different colours, yield and melting point of the complexes are high indicators of formation of new compounds from the reaction of the ligand with the metal salts. Comparing the conductivity of the ligand and complexes with KCl (1:1 electrolyte) and CuSO4 (2:2 electrolytes), it is very obvious that all the complexes with conductivity not close to KCl (1:1

electrolyte) and $CuSO_4$ (2:2 electrolytes) are non-electrolytes whereas the ligand is neutral. The formulae they are given also suggest this.

CHN analysis data

Carbon, hydrogen and nitrogen content of the ligand and complexes are given in Table 2.

Table 2: Elemental analysis of HL and its complexes

Elements		HL	$[Fe(HL)_2]Cl_3$	$[Co(HL)_2]Cl_2$	$[Cu(HL)_2]Cl_2$	$[Ni(HL)_2]Cl_2$
C	Found %	65.187	58.18	57.96	57.13	57.25
	Cal %	66.99	57.32	57.12	56.83	56.73
H	Found %	5.22	5.17	5.07	5.145	4.58
	Cal %	5.36	5.92	4.57	4.55	4.35
N	Found %	14.71	12.145	12.99	12.99	12.18
	Cal %	14.08	13.67.	12.69	12.69	12.72

The percentage of the elements present determined experimentally is compared with theoretical predictions. This is in very close agreement with the values thereby confirming synthesis of the compounds.

Ir spectra of HL and its $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, $[Cu(HL)_2]Cl_2$, and $[Ni(HL)_2]Cl_2$ complexes:

Ir spectra of a novel hydrazone ligand: 3-[2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)hydrazinylidene]-1-phenylbutandione(HL) and its complexes are presented in Table 3.

Table 3: Selected Infrared spectra assignments of HL and its complexes

HL	$Fe(HL)_2]Cl_3$	$[Co(HL)_2 Cl_2]$	$[Cu(HL)_2] Cl_2$	$[Ni(HL)_2]Cl_2$	Assignment
3415(br)	3417(br)	3409(br)	3408.33(br)	3444.98(br)	ν (N-H)
3032 (sh)		3050(w)	3061.13(w)	3074.63(w)	$\nu(C\text{-}CH_2)$
22929(sh)	2955(sh)	2928(sh)	2935.76(sh)	2920(w)	$\nu(N\text{-}CH_3)$
1810(sh),	1806(w)		1761.07(w)	1775(w)	ν(C=O) acetyl
1661(m)	1653(sh)	1670(w)	1650.20(sh)	1676.20(sh)	V(C=O) of pyrazolone
1643(m)	1630(sh)				
1597(s), 1578(m)	1596(w), 1554(m)	1595(w)	1595.18(s)	1525.74(s)	ν(C=N)
1522(s)	1502(w)	1575(m)			
1462(sh), 1447(m)	1400(sh)	1497(w)	1467.88(w)	1423.51(sh)	ν(C = C) of aromatic
1423(s)		1484(w)	1433.16(w)		
1357(sh), 1338(s)	1369(sh)	1399(s)	1368(sh)	1381(s)	Pyrazolone ring stretch
	512(w)	577(s)	569.02(sh)	553.59(sh)	(M-O) stretch
	488(w), 463(w)	488(sh)	449.43(sh)	457.14(sh)	(M-N) stretch
					(M-Cl) stretch

s= Strong, m- Medium, w- Weaker, br-Broad

A broad peak situated at 3415 cm^{-1} represents an (N-H) stretching vibration and this is in agreement with previous observations [11]. The peaks around 1810 and 1750 cm^{-1} were assigned to C=O of diketones, while the peaks at 1661 and 1643 cm^{-1} were assigned to the carbonyl (C=O) group of pyrazolone [12]. The rocking frequencies were observed as shoulder and weak peaks at 2929 cm^{-1} and 3032 cm^{-1} respectively. A strong peak at 1597 cm^{-1} was assigned to (C=N) stretching vibration.

It was observed that after HL complexed, the absorption peaks shifted to higher, lower, higher and even higher in the $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, $[Cu(HL)_2]Cl_2$, and $[Ni(HL)_2]Cl_2$ complexes spectrum respectively. This is in agreement with previous observations of other azopyrazolones complex [8]. A shoulder absorption band at 1462 cm^{-1} was observed and assigned to be due to (C=C) of the aromatic and after complexation the peaks almost remained the same in all the complexes. A characteristic peak assignable to C=C bending vibration of aromatic pyrazolone ring stretch was observed around 1462 cm^{-1}. In the spectra of the

complexes some of the peaks observed for (C=O) stretch in the ligand shifted to lower and higher frequencies in both complexes, showing an involvement of C=O carbonyl in the complexation. $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, $[Cu(HL)_2]Cl_2$, and $[Ni(HL)_2]Cl_2$ complexes had peaks around 512, 577, 569.02 and 553.59 cm^{-1} respectively. These peaks could be due to M-O vibration [13]. Other peaks around 463 cm^{-1}, 488 cm^{-1}, 449.43 cm^{-1} and 457.14 cm^{-1} in the spectrum of $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, $[Cu(HL)_2]Cl_2$, and $[Ni(HL)_2]Cl_2$ respectively could also be due to M-Cl vibrations [13].

Electronic Spectra

Table 4 shows the wavelength of maximum absorption as the molar absorptivity of ligand and complexes. The solution spectra were obtained in methanol.

Table 4: Electronic spectra of HL and its complexes

Compounds	λ max (nm)	λmax(cm^{-1})	ε($dm^3mol^{-1}cm^{-1}$)	Assignment
HL	413	24213	264.361	$\pi \rightarrow \pi^*$
	522	19157	67.6691	$n \rightarrow \pi^*$
$[Fe(HL)_2]Cl_3$	496.4	20145	62.3188	d←d
	560.7	17835	56.9565	d←d
	665	15037	40.8696	d←d
	775	12903	56.6667	d←d
$[Co(HL)_2]\ Cl_2$	351	28490	518.3110	$n \rightarrow \pi^*$
	430.35	23237	143.6162	$n \rightarrow \pi^*$
$[Cu(HL)_2]]\ Cl_2$	433	23095	230.5246	d←d
$Ni(HL)_2]\ Cl_2$	737.5	13559	22.9720	$^3A_{2g} \rightarrow {}^3T_g$ v(F)

Considering the electronic spectra of HL with that of its complexes in methanol (10-4 M) in Table 4 and with regards to band position and intensity, their spectra show much similarity. Two absorption bands, 413 and 522 nm, were observed. The bands could be attributed to $\pi \rightarrow \pi^*$ transitions of the conjugated bonds and $n \rightarrow \pi^*$ transitions of the non-bonding electrons in the ligand.

In the spectrum of $[Co(HL)_2]Cl_2$, two absorption bands were observed. The bands are 351 and 430.35 nm. These absorptions show a hypsochromic shift, indicating that the ligands are coordinates to metal ions via the oxygen of the carbonyl groups and with the hydrazone nitrogen [14]. These bands can be attributed to $\pi \rightarrow \pi^*$ transitions of the conjugated bonds and $n \rightarrow \pi^*$ transitions of the ligand. A shift from 413 nm of ligand HL to 351 nm in $[Co(HL)_2]Cl_2$

complex , showing evidence of complexation was observed in the ligand's spectrum in relation to the complex's spectrum.

Four absorption bands were observed in the spectrum of $[Fe(HL)_2]Cl_3$. The bands are 496.4 nm (10373 cm^{-1}), 560.7 nm (17835 cm^{-1}), 665 nm (15038 cm-1) and 775 nm (12903 cm^{-1}). The bands are mainly attributed to metal to ligand charge transfer or vice versa existing in this complex [14]. A shift from bands of ligand to bands of complex showing evidence of complexation was observed in the relation electronic spectrum of the complex.

$[Cu(HL)_2]Cl_2$ spectrum shows only one absorption band at 433 nm. The band is attributed to $n \rightarrow \pi^*$ of the ligand but the shift from 413 nm of HL to 433 nm in $[Cu(HL)_2]Cl_2$ suggests the coordination of ligand with Cu(II) ion [15]. This inner ligand transitions are common due to the presence of (C=O), (C=N) and (C=C) groups in the ligand structure [16]

$[Ni(HL)_2]Cl_2$ spectrum shows one strong absorption bands in the ultraviolet region, and it is 737.5 nm (13557 cm-1). This band could to be due to $^3A_{2g}$ _ ^3T_g ν (F) transition which suggested octahedral geometry for Ni(II) complex [16].

The molar conductance value of all the complexes revealed that $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$ and $[Ni(HL)_2]Cl_2$ are electrolytes, while $[Cu(HL)_2]Cl_2$ is non-electrolytes when compared to $CuSO_4$ and NaCl salts $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$ and $[Ni(HL)_2]Cl_2$ complexes were observed to have Cl^- inside co-ordination sphere with metal ion, while $[Cu(HL)_2]Cl_2$ was outside the coordination sphere [17].

^{1}H NMR and ^{13}C NMR Spectra of the Ligand HL

The proton nuclear magnetic resonance spectrum for HL and ^{13}C NMR are represented in Table 5.

The 1H NMR of HL was run in two places with different solvents. This shows that it existed in tautomeric form of azo and hydrazo form.

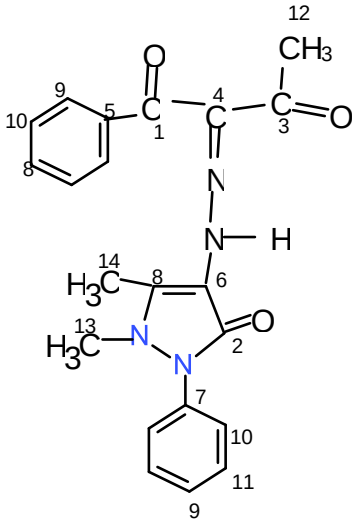
The azo form which was run in $CDCL_3$ as a solvent shows peaks at 1.6645ppm (1H, s), 2.0102ppm (3H,s) , 2.6265 (3H,s) and 3.0212 (3H,s) indicating the $CDCL_3$ solvent peak, CH_3 methyl carbon of acetyl, C-CH_3, and N-CH_3 methyl protons of the pyrazolone ring respectively. Peaks around 7.3179-7.5448(5H,m), 7.6188 &7.6223(1H,d) (4H,d) is due to phenyl protons. At 14.4375(1H, s), the peak indicated OH proton.

The hydrazo form of HL which was run in $CDCl_3$ + CD_3OD as solvent shows peaks at 1.88 and 1.99 ppm (3H,s) , 2.16 (3H,s) and 3.28 (3H,s) indicating CH_3 methyl carbon of acetyl, C-CH_3, and N-CH_3 methyl protons of the pyrazolone ring respectively. Another peaks around

4.88(1H,s), 6.35(1H, s) indicating solvent (HDO) peak and N-H proton. Peaks around 7.44 and 7.845(1H, d), 7.50 and 7.52(1H,s) were due to phenyl protons.

Table 5: Proton (1H) and ^{13}C- NMR spectra of HL [in ppm from TMS, $CDCl_3$]

Proton (^{1}H) data of HL		**^{13}C- NMR data HL**	
Peaks (δ)	Assignment.	Position of carbon	Chemical shift/ppm
1.6645	H_2O (trace impurity)	C1	197.22
2.0102(3H,s)	C- CH_3 methyl protons from acetyl	C2	194.16
2.6265(3H,s)	C- CH_3 methyl protons from pyrazolone	C3	182.78
3.0212(3H,s)	N- CH_3 methyl protons from pyrazolone ring	C4	178.36
7.3179-7.5448(5H,m) 7.6188 &7.6223(1H,d)	protons of phenyl ring	C5	133.4767
14.4375(1H,s)	N-H proton	C6	128.88
		C7	126.25
		C8	96.21
		C9	47.655
		C10	34.01
		C11	29.44
		C12	24.41
		C13	22.45
		C14	**9.67**



The 13C NMR spectrum of azo form of HL gave fourteen (14) peaks corresponding to the number of carbon atom in azo structure [18,19]. The peaks at 197.2550 and 192.7911 ppm were for the carbon atoms of the carbonyl groups. The peak at 158.8717 ppm and 143.1192 ppm were assigned to the carbon atoms of C-OH and C-N=N group respectively. The peaks for the carbon atoms of the aromatic rings substituent appeared at 139.3866 ppm, 124.0001 ppm, 114.6728 ppm, 77.0151 ppm. The peaks for carbon atoms of antipyrine appeared at

132.3844, 129.4933 and 127.8724 ppm. Peaks due to carbon atoms of the methyl rings appeared at 36.1439, 30.3369 and 10.9233 ppm respectively.

The ^{13}C NMR spectrum of hydrazo form of HL gave the same fourteen (14) peaks corresponding to the number of carbon atom in hydrazo structure. The peaks at 197.22 and 194.16 ppm were for the carbon atoms of the carbonyl groups. The peak at 182.78 ppm and 178.36 ppm were assigned to the carbon atoms of C=O of methyl and C=N group respectively. The peaks for the carbon atoms of the aromatic rings substituent appeared at 133.4767 ppm, 47.655 ppm, 34.01 ppm, 29.44 ppm. The peaks for carbon atoms of antipyrine ring appeared around 128.88, 126.25 and 96.21 ppm. Peaks due to carbon atoms of the methyl rings appeared at 24.41, 22.45 and 9.67 ppm respectively.

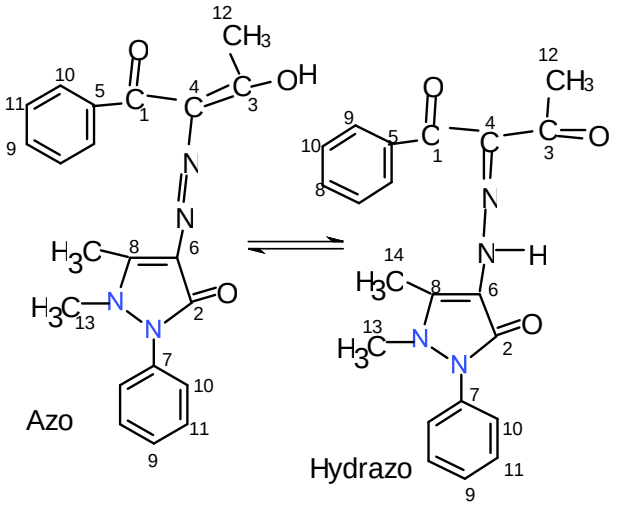


Fig 1: The tautomeric form of HL.

Mass spectrometry

Mass spectrometer showed signals at: m/z , 464.5385 (m.wt), m/z 384.336 indicated the removal of ($-C_6H_5$) of dibenzoyl methane in the ligand, m/z 306.4805 shows that fragmentation has taken place at the second benzoyl group. The mass spectrum shows a fragment at an m/z value of 922.872 suggesting that the ligand exists in a dimeric form probably H-bonding.

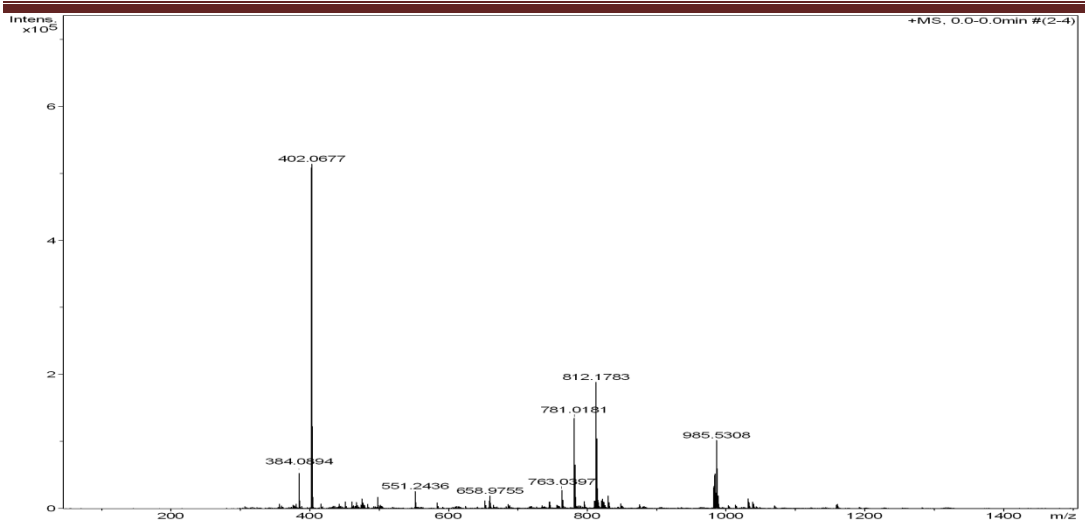


Fig 2: The mass spectral of HL.

Proposed structures:

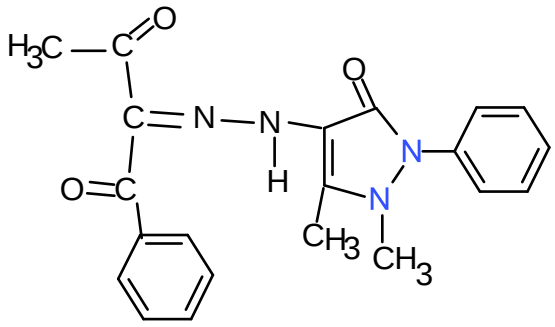


Fig 3: The structure of the ligand HL

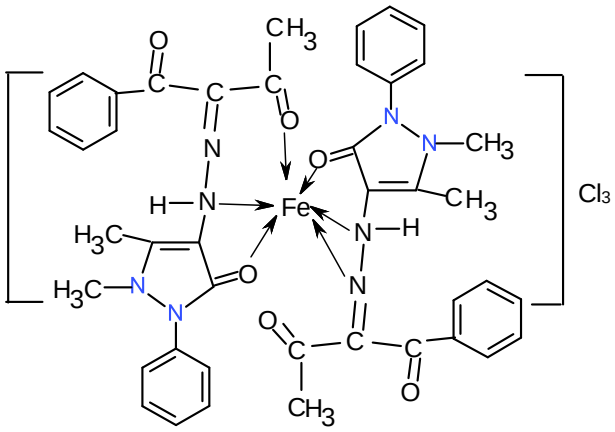


Fig 4: The structure of $[Fe(HL)_2]Cl_3$,

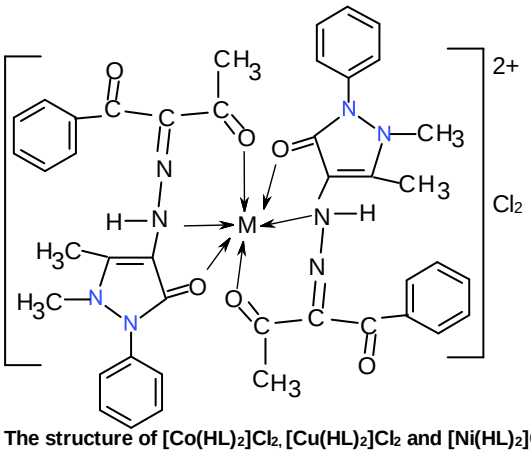


Fig 5: The structure of $[Co(HL)_2]Cl_2$, $[Cu(HL)_2]Cl_2$ and $[Ni(HL)_2]Cl_2$

M= Co, Cu and Ni

Antimicrobial activities

The results obtained from Antimicrobial activities and Minimum Inhibitory Concentrations are shown in Table 6 and 7 respectively.

Table 6: Sensitivity Test for HL and its complexes with some standard controls

Microorganism	HL	J	D	B	E	A	G	C
B.subtilis	23	-		-	-	0.625	0.16	0.16
S. pneumeniae	-	-		25	-	100	2.5	2.5
P. aeriginosa	-	-		-	-	100	50	50
E.coli(Eco 6)	-	-		20	-	100	6.25	6.25
E.coli (Eco 13)	-	-		21	-	100	50	50
S.aureus	-	-		-	-	2.5	2.5	2.5

Proteus	-	-	-	22	20	100	100	100
S.intermedius (G101)	-	-	23		-	2.5	2.5	2.5
K.pneumoniae	-	-			-	100	100	100
S.typhi	24					12.5	12.5	12.5

Where A= Ampicilin, G= Gentamicin and C= Ciprofloxacin, J= $[Co(HL)_2]Cl_2$, B= $[Cu(HL)_2]Cl_2$, D=$[Fe(HL)_2]Cl_3$, E=$[Ni(HL)_2]Cl_2$

Table 7: Minimum inhibitory concentration (MIC) of HL, $[Cu(HL)_2]Cl_2$ and $[Ni(HL)_2]Cl_2$ complexes

compounds	**Organisms**	**Zone of inhibition (mm).**					
		10 µg/ cm^3	**5** µg/ cm^3	**2.5** µg/ cm^3	**1.25** µg/ cm^3	**0.625** µg/ cm^3	**0.3125** µg/ cm^3
HL	*B. subtilis*	21	18	15	13	10	6
	S. typhi	20	18	15	13	11	9
$[Cu(HL)_2]Cl_2$	*S.pneumeniae*	22	20	19	14	13	12
	E. coli (Eco 6)	16	13	10	8	6	4
	P.mirabilis	17	15	13	10	7	6
	E.coli(Eco 13)	19	16	14	11	9	7
$[Ni(HL)_2]Cl_2$	*S.pneumonia*	17	15	12	10	7	5
	E.coli (Eco 6)	21	18	16	14	10	7
	P.mirabilis	17	15	13	10	7	Nil
	E.coli (Eco 13)	20	17	15	12	9	Nil
	B. subtilis	20	18	16	13	9	Nil

In Table 7, HL ligand, $[Cu(HL)_2]Cl_2$ and $[Ni(HL)_2]Cl_2$ complexes had activities against *B. subtilis, S. typhil, S. pneumeniae, Eco 6* and *Eco 13, Protous.* HL and $[Ni(HL)_2]Cl_2$ had good activities on *B.subtilis* and Eco 6 respectively up to concentration 0.3125 µg/cm^3. HL ligand

and $[Cu(HL)_2]Cl_2$, at concentration 0.3125µg/ cm^3 had activities on *S. typhil* and *S. pneumeniae* respectively. At 1.25µg/cm^3 $[Ni(HL)_2]Cl_2$ was seen to have activities on Eco 6 & 13, *Protous.*

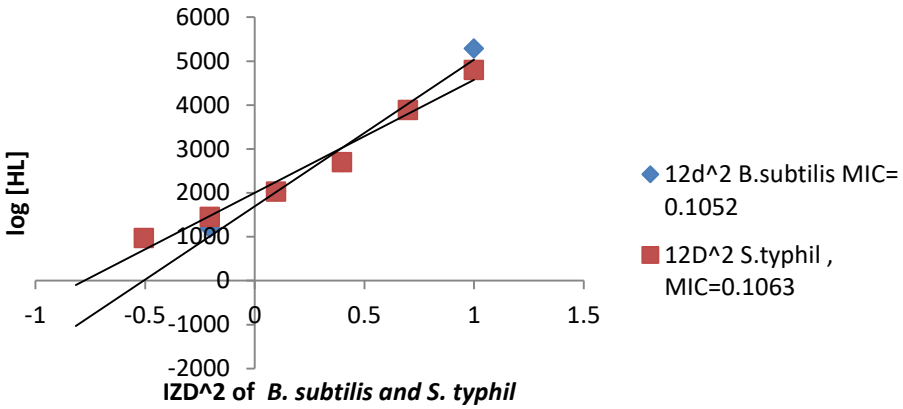


Fig 6: Plot of log[HL] against IZD^2 of *B.subtilis and S.typhi*

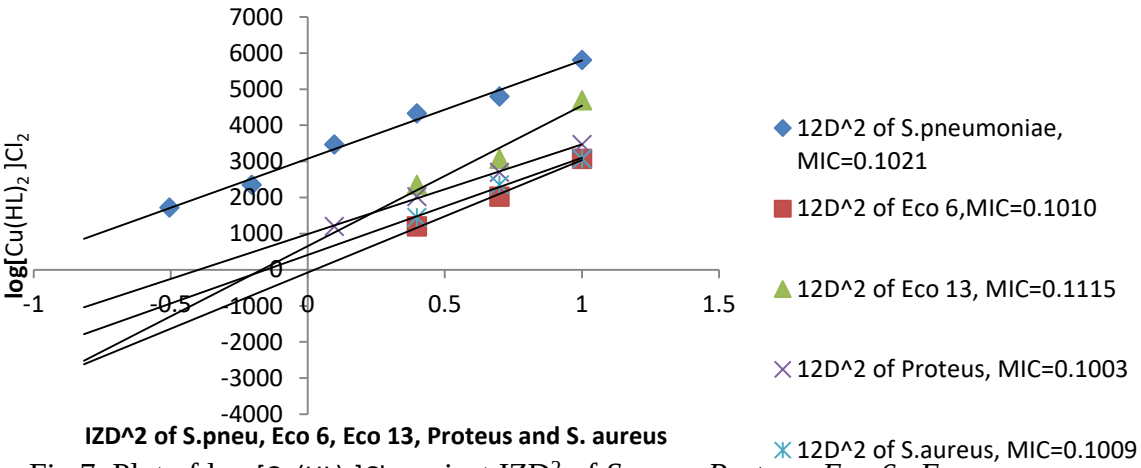


Fig 7: Plot of log $[Cu(HL)_2]Cl_2$ against IZD^2 of *S.pneu*, *Proteus*, *Eco 6* , *Eco 13 and S. aureus.*

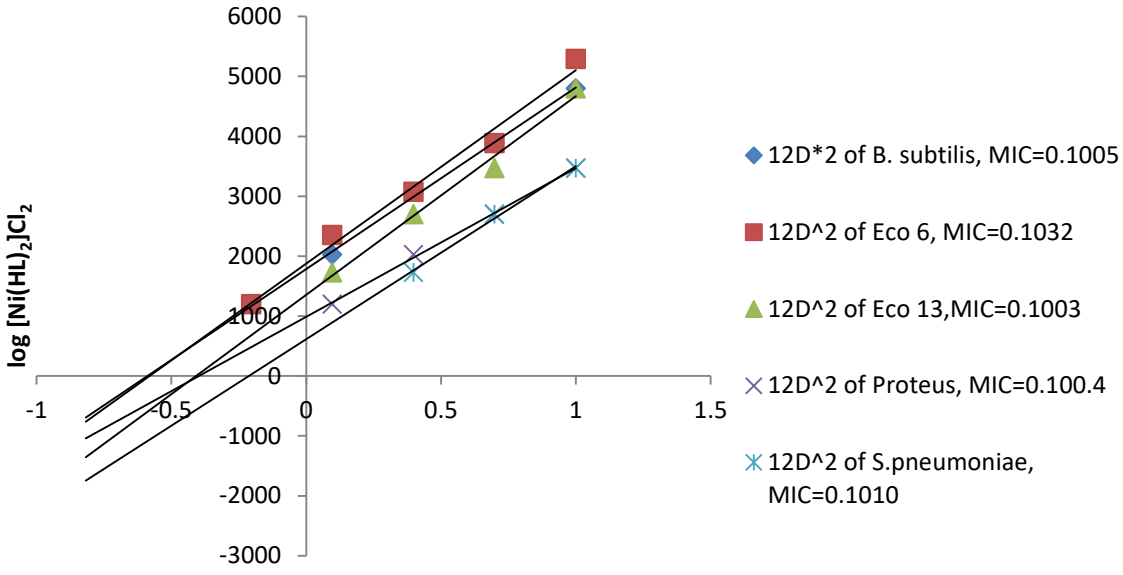


Fig 8: Plot of log[Ni(HL)$_2$]Cl$_2$ against IZD^2 *B.sub,Eco 6&13,proteus and S.pneumoniae*

CONCLUSIONS

The Ligand and its complexes were successfully synthesized. They were characterized by spectral, molar conductance and biological activities data. The spectral data showed the absence of bonding interaction in the complexes. Bonding of the ligands to the central metal atom in the complexes most probably occurred by -bonding through the participation of carbonyl oxygens of pyrazolone and diketones and hydrazo nitrogen group. Based on these data, an octahedral geometry have been assigned to [Co(HL)$_2$]Cl$_2$, [Fe(HL)$_2$]Cl$_3$, [Ni(HL)$_2$]Cl$_2$, [Cu(HL)$_2$]Cl$_2$ complexes. The molar conductivity values revealed that [Fe(HL)$_2$]Cl$_3$, [Co(HL)$_2$]Cl$_2$ and [Ni(HL)$_2$]Cl$_2$ complexes are all electrolytes while only Cu(II) behaved as non-electrolyte when compared with NaCl and CuSO$_4$ salts. Based on the data obtained the chemical structure of Ligand and its complexes are shown in Figures 3-5. The antimicrobial tests reveal that HL ligand, [Cu(HL)$_2$]Cl$_2$ and [Ni(HL)$_2$]Cl$_2$ complexes had activities against B. *subtilis*, S. *typhil*, S. *pneumeniea*, E. *coli* (Eco 6 &13) and *Proteus moratis*. HL and [Ni(HL)$_2$]Cl$_2$ had good activities on B. *subtilis* and Eco 6 respectively up to concentration 0.3125 µg/cm^3. HL ligand and [Cu(HL)$_2$]Cl$_2$, at concentration 0.3125µg/ cm^3 had activities on S. *typhil* and S. *pneumeniea* respectively. At 1.25 µg/cm^3 [Ni(HL)$_2$]Cl$_2$ had activities on Eco 6 & 13, *Proteus.*, HL, [Cu(HL)$_2$]Cl$_2$ and [Ni(HL)$_2$]Cl$_2$ and can be used as anti-bacteria drugs in pharmaceutical industry.

REFERENCES

1. Aljamali, N. M., Aati, S. A., Obaid, N. H. & Wannas, F. A. (2019). A Literature Review on Antipyrinein Chemistry Fields. *American International Journal of Sciences and Engineering Research*, 2(1), 9–21. https://doi.org/10.46545/aijser.v2i1.41.
2. Ipsita, M. & Jyotsna, M. (2014). Synthesis and Biological Activities of 4-Aminoantipyrine Derivatives Derived from Betti-Type Reaction, *International Scholarly Research Notices*, Vol. 2014, Article ID 639392, 7 pages, https://doi.org/10.1155/2014/639392.
3. Khushbu. K. M., Sunil, T. P. & Asha D. P. (2017). Studies on Chelating Properties of Antipyrine Based Azo Ligands and Its Coordination Compounds. Der PharmaChemica, 9(12),79-82.
4. Agarwal, R. K., Singh, L & Sharma, D. K. (2006). Synthesis, spectral, and biological properties of copper (II) complexes of thiosemicarbazones of Schiff bases derived from 4-aminoantipyrine and aromatic aldehydes. *Bioinorganic Chemistry and Applications*, Volume 2006, Article ID 59509, Pages 1–10.
5. Magaldi, S., Mata-Essayag, S., De Capriles, C. H., Pérez, C., Colella, M. T., Olaizola, C. & Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases*, 8(1), 39-45.
6. Valgas, C., Souza, S. M. D., Smânia, E. F. & Smânia, J. A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, 38, 369-380.
7. Heinosuke Yasuda (1967). Infrared Analysis of 2-pyrazolin-5-one Derivatives., Applied Spectroscopy, Vol 23, 1969.
8. El.Saied, F.A., Ayad, M.I., Issa, R.M. & Aly S.A. (2001): Synthesis and charactersation of Iron(III), Cobalt (II),Nickel(II) and Copper(II) Complexes of 4-formylazoanilne Antipyrine, *Polish.* Journal *of Chemistry*, 75, 774.
9. Heatley, N. G. (1944). A method for the assay of penicillin. *Biochemical Journal*, 38(1), 61.
10. Balouiri, M., Sadiki, M. & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 6(2), 71-79.
11. Altaf, S.., Ali, S., Ghada, E.A., Ali, E. & Ehab, A.L (2023)., Synthesis of some new Antipyrine-Thiophene hybrids and their evaluations as antioxidant and antibacterial

agents., *Bulletin of the Chemical. Society of Ethiopia*, 37(1), 123-140. DOI: https://dx.doi.org/10.4314/bcse.v37i1.11.

12. El-Saied, F. A., Aly, M. M., & Anwar, A. M. (1993). β-diketone complexes of manganese (II), cobalt (II), nickel (II) and palladium (II). *Transition Metal Chemistry*, 18, 588-590.
13. Ajayeoba, T.A., Akinyele, O.F. & Oluwole, A. O. (2017). Synthesis, Characterisation and Antimicrobial Studies of mixed Nickel(II) and Copper(II) complexes of Aroylhydrazones with 2,21-bipyridine and 1,10-phenanthroline, *Ife Journal of Science*, 19(1),119-132.
14. Lopes, A.B., Miguez, E., Kümmerle, A.E., Rumjanek, V.M., Fraga, C.A.M. & Barreiro, E.J. (2013). Characterization of Amide Bond Conformers for a Novel Heterocyclic Template of N-acylhydrazone Derivatives. *Molecules*, 18, 11683-11704. https://doi.org/10.3390/molecules181011683.
15. Özlem, A., Erbay, K., Feyzi, S.T., Zehra, C. & Engin, Ş. (2023). Design, synthesis, antioxidant and anticholinesterase activities of novel isonicotinic hydrazide-hydrazone derivatives, *Journal of Molecular Structure*, Volume 1279, 2023, 135037. https://doi.org/10.1016/j.molstruc.2023.135037.
16. Tharmaraj, P., Kodimunthiri, D., Sheela, C.D. & Shanmugapriya, C.S. (2009) Synthesis, Spectral Studies and Antibacterial Activity of Cu(II), Co(II) and Ni(II) Complexes of 1-(2-Hydroxyphenyl)-3-phenyl-2-propen-1-one)N2-[(3,5-dimethyl-1H-pyrazol-1-yl)methyl] Hydrazone. *Journal of the Serbian Chemical Society*, 74, 927-938. http://dx.doi.org/10.2298/JSC0909927T.
17. Imran, A., Waseem, A. W. & Kishwar, S. (2013). Empirical Formulae to Molecular Structures of Metal Complexes by Molar Conductance, Synthesis and Reactivity in Inorganic, *Metal-Organic, and Nano-Metal Chemistry*, 43(9), 1162-1170.
18. Sharma, P.C., Sharma, D, Sharma, A., Saini, N., Goyal, R., Ola, M., Chawla, R. & Thakur, V.K. (2020), Hydrazone comprising compounds as promising anti-infective agents: chemistry and structure-property relationship, *Materials Today Chemistry*, Volume 18, 2020,100349. https://doi.org/10.1016/j.mtchem.2020.100349.
19. Jyotirmaya, S., Chita, R. S., Priyambada, K.N., Shakti, K. P., Rabindra, N. P. & Sudhir, K. P. (2020). Molecules with versatile biological activities bearing antipyrinyl nucleus as pharmacophore, *European Journal of Medicinal Chemistry*, Volume 186, 111911, https://doi.org/10.1016/j.ejmech.2019.111911.