Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(3):105-116
(http://derpharmachemica.com/archive.html)

Facile Eco-friendly Synthesis, Characterisation and Evaluation of Anti-microbial Activity of Cu(II) Complexes of Tridentate Ligands

K. P. Srivastava*, Om Prakash Putul and Nagendra Kumar

Department of Chemistry, N. L. S. College, Jaitpur-Daudpur, Saran (Bihar), India

---

ABSTRACT

A facile, fast, simple, highly efficient, environmentally safe and economical method has been used for the synthesis of the two new biologically active 1,2-dihydroquinazolin-4(3H)-ones (L_1 & L_2) in single step by treating o-aminobenzoylhydrazone (o-ABH) with aromatic aldehydes under normal conditions and other two new hydrazones (L_3 & L_4) by condensing o-ABH with same aromatic aldehydes with superior yields in polyethylene glycol (PEG) as an alternative solvent. The Cu(II) complexes of synthesed ligands have been prepared in an environmentally benign microwave protocol and characterized by elemental analysis, conductivity measurements, magnetic moment, spectral and thermogravimetric analysis. The antimicrobial activity of the free ligands and their Cu(II) complexes clearly indicates that the ligands have both an antibacterial and antifungal potency against the organisms tested. In most cases, the complexes were found to be more active than the free ligands, but in some cases, an equal activity was displayed.

Keywords: Coordination Complexes; Quinazolinone; antimicrobial activity; PEG-300; Cu(II)

INTRODUCTION

Quinazolinones are one of the most important heterocyclic compounds having varied biological activities and still of great scientific interest now a days. They are widely found in bioorganic and medicinal chemistry with application in drug discovery. As drug has various functional groups which can bind to receptor or enzyme or metal ions present in the body, they can confirm many type of complexes and can enhance the activity of drugs. The metal complexes of drugs play an important role in drug action and metabolism [1, 2]. Metal complexes of drugs are found to be more potent than parent drugs. Metal complexes are widely used in various fields, such as biological processes, pharmaceuticals, analytical processes, separation techniques etc [3-5]. The therapeutic, diagnostic and other significant properties of transition metal complexes provide considerable attention leading to their application in many areas of modern medicine [1].

Application of green and sustainable chemistry protocols has seen enormous surge in recent times for the development of novel and eco-friendly methodologies towards the synthesis of valuable synthetic scaffolds and drug intermediates. Polyethylene glycol (PEG) has gained wide popularity as alternative solvent in contributing to such green methodologies by successfully plummeting the generation of industrial waste.

In the present protocol it has been found possible to highlight comparative study on the yield ratio and characterization of some 4-substituted analogues of benzoquinazoline derivatives. These observations have encouraged us to synthesize some new products containing the benzoquinazoline moiety hoping to obtain new compounds with potential biological activity. All the reactions involved are highly efficient to give the desired compounds in high yield and high purity. Subsequently, this adopted procedure is simple, rapid and eco-friendly due to easy experimental procedures. The versatility of this methodology can be extended to develop a stream-lined approach to other drug like heterocycles in a combinatorial fashion.

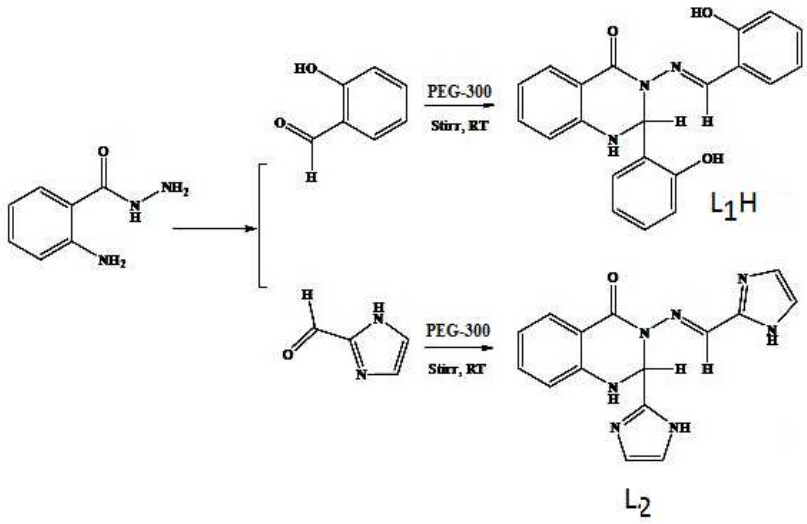
As part of our ongoing program [7-9] to develop more efficient and environmentally benign methods for organic / inorganic syntheses using economic and eco-friendly materials as solvents, we have looked into the synthesis of 1,2-dihydroquinazolin-4(3H)-ones (L_1 & L_2) and two new hydrazones (L_3 & L_4) as ligands and their Cu(II)-complexes using PEG-300 as green solvent with excellent yields under microwave irradiation.

MATERIALS AND METHODS

All the chemicals and solvents used for the synthesis were of analytical grade. The solvents were purified by standard methods. The infrared spectra of the ligands and metal complexes were run as KBr discs in the range 4000-400 cm^{-1} on a Shimadzu Infrared Spectrophotometer. Electronic spectra in the solid state as well as in solution were recorded on a Shimadzu UV-160, UV-visible spectrophotometer. Conductivity measurements of the metal complexes were done in DMF bridge model PW 9501 using Philips PW 9515/10 conductivity cell. Melting points were measured on an Electro-thermal 9100 apparatus and are uncorrected. Elemental analyses were recorded on a Carlo-Erba EA1110CNNO-S analyzer. The copper content was determined gravimetrically as copper salicylaldoximate and chloride content was determined gravimetrically as AgCl using $AgNO_3$ as precipitating agent. Magnetic susceptibility of the complexes was carried out by Gouy's method using $Hg[Co(NCS)_4]$ as standard. The EPR spectra of polycrystalline Cu(II) complexes were recorded at room temperature on a Varian E-109 X-band spectrometer using TCNE (tetracyanoethylene) as 'g' marker (g = 2.00277) at a frequency of 9.1 GHz under the magnetic strength of 3000 G. ^1H NMR spectra of ligand and Cu(II) complexes were recorded either in DMSO-d_6 on Bruker AMX-300 MHz and AMX-400 MHz operating at 400.23 MHz for ^1H NMR and 100.63 for ^{13}C with $^1H/^{13}C$ dual probe using tetramethylsilane (TMS) as an internal standard. Thermo gravimetric studies were carried out in the temperature range 25-1000 ^0C using a TGA7 ANALYSER, Perkin-Elmer with a heating rate of 10 ^0C per min in a nitrogen atmosphere. Microwave assisted synthesis were carried out in open glass vessel on a modified microwave oven model 2001 ETB with rotating tray and a power source 230 V, at output energy of 800W and 2450 MHz frequency. A thermocouple device was used to monitor the temperature inside the vessel of the microwave. The microwave reactions were performed using on/off cycling to control the temperature.

Synthesis of Ligands (L_1H & L_2)

Two molar quantity of aldehydes (salicyldehyde, 2.31g or 20 mmol) for L_1H ***{*** *(E)-3-(2-hydroxybenzylideneamino)-2-(2-hydroxyphenyl)-2,3-dihydroquinazolin-4(1H)-one}* or (imidazole-2- carbaldehyde, 1.92g or 20 mmol) for L_2 {*(E)-3-((1H-imidazol-2-yl)methyleneamino)-2-(1H-imidazol-2-yl)-2,3-dihydroquinazolin-4(1H)-one}* was added to 50 mL PEG-300 solution of o-aminobenzoylhydrazide (1.51g or 10 mmol) and stirred for 2-3 hours till light yellow or yellow solid separated. The separated coloured solids were filtered, washed repeatedly with methanol and dried in air. The coloured compounds were recrystallised from hot methanol and their purity were checked by TLC on pre-coated silica gel plate (Scheme-1).

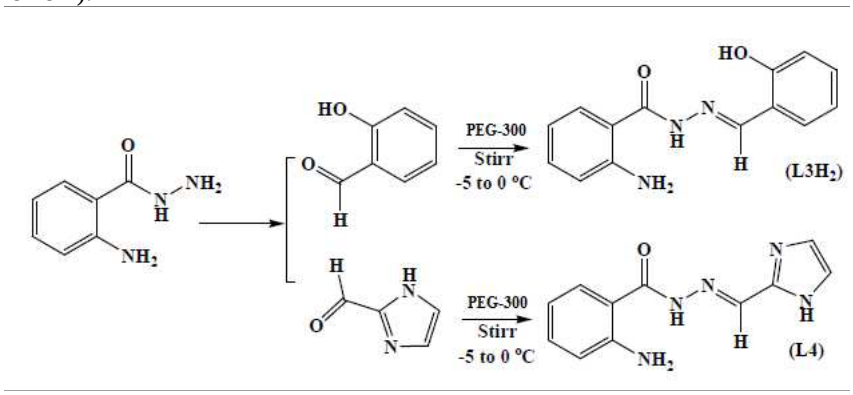


Scheme-1: Synthesis of ligands L_1H & L_2

Synthesis of Ligands (L_3H & L_4)

Salicyldehyde (25.00 mmol) or imidazole-2-carbaldehyde (25.00 mmol) was gradually added over a cooled (-5 to 0^0C) solution of o-aminobenzoylhydrazide (25.00 mmol) in PEG-300 (15 mL). The yellow solution was kept stirring at the same temperature for about 3 hours. The bright yellow solid (L_3H & L_4) formed was filtered, washed

thoroughly with cold methanol and dried in vacuo. The purity of the compound was checked by TLC on pre-coated silica gel plate (Scheme-2).



Scheme-2: Synthesis of ligands L_3H_2 & L_4

Synthesis of Cu(II) complexes of L_1H & L_2

The ligands L_1H & L_2 (1 mmol) were stirred initially with $CuCl_2.2H_2O$ in1:1 molar ratio in PEG-300 solution and then the reaction mixtures were refluxed in microwave oven for over 5-8 minutes. The resulting complexes were washed with methanol and then with chloroform and air dried. The physical parameters of the synthesised complexes are presented in Table-1.

Synthesis of Cu(II) complexes of L_3H_2 & L_4

1 mmol $CuCl_2.2H_2O$ was slowly added to 20 mL PEG-300 solution of L_3H_2 or L_4 (1mmol) with constant stirring at room temperature and then irradiated in microwave oven for over 5-8 minutes. The product thus obtained was filtered, washed several times with methanol, ether and dried under vacuo. The physical parameters of the synthesised complexes are presented in Table-1.

Biological Evaluation

The *in vitro* biological activity of the investigated ligands ($L_1 - L_4$) and their Cu(II) complexes were tested against two Gram positive bacteria namely *Staphylococcus aureus (SA)* & *Enterococcus faecalis (EF),* two Gram negative bacteria namely *Escherichia coli (EC) & Streptococcus mutans (SM)* and the fungal strains *Candida albicans (CA)* & *Aspergillus niger (AN)* by disc diffusion method [10] using nutrient agar as medium. Chloramphenicol was used as a standard reference in the case of bacteria, while Griseofulvin was used as a standard for antifungal reference.

The tested compounds were dissolved in DMSO (no inhibition activity) to get concentration of 1 mg/mL. The test was performed on nutrient agar medium for antibacterial activity and Sabraoud dextrose agar medium for antifungal activity [11]. Sterile disks were soaked in test compounds and carefully placed on incubated agar surface. The petridishes were incubated for 24 h at 37°C in the case of bacteria and for 48 h at 37°C in the case of fungi. Finally, the zone of inhibition was carefully measured. Each test was performed in triplicate in individual experiments and the average is reported (Table-4).

RESULTS AND DISCUSSION

As a result of microwave assisted synthesis, it was observed that the reaction was completed in a short time with higher yields compared to the conventional method. In the microwave method homogeneity of reaction mixture was increased by the rotating of reaction platform tray. The confirmation of the results was also checked by the repeatition of the synthesis process. Comparative study results obtained by microwave assisted synthesis; versus conventional heating method is that some reactions which required 2-3 h. by conventional method, was completed within 5-8 min. by the microwave irradiation technique, yields have been improved from 37-48% to 73-89%.

All the complexes are coloured, solid and stable towards air and moisture at room temperature. They do not possess sharp melting points and decompose on heating at higher temperature than 300^0C. The complexes are soluble in common organic solvents. The comparative results of conventional and microwave methods, analytical data of the compounds, together with their physical properties are consistent with proposed molecular formula are given in **Table-1**. The micro-analytical data suggest that the composition of all the metal complexes corresponds to 1:1 (metal: ligand) stoichiometry and have one or two chlorine atoms. The observed molar conductance values (0.47 – 3.1 $ohm^{-1}cm^2mol^{-1}$) are too low to account for any dissociation of the complexes in DMF at room temperature, indicating non-electrolytic nature of the complexes [12].

Table-1 The comparative results of conventional and microwave methods, analytical and physical data of the compounds under investigation

Compounds (Colour)	Reaction Time	Yield (%)	Elemental analysis Found (Calculated) %					μ_{eff} (BM)	Conductance (ohm^{-1} cm^2 mol^{-1})
	CM (MM)	CM (MM)	C	H	N	Cl	Cu		
$C_{21}H_{17}N_3O_3$ Light yellow **L_1H**	2h	54 (87)	70.13 (70.18)	4.78 (4.77)	11.65 (11.69)	--	--	--	--
$C_{15}H_{13}N_7O$ Yellow **L_2**	2h	57 (89)	58.66 (58.62)	4.23 (4.26)	31.81 (31.90)	--	--	--	--
$C_{14}H_{13}N_3O_2$ Light yellow **L_3H_2**	3h	56 (90)	65.83 (65.87)	5.03 (5.13)	16.41 (16.46)	--	--	--	--
$C_{11}H_{11}N_5O$ Light yellow **L_4**	3h	51 (85)	57.65 (57.63)	4.89 (4.84)	30.51 (30.55)	--	--	--	--
$[Cu(C_{21}H_{17}N_3O_3)Cl]$ Dark green	2.5h (7m)	56 (82)	55.12 (55.15)	3.54 (3.53)	9.31 (9.35)	7.73 (7.75)	9.24 (9.19)	1.76	3.10
$[Cu(C_{15}H_{13}N_7O)Cl_2]$ Dark green	2.5h (6m)	59 (87)	40.70 (40.78)	2.93 (2.97)	22.11 (22.19)	16.13 (16.05)	14.36 (14.3)	1.67	2.35
$[Cu(C_{14}H_{14}ClN_3O_3)Cl].H_2O$ Dark green	2.5h (5m)	61 (78)	45.24 (45.29)	3.77 (3.80)	11.36 (11.32)	9.59 (9.55)	17.17 (17.1)	1.69	0.65
$[Cu(C_{11}H_{11}N_5O)Cl_2]$ Dark green	2.5h (8m)	65 (83)	36.36 (36.33)	3.13 (3.05)	19.24 (19.26)	19.48 (19.50)	17.43 (17.4)	1.74	0.47

(CM = Conventional method, time in hours; MM = Microwave method, time in minutes)

IR Spectral Studies

The data of the IR spectra of investigated Schiff base ligands and their metal complexes are listed in **Table-2**.The IR spectra of the complexes were compared with those of the free ligand in order to determine the involvement of coordination sites in chelation. Characteristic peaks in the spectra of the ligand and complexes were considered and compared. The FT-IR spectra of the investigated complexes contained all the absorption bands from the ligands and some new absorption bands indicative of coordination of the ligands with metal ion through N & O.

The C=O stretching vibration was observed in the region 1647 cm^{-1} for L_1H and 1655 cm^{-1} for L_2. The appearance of carbonyl stretching vibration at lower wave number compared to the similar structures of earlier reports for 2,3-disubstituted quinazolin- 4(3H)-ones [13], might be due to strong intermolecular hydrogen bonding between carbonyl oxygen and hydrogen of quinazoline ring nitrogen of another molecule. The ν(N-H) band was observed at 3310 and 3413 cm^{-1} in **L_1H** and **L_2** respectively. This has shifted to higher frequency side in the complexes due to the breakdown of the intermolecular hydrogen bonding after complexation [14-15]. The stretching frequency of C=N was observed in the region 1609 cm^{-1} for **L_1H** and 1610 cm^{-1} for **L_2**. The shift of ν(C=O) and ν(C=N) bands to lower frequency region by 35-47 cm^{-1} and 26-43 cm^{-1} respectively, in the respective complexes indicate the involvement of carbonyl oxygen and azomethine nitrogen in the coordination with metal ions. The ν(C-O) observed in the region 1365 cm^{-1} for the ligand (**L_1H**) has shifted to higher frequency region in all its complexes, indicating the involvement of phenolic oxygen via deprotonation. Only three bands of ring stretching vibrations of imidazole moiety were observed at 1572, 1484 and 1469 cm^{-1}. All these bands show slight changes in the spectra of all the complexes indicating the involvement of imidazole ring nitrogens in coordination.

On the basis of these criteria and the down field shift of ring carbonyl stretching frequencies of **L_1H** and **L_2**, it is concluded that, in the respective complexes, the ligand **L_1H** is coordinated to the metal through ring carbonyl oxygen, azomethine nitrogen and the azomethine group linked phenolic oxygen via deprotonation and the ligand **L_2** is coordinated to the metal through ring carbonyl oxygen, azomethine nitrogen and nitrogen of the imidazole moiety linked to the azomethine group.

Thus, IR spectral data suggest monobasic tridentate ONO ligational behaviour of **L_1H** and neutral tridentate ONN ligational behaviour of **L_2**.

Further, In the IR spectrum of **L_3H_2**, the characteristic ν(C=O), δ(N-H), ν(C=N) bands appear at 1657, 1614 and 1581 cm^{-1} respectively. A strong band at 3413 cm^{-1} and comparatively weak band at 3273 cm^{-1} were assigned to phenolic O^1H and amide N_2H stretching vibrations respectively. The presence of asymmetric and symmetric modes of the ν(N^3H_2) bands were observed at 3371 and 3325 respectively. Coordination sites of the ligand **L_3H_2** is elucidated by comparison of its IR spectrum with those of the respective complex. The absence of band due to phenolic O1H group in the spectra of the respective complex suggests the coordination of ligand to the metal via deprotonation. The bands due to ν(C=N) and ν(C=O) of the free ligand have shifted to lower frequency on complexation, indicating the involvement of azomethine nitrogen and carbonyl oxygen in coordination [16]. The presence of asymmetric and symmetric modes of the ν(N^3H_2) bands in the complex clearly indicates the non-

involvement of -NH_2 in coordination [17]. The presence of lattice held water molecule was confirmed by the NMR spectral study of the Cu(II) complex of **L_3H_2.**

In the Infrared spectral data of ligand **L_4,** sharp bands of medium intensity at 3371 and 3325 cm^{-1} are due to asymmetric and symmetric stretching vibrations of –NH_2 group [18]. A sharp absorption band at 3391 cm^{-1} was attributed to ν(-NH) stretching [19]. The absorption bands characteristic of ν(C=O) vibrations appear at 1657 cm^{-1} [20] and the absorption band at 1560 cm^{-1} was assigned to azomethine ν(C=N) frequency. The amide carbonyl stretching frequency is observed at lower frequency region as compared to ν(C=O) of aromatic acid hydrazides without –NH_2 group at *ortho* position in which it has appeared at 1673 cm^{-1} [21]. Another important band appeared at 1575 cm^{-1} is characteristic of C=N stretching vibrations of imidazole ring. In the IR spectra of all the complexes, ν(C=N) stretching frequency of imidazole ring has decreased by 7-17 cm^{-1}, indicating the involvement of imidazole ring nitrogen in the coordination. Similarly on complexation, ν(C=O) of amide and ν(C=N) of azomethine bands have also shifted to lower frequency by 18-29 cm^{-1} and 9-19 cm^{-1} respectively, indicating the involvement of amide carbonyl oxygen and azomethine nitrogen in coordination. The stretching vibrations due to –NH_2 group in complexes remains unchanged as compared to ligand and confirms it's non-involvement in the coordination. The above assignments suggest that the ligand **L_4** has coordinated through carbonyl oxygen, azomethine nitrogen and imidazole ring nitrogen.

Thus, the IR spectral data clearly reveals that **L_3H_2** acts as monobasic, neutral tridentate ONO ligand and **L_4** acts as neutral tridentate ONN ligand.

Table-2 Observed IR bands (cm^{-1}) of Ligands and their Cu**-complexes**

Compound	ν(NH_2)	ν(NH)	δ(NH)	ν(OH)	ν(C=O)	ν(C=N)	ν(C-O)	ν(C=N) Imid. ring	Imidazole Ring Vibrations
L_1H	--	3310s	--	3227b	1647s	1610s	1365m	--	--
L_2		3413s	--	--	1655s	1609s	--		1572w, 1484s, 1469s
L_3H_2	3371w, 3325sh	3273w	1614sh	3413s	1657s	1581sh	1153s	--	--
L_4	3375w, 3317sh	3391s	1604sh	--	1657s	1560s	--	1575m	1516 s, 1462s, 1437m, 1416m
$[Cu(C_{21}H_{17}N_3O_3)Cl]$		3264s	--	n.o.	1612s	1593s	1378s	--	--
$[Cu(C_{15}H_{13}N_7O)Cl_2]$		3404s	--	--	1612s	1593s	--		1579s, 468s, 1460s
$[Cu(C_{14}H_{14}ClN_3O_3)Cl].H_2O$	3456b, 3336w	3185b	1615sh	n.o.	1635s	1574w	1152s	--	--
$[Cu(C_{11}H_{11}N_5O)Cl_2]$	3456b, 3336w	3364s	1618sh	--	1632s	1543s	--	1672s	1506 s, 1469s, 1448m, 1424m

(Where s= strong, sh= sharp, m = medium & b = broad & n.o. = not observed)

Electronic Spectral Studies& Magnetic Properties

Electronic spectra of ligands and their complexes were recorded in order to assign the plausible geometry around the metal ions. The electronic spectra of all the compounds in DMF were scanned in the region 200-1000 nm.

The electronic spectra of ligands **L_1H** and **L_2** exhibit strong absorptions at 333 and 325 nm respectively and are assigned to π→π * transitions and absorptions at 325 and 372 nm are assigned to n→π* transitions. A broad band in the electronic spectrum of Cu(II) complex (with **L_1H)** with peak maxima at 627 nm was assigned to the combination of $^2B_{1g}\rightarrow{}^2A_{1g}$ and $^2B_{1g}\rightarrow{}^2E_g$ transitions as for a *square-planar configuration* around the metal ion [22-23]. The electronic spectrum of the Cu(II) complex (with **L_2**) shows broad absorption at 723 nm attributed to the $^2A^I{}_1\rightarrow{}^2E^{II}$ which illustrates the *trigonal bipyramidal geometry* around Cu(II) ion.

The electronic spectral data of the ligand **L_3H_2** exhibit three peaks in the UV region. The peak appearing in the range of 316 nm is attributed to π→π* transition of the benzenoid moiety of the ligand. The peak around 348 nm can be assigned to intra ligand π→π* transition. The other peak observed in the region of 351 nm is attributed to n→π* electronic transitions [24-25]. The appearance of two peaks at 555 and 369 nm in the electronic spectrum of Cu(II) complex (with **L_3H_2** is consistent with the *square planar geometry* [26]. The magnetic moment value observed for **L_3H_2** complex supports the electronic transitions. An effective magnetic moment of 1.69 BM observed for Cu(II) complex is close to 1.73 BM expected for discrete magnetically non-coupled spin only value for Cu(II) ion [27].

The electronic spectrum of ligand **L_4** exhibit strong absorptions at 332 and 372 nm and are assigned to π→π * and n→π * transitions respectively [28].The electronic spectrum of the Cu(II) complex (with **L_4)** shows broad absorption at 623 nm attributed to the $^2A^I{}_1\rightarrow{}^2E^{II}$ which illustrates the *trigonal bipyramidal geometry* around Cu(II) ion with D_{3h} symmetry and is further supplemented by its magnetic moment value of 1.74 BM [22-23]. Thus, the magnetic

moments of Cu(II) complexes are in consistent with one unpaired electron and indicates the mononuclear nature of the investigated complexes.

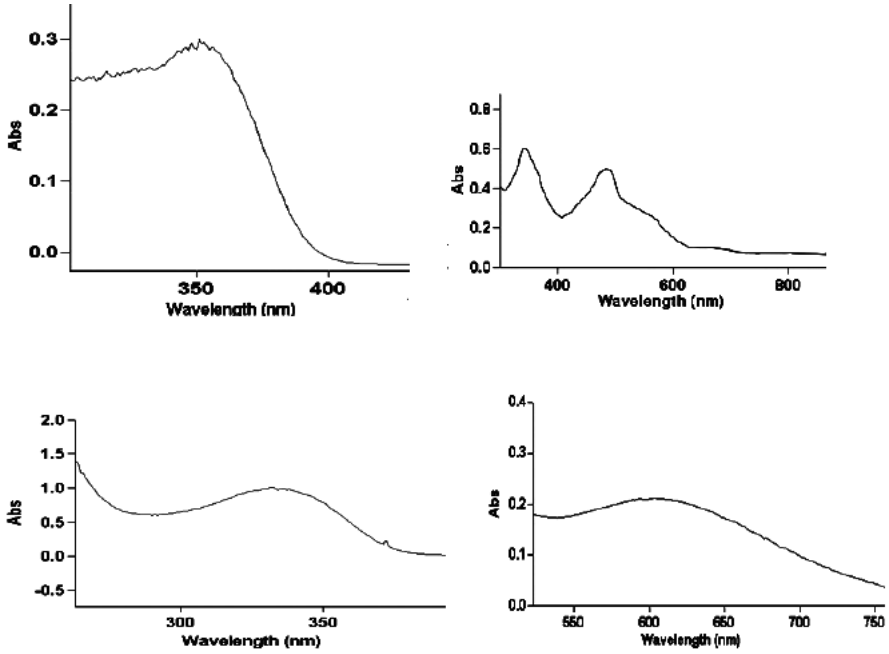


Figure-1: Electronic absorption spectra of L_3H_2 & its complex and L_4 & its complex

NMR studies

The NMR (^{1}H & ^{13}C) spectral studies of ligands aids the complete assignment of chemical shifts of protons directly attached to carbon and in turn confirms the formation of 1,2-dihydroquinazolinone instead of Schiff base. Thus, NMR studies were successfully used to prove the formation of 1,2-dihydroquinazolinone derivatives **L_1H** and **L_2**.

The numbering scheme for the assignment of carbons and corresponding protons for **L_1H** and **L_2** is given in **Figure-2 (a)** and **(b)** respectively. The spectral assignments were made based on the comparison with NMR assignments of *o*-ABH [29], salicylaldehyde [30] and imidazole-2-carbaldehyde [31].

In the ^{1}H NMR spectrum of **L_1H** the two non-equivalent O^2H and O^3H protons resonate as broad singlets in the downfield region 11.41 and 10.28 ppm respectively. The resonance due to N^1H, C^1H and azomethine proton ($N=C^{15}H$) appeared as singlets at 7.49, 7.81 and 8.48 ppm respectively. The aromatic protons were observed as multiplets in the region 6.75-7.51 ppm. In the ^{1}H NMR spectrum of Cu(II) complex, signal corresponding to O^2H proton is absent which is the clear indication of involvement of this phenolic oxygen in the coordination by losing its proton. The presence of other phenolic O^3H proton is observed at 9.78 ppm and indicates its non participation in the coordination. The azomethine proton has shifted down-field in the respective complex compared to that observed in L_1H suggesting the coordination through azomethine nitrogen. The signals due to remaining protons were unperturbed. In ^{1}H NMR of L_2, the N^1H, N^6H and N^7H protons were observed as singlet at 14.28, 12.94 and 7.38 ppm respectively. Two singlets appearing at 8.82 and 6.46 ppm were due to C^1H proton and azomethine ($N=C^{12}H$) protons respectively.

Remaining aromatic protons were observed in the expected region. The ^{1}H NMR spectra of Cu(II) complex show a slightly downfield shift of imidazole ring protons indicating the involvement of imidazole ring nitrogen (N^2) in the coordination. Downfield shift of azomethine and carbonyl carbons clearly support the coordination through azomethine nitrogen and carbonyl oxygen. ^{13}C NMR of **L_1H** showed a signal at 66.00 ppm assigned to sp3 hybridized C^1 carbon giving direct evidence for the formation of 1,2 dihydroquinazolinone rather than hydrazone. Carbonyl carbon, azomethine carbon and carbons attached to OH groups were observed at 156.8, 150.0 and 157.4 (C^{10}), 160.1 (C^{21}) ppm respectively. ^{13}C NMR spectrum of Cu(II) complex showed a downfield shift of signals corresponding to carbonyl, azomethine and C^{21} carbons suggesting the coordination through carbonyl oxygen, azomethine nitrogen and phenolic oxygen.

The ^{13}C NMR spectra of **L_2** exhibit a signal around 66.9 ppm due to sp^3 hybridized carbon C^1 which clearly indicates the formation of 1,2-dihydroquinazolinone. The peaks due to carbonyl carbon and azomethine carbons

resonate at 160.1 and 152.3 ppm respectively. The ^{1}H NMR spectra of Cu(II) complex show a slightly downfield shift of imidazole ring protons indicating the involvement of imidazole ring nitrogen (N^{2}) in the coordination. Downfield shift of azomethine and carbonyl carbons clearly support the coordination through azomethine nitrogen and carbonyl oxygen.

The ^{1}H and ^{13}C NMR spectral data of **L_1H, L_2, L_3H_2** and **L_4** and their complexes obtained in DMSO-d_6 are given in **Table-3 & 4.** The numbering scheme for the ligands is shown in **Figure-2.**

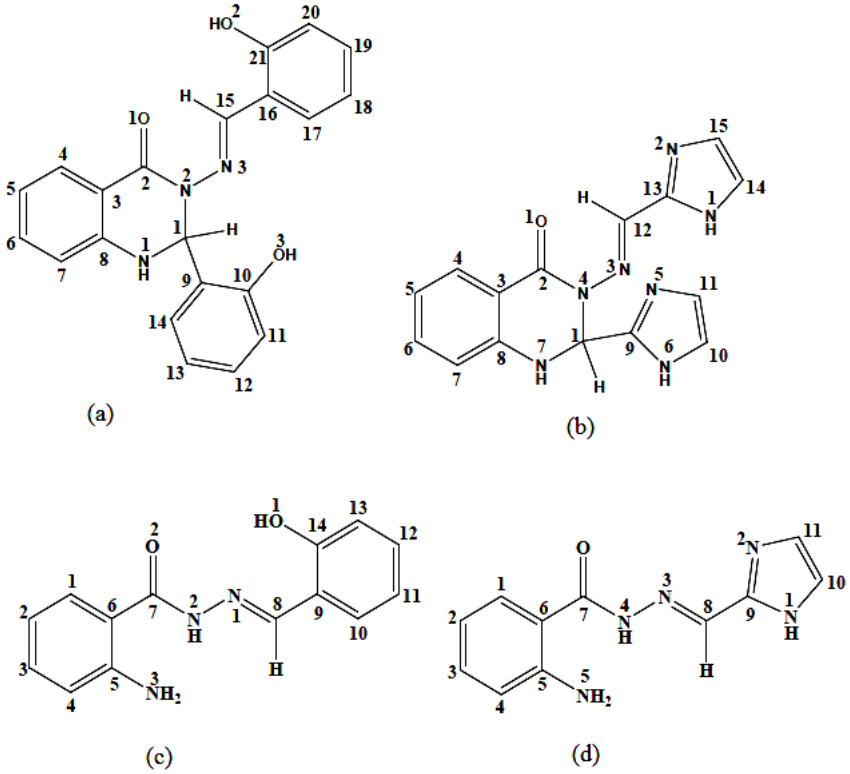


Figure-2: ***Numbering of (a) L_1H (b) L_2 (c) L_3H_2 and (d) L_4***

It is evident from IR and NMR spectra that the ligand **L_3H_2** coordinates through amide carbonyl oxygen, azomethine nitrogen and phenolic OH via deprotonation. The ligand **L_4** coordinates through amide carbonyl oxygen, azomethine nitrogen and imidazole ring nitrogen.

Table-3 ^{1}H and ^{13}C NMR chemical shifts of L_1H, L_2, L_3H_2 and L_4 Ligands

Position	L_1H		L_2		L_3H_2		L_4	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
C^1	66.00	7.81 (s, 1H)	66.9	6.46 (s, 1H)	129.0	7.63 (dd, *J* = 7.6, 1.5 Hz, 1H)	129.0	7.63 (m, 3H),
C^2	156.8	--	160.1		117.6	6.79 (td, *J* = 7.6, 1.5 Hz, 1H)	117.6	6.79 (td, *J* = 7.6, 1.5 Hz, 1H)
C^3	114.3	--	114.3		133.7	7.10 (td, *J* = 7.6, 1.4 Hz, 1H)	133.7	7.10 (td, *J* = 7.6, 1.4 Hz, 1H)
C^4	126.1	7.68 (dd, *J* = 7.4, 1.5 Hz, 1H)	126.1	7.68 (m, 2H)	117.0	6.69 (dd, *J* = 7.4, 1.4 Hz, 1H)	117.0	6.69 (dd, *J* = 7.4, 1.4 Hz, 1H)
C^5	121.2	6.75 (td, *J* = 7.1, 1.2 Hz, 1H)	121.2	6.75 (m, 2H)	151.1		151.1	
C^6	135.8	7.44 (td, *J* = 7.3, 1.4 Hz, 1H)	119.8	7.44 (td, *J* = 7.4, 1.4 Hz, 1H)	110.5		110.5	
C^7	114.8	7.00 (dd, *J* = 7.4, 1.6 Hz, 1H)	114.8	7.00 (m, 2H)	167.2		167.2	
C^8	145.6		129.6		147.4	8.74 (s, 1H)	132.4	7.22 (s, 1H)
C^9	124.0		140.2		119.9		146.1	
C^{10}	157.4		118.4	6.92 (d, *J* = 7.6 Hz, 1H)	129.2	7.51 (dd, *J* = 7.4, 1.4 Hz, 1H)	121.9	6.57 (s, 1H)
C^{11}	116.2	6.83 (m, 5H)	124.5	7.02 (m, 2H)	121.0	6.91 (dd, *J* = 14, 1.4 Hz, 2H)	126.2	6.57 (s, 1H)
C^{12}	132.2	7.08 (m, 2H)	151.0	8.82 (m, 2H)	130.6	7.32 (td, *J* = 7.4, 1.4		

						Hz, 1H)		
C^{13}	118.9	6.88 (m, 2H)	146.4		116.0	6.93 (dd, *J* = 14, 1.4 Hz, 1H)		
C^{14}	129.5	7.13 (m, 2H)	119.9	6.57 (m, 2H)	159.5			
C^{15}	150.0	8.48 (s, 1H)	126.2	6.57 (m, 2H)				
C^{16}	119.6	--						
C^{17}	129.7	7.51 (dd, *J* = 7.1, 1.5 Hz, 1H)						
C^{18}	121.0	6.91 (m, 5H)						
C^{19}	131.0	7.32 (td, *J* = 7.3, 1.4 Hz, 1H)						
C^{20}	116.3	6.93 (m, 5H)						
C^{21}	160.1							
$N^{1}H$	--	7.49 (s, 1H)	--	14.28 (s, 1H)				7.63
$O^{2}H$	--	11.41 (s, 1H)						
$O^{3}H$	--	10.28 (s, 1H)						
$N^{4}H$								10.87 (s, 1H)
$N^{5}H_2$								5.58 (s, 1H)
$N^{6}H$			--	12.94 (s, 1H)				
$N^{7}H$			--	7.38 (s, 1H)				
$O^{1}H$						11.01 (s, 1H)		
$N^{2}H$						11.86 (s, 1H)		
$N^{3}H_2$						5.58 (s, 1H)		

(m = multiplet, d = doublet, s = singlet, dd = doublet of doublet, td = triplet of doublet)

Table-4 ^{1}H and ^{13}C NMR chemical shifts of Cu(II) complexes with Ligands

Position	**[Cu(L_1Cl)]**		**[Cu(L_2Cl_2)]**		**[Cu(L_3Cl)]**		**[Cu(L_4Cl_2)]**	
	^{13}C	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**
C^{1}	65.80	7.81 (s, 1H)	60.4	6.26 (s, 1H)	128.9	7.15	128.9	7.15 (dd, *J* = 7.2, 1.9 Hz, 1H)
C^{2}	155.8	--	173.7		117.5	6.88 (m, 3H)	117.5	6.88 (m, 2H)
C^{3}	113.9	--	117.5		133.8	6.85 (m, 3H)	133.8	6.85 (m, 2H)
C^{4}	125.8	7.68 (dd, *J* = 7.4, 1.5 Hz, 1H)	125.4	7.20 (m)	117.5	6.55 (dd, *J* = 7.1, 1.8 Hz, 1H)	117.5	6.55 (dd, *J* = 7.1, 1.8 Hz, 1H)
C^{5}	122.2	6.75 (td, *J* = 7.1, 1.2 Hz, 1H)	121.4	6.84 (dd, *J* = 7.6, 1.7 Hz, 3H)	152.3		151.3	
C^{6}	136.8	7.44 (td, *J* = 7.3, 1.4 Hz, 1H)	120.8	7.19 (t, *J* = 7.4 Hz, 2H)	106.4		106.4	
C^{7}	113.8	7.00 (dd, *J* = 7.4, 1.6 Hz, 1H)	115.1	6.82 (m)	168.5		167.1	
C^{8}	146.6		129.3		147.4	8.83 (s, 1H)	140.8	7.22 (s, 1H)
C^{9}	125.0		144.9		121.9		147.9	
C^{10}	156.4		122.4	6.92 (d, *J* = 7.5 Hz, 1H)	126.6	7.13 (m, 2H)	129.4	6.57 (s, 1H)
C^{11}	115.2	6.83 (m, 5H)	124.5	7.02 (d, *J* = 7.3 Hz, 1H)	118.0	6.98 (td, *J* = 7.5, 1.4 Hz, 1H)	124.3	6.67 (s, 1H)
C^{12}	133.2	7.08 (m, 2H)	152.3	8.92 (m)	132.2	7.22 (td, *J* = 7.5, 1.4 Hz, 1H)		
C^{13}	117.9	6.88 (m, 2H)	149.5		119.1	6.81 (m, 3H)		
C^{14}	128.5	7.13 (m, 2H)	131.3	6.57 (m, 2H)	162.4			
C^{15}	151.0	8.48 (s, 1H)	129.4	6.57 (m, 2H)				
C^{16}	118.6	--						
C^{17}	128.9	7.51 (dd, *J* = 7.1, 1.5 Hz, 1H)						
C^{18}	122.0	6.91 (m, 5H)						
C^{19}	132.0	7.32 (td, *J* = 7.3, 1.4 Hz, 1H)						
C^{20}	115.9	6.63 (m, 5H)						
C^{21}	161.1							
$N^{1}H$	--	7.59 (s, 1H)		13.49 (s, 1H)				7.63 (s, 1H)
$N^{2}H$						11.86 (s, 1H)		
$N^{3}H_2$						5.07 (s, 1H)		
$N^{4}H$								1.90 (s, 1H)
$N^{5}H_2$								4.07 (s, 1H)
$O^{2}H$	--	11.51 (s, 1H)						
$O^{3}H$	--	10.38 (s, 1H)						
$N^{6}H$				12.56 (s, 1H)				
$N^{7}H$				7.20 (s, 1H)				

EPR spectral studies

The EPR spectrum of metal chelates provides information about hyperfine and superfine structures which are the important parameters in studying the metal ion environment in complexes i.e. the geometry, nature of the ligating sites and the degree of covalency of the metal–ligand bonds [31-32]. In the present study the X-band EPR spectra of Cu(II) complexes were recorded for polycrystalline sample at room temperature.

From the observed g values $g_{\parallel} > g_{\perp} > g_e$ (2.0023), it is evident that the unpaired electron lies predominantly in $d_{x^2-y^2}$ orbital with the possibility of some d_{z^2} character being mixed with it because of low symmetry [33-35].

In the present investigation all complexes (with **L_1H** and **L_2)** show G values more than 4 ruling out the interaction between copper centers and are further supported by the magnetic moment values which correspond to one unpaired electron. The $g_{\parallel}$ values for Cu(II) complexes in the present investigation are less than 2.3, as 2.034 and 2.019 impute that the complexes are covalent in nature.

The EPR spectra of the Cu(II) complex (with **L_3H_2**) at 300 shows a broad absorption band, which is isotropic due to the tumbling motion of the molecules. The 'g_{iso}' values at 300 are 2.11. Similarly, Cu(II) complex with **L_4** has also exhibited a broad isotropic signal with 'g_{iso}' values at 2.073.

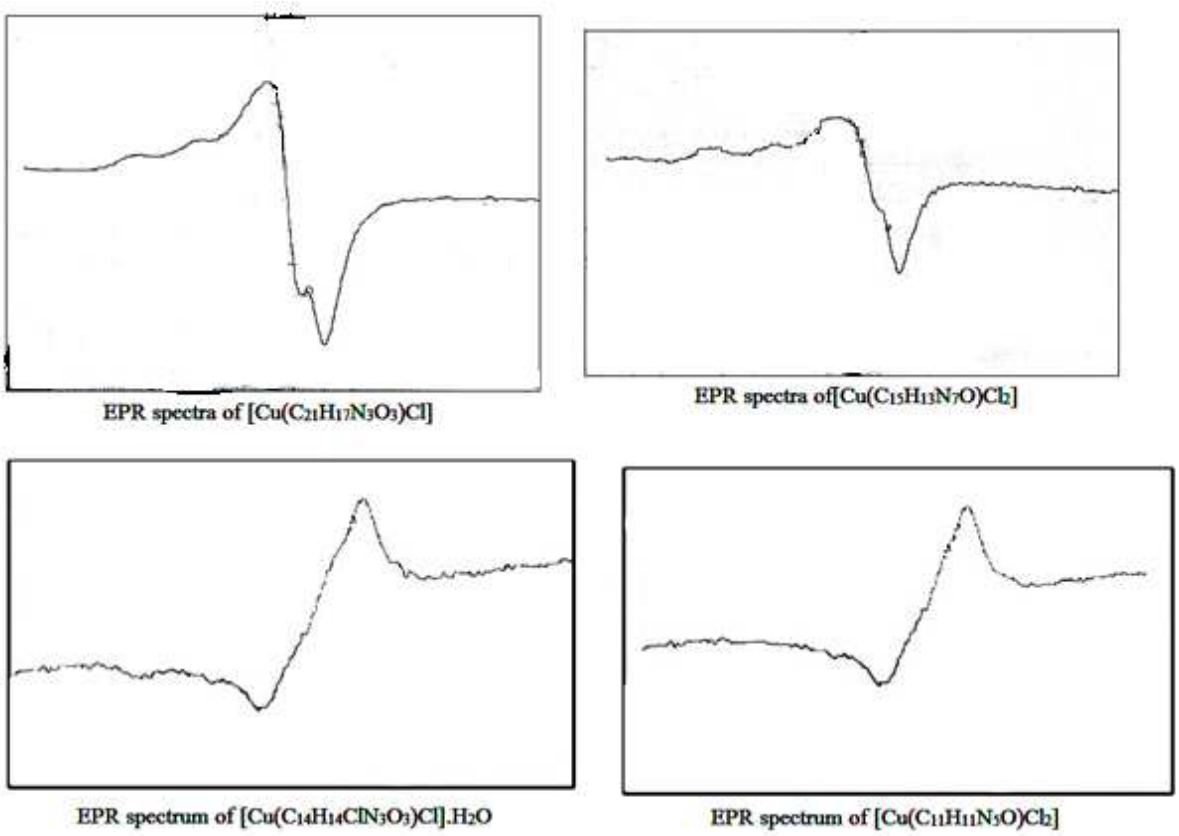


Figure-3: EPR spectra of investigated Cu(II) complexes

Thermal studies

Thermal studies were undertaken in order to study the thermal stability of complexes and also as supportive data for the proposed molecular formulae. Details of the thermal decomposition of complexes are summarized in **Table-5.**

Table-5 Details of the thermal decomposition of the investigated complexes

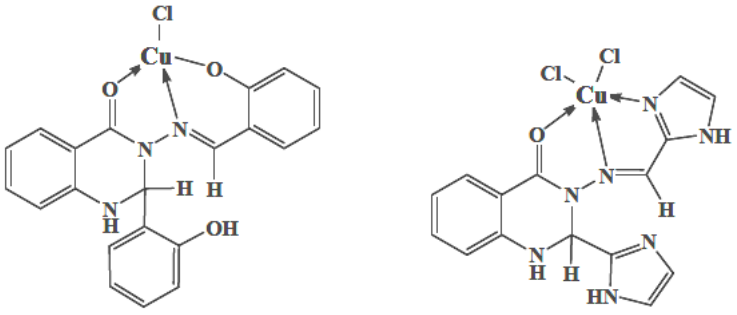
Complex	Temp (0C)	Wt. loss corresponds to	Mass	
			Calcd.	Expt.
$[Cu(C_{21}H_{17}N_3O_3)Cl]$	368-700 >700	1 ligand and 1 chlorine Decomposition of coordination sphere	86.20	86.13
$[Cu(C_{15}H_{13}N_7O)Cl_2]$	217-276 277-759 >759	two coordinated chlorides Loss of ligand	15.90 69.80	15.87 69.85
$[Cu(C_{14}H_{14}ClN_3O_3)Cl].H_2O$	40-90 90- 470 >470	one coordinated water molecule two coordinated chlorides one ligand molecule	4.83	4.85
$[Cu(C_{11}H_{11}N_5O)Cl_2]$	30-280 281-543	two coordinated chlorides one ligand molecule	19.38 63.13	19.32 63.29

The complex with $\mathbf{L_1H}$ is stable up to 367 ^{0}C. The sharp decomposition of 86.13 % (86.20 %) at 368-700 ^{0}C is associated with loss of one chloride and the ligand molecule. The final product of decomposition above 700 ^{0}C resulted in the formation of stable copper oxide.

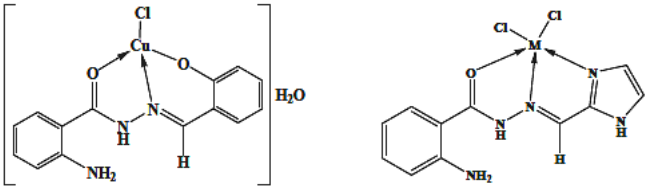
The complex with $\mathbf{L_2}$ also follows the two step decomposition and shows a weight loss of 15.87 % (15.90 %) respectively in the first step around 209-276 °C and corresponds to the loss of two coordinated chlorides. In the second step, the mass loss 69.85 % (69.80 %) around 243-810 °C corresponds to the loss of ligand molecule. A plateau obtained above 810 °C corresponds to the formation of stable metal oxide of the complex. The metal content calculated from the residual weight is in agreement with the metal analysis of complex.

In case of Cu(II) complex (with $\mathbf{L_3H_2}$), the weight loss of 4.83 % (4.85 %) at about 90 ^{0}C corresponds to the loss of lattice held water molecule while the 2nd and 3rd decomposition stages resembles the previous complexes.
The thermogram of complex (with $\mathbf{L_4}$) shows a weight loss of 19.32 and 19.27 %) in the region 30-280 ^{0}C and corresponds to the losses of two chlorides coordinated to the central metal ion. The weight loss of 63.15% (63.12 %) in the region 281-543 ^{0}C in the thermogram of complex corresponds to the loss of one ligand molecule. The plateau obtained after heating the complex above 543 ^{0}C corresponds to the formation of stable metal oxide.

Proposed Structures
On the basis of the above observations, it is tentatively suggested that Cu(II) investigated complexes show various geometries [**Figure-4**] in which the ligands act as tridentate ligands.



(With L_1H) **(With L_2)**



(With L_3H_2) **(With L_4)**

Figure-4: Proposed structures for investigated Cu(II) complexes

Anti-Microbial Activity

The difference in anti-microbial activities of the investigated complexes and ligands were studied and the results are presented in **table-6**.

A comparative study of MIC (Minimum Inhibitory Concentration) values of the ligands and their complexes (**Table-6**) indicates that the metal complexes generally have a better activity than the free ligands. Such an increased activity of the metal complexes is probably due to the greater lipophilic nature of the complexes which can be explained based on chelating theory [36]. The antimicrobial results evidently show that the activity of the ligands has enhanced on coordination to the metal ion.

The antimicrobial activity reveals that the compounds exhibited better activity against the bacterial strains tested. The bacterium, *Staphylococcus Aureus (SA)* is found to be most susceptible one. Similarly, *Candida Albicans (CA)* is most susceptible among the fungal strains. From the *in vitro* antimicrobial assay, it is thus found that the tested compounds possess excellent antimicrobial activities even when compared with the standards used.

Table-6 *In vitro* Antimicrobial Activity of the ligands and their Cu-complexes (in μgmL^{-1})

Compound	MIC(μg/mL)					
	Bacteria				Fungi	
	Gram positive		*Gram negative*			
	SA	*EF*	*EC*	*SM*	*CA*	*AN*
L_1H	13	11	9	12	14	12
L_2	9	6	10	7	16	11
L_3H_2	7	11	12	9	12	16
L_4	10	7	16	8	13	19
$[Cu(C_{21}H_{17}N_3O_3)Cl]$	4	6	4	6	8	9
$[Cu(C_{15}H_{13}N_7O)Cl_2]$	5	5	7	7	9	10
$[Cu(C_{14}H_{14}ClN_3O_3)Cl].H_2O$	4	6	4	7	5	8
$[Cu(C_{11}H_{11}N_5O)Cl_2]$	3	4	6	4	7	7
Chloramphenicol	0.25	1.5	2.3	1.7	3.2	3.5
Griseofulvin	--	--	--	--	8	11

CONCLUSION

An efficient synthesis of the two new biologically active 1,2-dihydroquinazolin-4(3H)-ones (L_1 & L_2) in single step by treating *o*-aminobenzoylhydrazone (o-ABH) with aromatic aldehydes under normal conditions and other two new hydrazones (L_3 & L_4) by condensing o-ABH with same aromatic aldehydes with superior yields in polyethylene glycol (PEG) as an alternative solvent and their Cu(II) complexes carrying potential pharmacophores have been prepared in an environmentally benign microwave protocol. The yields of the products formed under MWI were high in comparison to classical method and time required for completion of these reactions was also less in comparison to classical method.

The synthesized ligands coordinated with the Cu(II) ion in a tridentate manner. On the basis of elemental analysis, molar conductance, magnetic susceptibility measurements, electronic, IR, (^{1}H & ^{13}C) NMR and ESR spectral observations, various geometries with various coordination numbers (Fig.4) have been proposed for the Cu(II) complexes. Antimicrobial data suggests that the metal complexes are better antibacterial and antifungal agents as compared to their ligands.

In conclusion, this paper describes a simple, proficient and green approach for the synthesis of ligands and their Cu(II) complexes in green solvent media under MWI as green methodology. Present methodology offers very attractive features such as simple experimental procedure, higher yields and economic viability, when compared with other method as well as with other methodologies and solvents, and will have wide scope in organic/inorganic syntheses.

REFERENCES

[1] M.S.Karthikeyan, D.J.Prasad, B.Poojary, K.S.Bhat, B.S. Hollaa, N.S.Kumari, *Bioorg. Med. Chem.*, **2006, 14,** 7482-7489.
[2] B.S.Holla, B.Veerendra, M.K.Shivananda, B.Poojary, *Eur. J. Med. Chem.*, **2003, 38,** 759-76.
[3] T.Liu, J.Lv, S.Cai, X.Wang, L.Liu, Y.Wang, *J. Inorg. Biochem*, **2006**, **100,** 1888-1896.
[4] K.Singh, M.S.Barwa, P.Tyagi, *Eur. J. Med. Chem.*, **2007, 42,** 394-402.
[5] K.Dhara, J.Ratha, M.Manassero, X.Y.Wang, S.Gao, P.Banerjee, *J. Inorg. Biochem.* **2007, 101,** 95-103.

[6] X.B.Yang, L.Wang, J.Zhang, Z.W.Zhang, H.H.Lin, L. H.Zhou, X. Q.Yu, *J. Enzym. Inhib. Med. Chem.*, **2009, 24,** 125-130.
[7] K.P.Srivastava, et al. *Der Chemica Sinica*, **2011, 2** (2), 66-76**.**
[8] K.P.Srivastava, et al. *ISOR Journal of Applied Chemistry*, **2014, 7**(4), 16-23.
[9] K.P.Srivastava, et al. *Der Pharma Chemica***, 2015, 7**(1):121-127**.**
[10] D.Greenwood, R.Snack, J.Peurtherer, *Medical microbiology: A guide to microbial infections: Pathogenesis, immunity, laboratory diagnosis and control*, 15th edn. **1997**.
[11] R.Vijayalakshmi, M.Kanthimathi, V.Subramanian, B.U.Nair, *Biochem, Biophys. Acta.* **2000, 1475,** 157-164.
[12] W.J.Geary, *Coord. Chem. Rev*., **1971, 7**, 81.
[13] Z.Guo, P. J.Sadler, *Angew. Chem. Int. Ed*., **1999, 38**, 1512, and references therein.
[14] C. J.Morris, *Meth. Mol. Biol.*, **2003, 225**, 115.
[15] S.Dhar, M.Nethaji, A. R.Chakravarty, *Inorg. Chem.*, **2006, 45,** 11043-11050 ().
[16] K. Nakamoto, *Infrared spectra of Inorganic and Coordination Compounds*; 2nd edn. Wiley-Interscience, New York, **1970**.
[17] L. J. Bellamy, *the Infrared Spectra of complex molecules*; 3rd edn. Chapman and Hall, London, Vol. 233, **1975**.
[18] K. B. Gudasi, R. S. Vadavi, R. V. Shenoy, M. S. Patil, S. A. Patil, M. Nethaji, *Inorg. Chim. Acta*, **2005**, 358, 3799.
[19] K. B. Gudasi, R. S. Vadavi, R. V. Shenoy, S. A. Patil, *J. Coord. Chem*., **2007**, 1.
[20] M. C. Rodrı´guez-Arguelles, S. Mosquera-Va´zquez, P. Touro´n-Touceda, J. S.Matalobos, A. M. Garcı´a-Deibe, M. Belicchi-Ferrari, G. Pelosi , C. Pelizzi, F. Zani, *J. Inorg. Biochem.* **2007**, 101, 138.
[21] M. P. Satisha, V. K. Revankar, K. S. R. Pai, *Metal Based Drugs*, **2008**, doi:10.1155/2008/362105, Article ID 362105.
[22] D. N. Sathynarayana, *Electronic absorption spectroscopy and Related Techniques*, Universities Press Ltd., India, **2001**.
[23] G. Wilkinson, R. D. Gillard. J. A. McCleverty, *Comprehensive Coordination Chemistry (Late Transition Metals)*, Pergamon press, Oxford, Vol-5, **1987**.
[24] S. Naskar, S. Biswas, D. Mishra, B. Adhikary, L. R. Falvello, T. Soler, C. H. Schwalbe, S. K. Chattopadhyay, *Inorg. Chim. Acta*. **2004**, 357, 4257.
[25] D. Maiti, H. Paul, N. Chanda, S. Chakraborti, B. Mondal, V. G. Puranik, G. K. Lahiri, *Polyhedron*, **2004**, 23, 831.
[26] C. Biswas, S. Chattopadhyay, M. G. B. Drew, A. Ghosh, *Polyhedron*, **2007**, 26, 4411.
[27] R. Gup, B. Kırkan, *Spectrochim. Acta Part A*, **2005**, 62, 1188.
[28] B. Prabhakar, K. Laxmareddy, P. Lingaiah, *Indian J. Chem*., **1988**, 27A, 217.
[29] R. Dinda, P. Sengupta, S. Ghosh, T. C. W. Mak, *Inorg. Chem.*, **2002**, 41, 1684.
[30] M. T. H. Khan, R. Khan, Y. Wuxiuer, M. Arfan, M. Ahmed, I. Sylte, *Bioorg. Med. Chem.*, **2010**, 18, 4317.
[31] E. Galeazzi, A. GuzmBn, J. L. Navaz, Y. Liu, M. L. Maddox, and J. M. Muchowski, *J. Org. Chem.*, **1996**, 60, 1090.
[32] E. Pereia, L. Gomes, B. Castro, *J. Chem. Soc. Dalt. Trans*., **1998**, 629.
[33] H. S. Seleem, B. A. El-Shetary, S. M. E. Khalil, M. Mustafa, Ma. Shebl, *J. Coord. Chem*., **2005**, 58, 479.
[34] I. N. Procter, B. J. Hathway, P. Nicholls, *J. Chem. Soc. A*, **1968**, 1678.
[35] A. A. G. Tomlinson, B. J. Hathway, D. E. Billing, P. Nicholls, *J. Chem. Soc. A*, **1969**, 65.
[36] T. M. Aminbhavi, N. S. Biradar, V. L. Roddabasangoudar, W. E. Rudzinski, D. E. Hoffman, *Inorg. Chim. Acta.* **1986**, 121, 145.