Design, Synthesis And Bioassay Of Novel Complexes Of 3-Amino-2-Methyl-7-Chloro-Quinazolin4 (3h)-One

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ABSTRACT: A novel Ligand of 3-Amino-2-Methy-7-Chloro-Quinazolin 4(3H)-one (L) has been synthesized in good yield by the reaction of methyl-2-amino-4- chlorobenzoate with acetic anhydride then replaced the oxygen with nitrogen of hydrazine. When the Ligand react with Co (II), Zn (II) and Cu (II) new complexes are formed. The chemical structure of all prepared compounds were characterize by IR, UV/Visible, ^{1}H-NMR, ^{13}C-NMR, GCMS and elemental analysis, moreover, molar ratio M:L were also determined. The free Ligand and their metal complexes have been tested in vitrol against a number of microorganisms gram positive bacteria (*Staphylococcus aureus Bacillus species and Enterococcus feasalis)*, gram negative bacteria *(Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa)* and fungi (*Candida albicans*) in order to assess their antimicrobial properties. All our complexes should considerable activity against all bacteria.

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INTRODUCTION

Of the many derivatives of quinazoline system known so far, keto-quinazolines also called quinazolinones are the most important compounds. Depending upon the position of the keto or oxo group, these compounds may be classified into two. The two structural isomers are 2-quinazolinone and 4-quinazolinone, with the 4-isomer being the most common (Wikipedia, 2010).

Quinazolines are a large class of active chemical compounds exhibiting a broad spectrum of biological activities in animals as well as man. These activities include antihypertensive, diuretic, antimicrobial, pesticidal, central nervous system activities such as anticonvulsant, anaesthetic and sedative activities, anti-malarial, and anti-diabetic as well as analgesic activities. (Rajput and Mishra, 2012).

4(3H)-quinazolinone ring have been reported to possess different biological activities such as antibacterial (Nesrin *et al.,* 2009), antifungal (Bantroliet *et al.,* 1998, Aysel *et al.,* 2005), antitubercular (Kumar *et al.,* 1983), antiviral (Corbett *et al.,* 2000), anticancer (Hour *et al.,* 2000, Hamel *et al., 1996*). Quinazolinone hydrazones exhibit antimicrobial (Radhakrishnan *et al.,* 1984; Karali *et al.,* 1998) and anticonvulsant (Aysel *et al.,* 2005; Abdel-Hamide *et al.,* 2007) activity.

Privileged scaffolds increase hit rates for biological targets of intenset, leading to the discovery of other biologically active targets and generating leads with enhanced drug-like properties (Horton *et al.,* 2003, De Simone, 2004).

EXPERIMENTALS

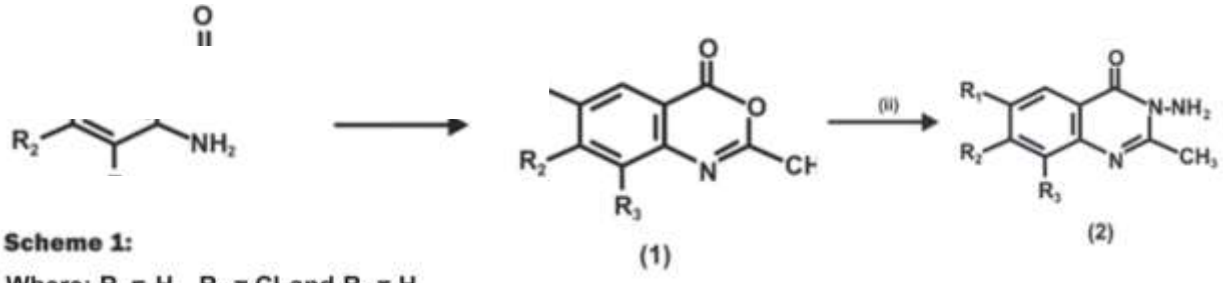
All reagents and solvents were purchased from Sigma-Aldrich chemical supplier in Germany. Melting points were

determined on a Kofler hot Sttage apparatus and are uncorrected. IR spectra were recorded on a Buck scientific IR. M500 instrument. The ^{1}H and ^{13}C NNM spectra were recorded in DMSO at 400 MHz with HAZ VOLATILE V2M. Chemical shifts one reported in ppm relative to tetramethylsilane. Gas Chromatography Mass spectra were obtained on a HAZ VOLATILE V2M (400 MHz) and Chemical shifts are reported in ppm relative to tetramethylsilane as reference standard. Elemental analysis agreed favourable with the calculated values. Analystical thin layer chromatography (TLC) were used to monitor the reactions. Synthesis of 2-methyl-7-chloro-H-benzo [1,3]-oxazin-4-one(1) and Synthesis of 3-amino-2-methyl-7-quinazolin4(3H)one(2).

This involved the condensation of 1.97g (0.005mol) Methyl-2-amino-4-chlorobbenzoate or 4-chloroanthranilate with 10mL, 1.02g (0.01mol) acetic anhydride in 30ml ethanol medium. The reaction was heated under neflux with stirring using a magnetic stirrer until the reaction mixture showed no trace of starting material when the TLC was developed (2hours). Ethanol was removed in vacuum and the crude mixture was poured into 50ml of ice water on a cold water bath. The mixture was stirred for 30minutes filtered and extracted into ethyl acetate and allowed to evaporate at room temperature to give solid products which were incrystallized from hexane or dichloromethane-hexane mixture. Yield 0.81g (92%) mp:87=89^{0}C.

The condensation of equimolar amounts (1.59, 0.005mol) of 2-methyl-2-chloro-4H-benzo[1,3]-oxazin-4-one and hydrazine hydrate (0.93g, 0.001mol) in 30ml boiling ethanol with stirring using a magnetic stirrer until the reaction mixture showed no trace of stating materials when the TLC was developed (3hours). At the end of the reaction, the reaction mixture was concentrated in vacuum under reduced pressure using rotary evaporator. The white precipitate formed was then filtered, washed three times with 20ml of distilled water (20ml x 3). The white crystals were dried and recrystallized from dimethylform-amide (DMF) to give pure 3-Aimo-2-methyl-7-chloro-quinazolin 4(3H)-one. Yield was 1.48g (92%) mp: 114-116^{0}C.

Synthesis of 2-methyl 7-chloro-4H-benzo (1, 3)-oxazin-4-one and Synthesis of 3-amino-2-methyl 7-chloro quinazolin-4 (3H)-one.



Scheme 1:

Where: R_1 = H, R_2 = Cl, and R_3 = H

i = Acetic anhydride

ii = Hydrazine hydrate

Scheme of complexes

A hot ethanolic solution (20ml) of corresponding metal salt (0.005mol) was mixed with hot ethanolic solution of the Ligand (0.01mole). The mixture was nefluxed for 5hours on a water bath on cooling the content, the coloured complex separated out in each case. The same was filtered, washed with 50% ethanol and dried in vacuum over P_4O_{10}. Purity of the complexes was checked by TLC.

TABLE 1: Physical Data of Complexes

No	Complexes	Colour	M.P ^{0}C	Yields (%)	M : L
C_1	CoL_2U_2	Green	248-250	82	1 : 2
C_2	CuL_2U_2	Brown	250-252	88	1 : 2
C_3	ZnL_2U_2	White	244-246	84	1 : 2

Study of formation of complexes in solution

Complexes of Ligand with metal ions were studies in solution using DMF as solvent in order to determine (M : L) ratio in the complex following the molar ratio method (Nada *et al.,* 2001). A series of solutions were prepared having a constant concentration (10^3M) of metal ion and Ligand (L). The (M/L) ratio was determined from the relationship between the absorbance and the mole ratio of (M/L). The results are listed in Table 1.

Antimicrobial analysis

Agar wall diffusion method was utilized for the antimicrobial activities (Okeke, 2001). Seven species: *Staphylococcus aureus, Bacillus species, Enterococcus feacalis, Esherishia coli, Klebsiella pneumonia,. Pseudomonas aeruginosa* and *Candida albicans* stock cultures were used. The test organisms were supplied by the pharmaceutical Microbiology Department of the University of Benin. The test organisms were cultured overnight in nutrient broth, diluted to the turbidity of Marcfolon standard. 0.2ml of the broth culture is seeded on nutrient agar and is allowed to dry. The various concentrations of the extract were introduced. The culture plates are tehn incubated at 37^{0}C for 24hours and the result was taken by considering the zone of inhibition by the test extract (Mackie and McCartney, 1989). Activity and inactivity were observed in accordance with the standard and accepted method. The results are shown in Figures 1, 2, 3, and 4.

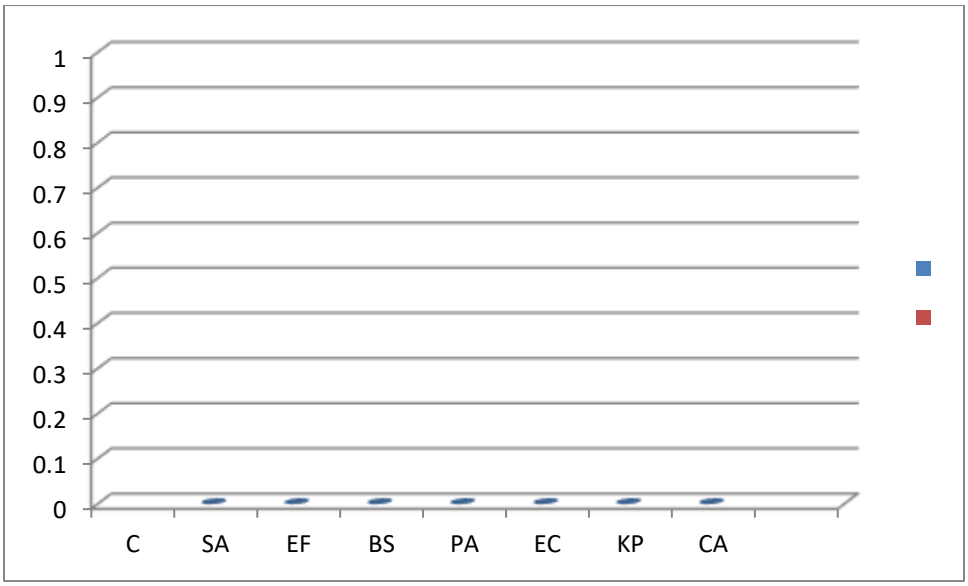


Figure 1; The effect of Ligand toward studied bacteria. SA=Staphylococcus aureus, BS=Bacillus species, EF=Enterococcus feacalis, EC=Escherichia coli, KP=Klebsiella pneumonia,PA= pseudomonas aeruginosa and CA=candida albicans (4c = L)
Significantly different from Ligand at P< 0.05, values are in mm

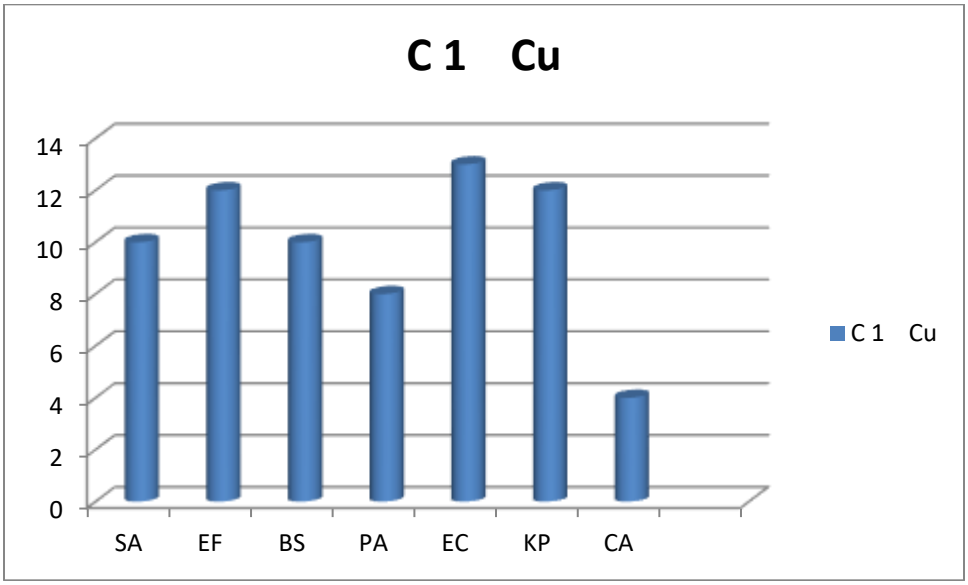


Figure 2. The effect of the complex (C_1) toward studied bacteria. SA=Staphylococcus aureus, BS= Bacillus species, EF=Enterococcus feacalis, EC=Escherichia coli, KP=Klebsiella pneumonia, PA= pseudomonas aeruginosa and CA=candida albicans
Significantly different from Ligand at P<0.05, Values are in mm.

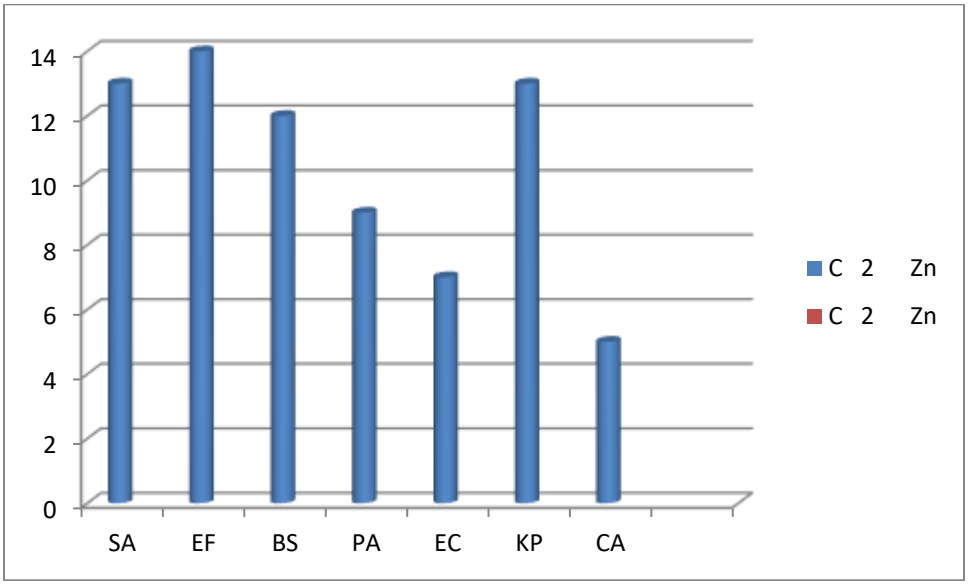


Figure 3. The effect of the complex (C_2) toward studied bacteria. SA=Staphylococcus aureus, BS=Bacillus species, EF=Enterococcus feacalis, EC=Escherichia coli, KP= Klebsiella pneumonia, PA= pseudomonas aeruginosa and CA=candida albicans
Significantly different from Ligand at P<0.05, Values are in mm.

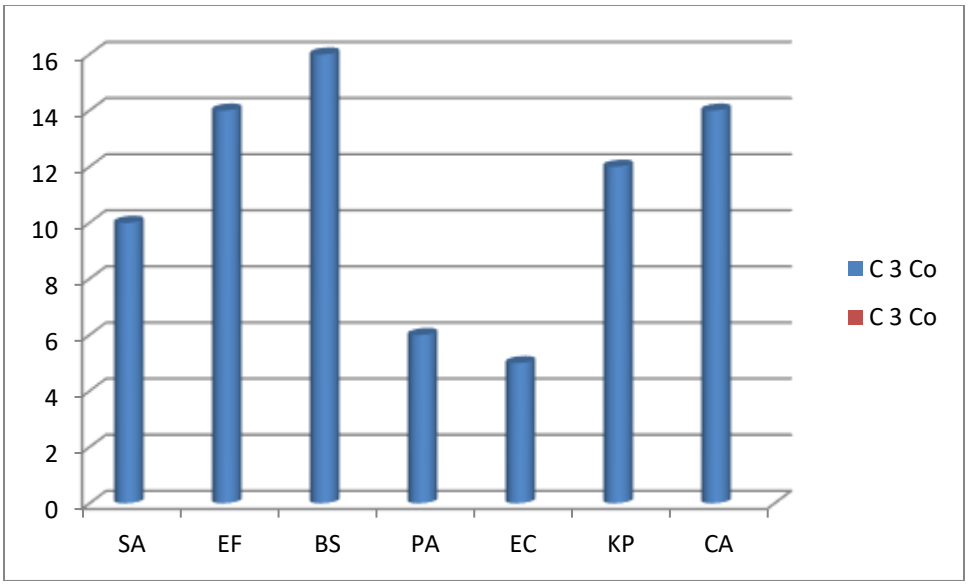
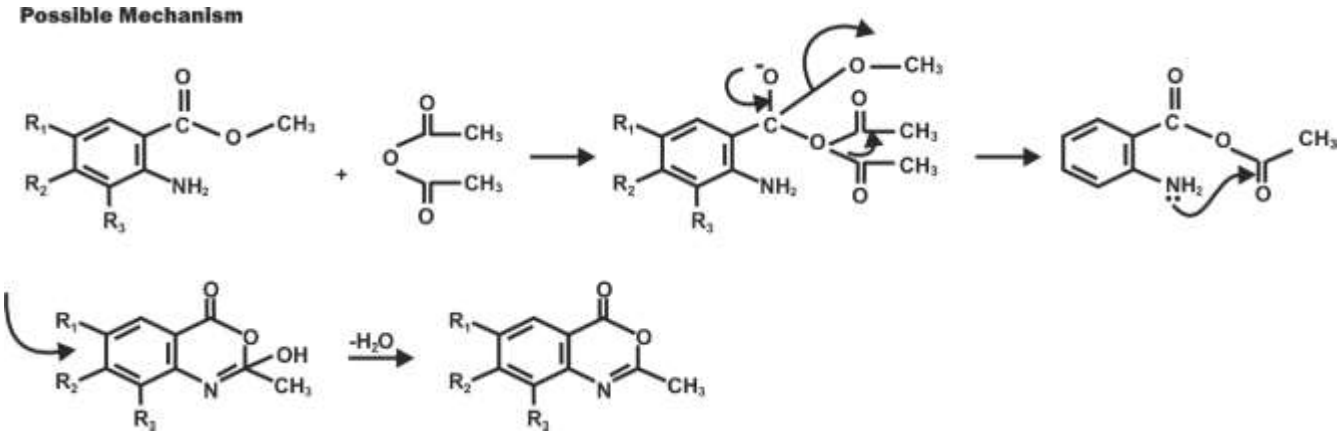


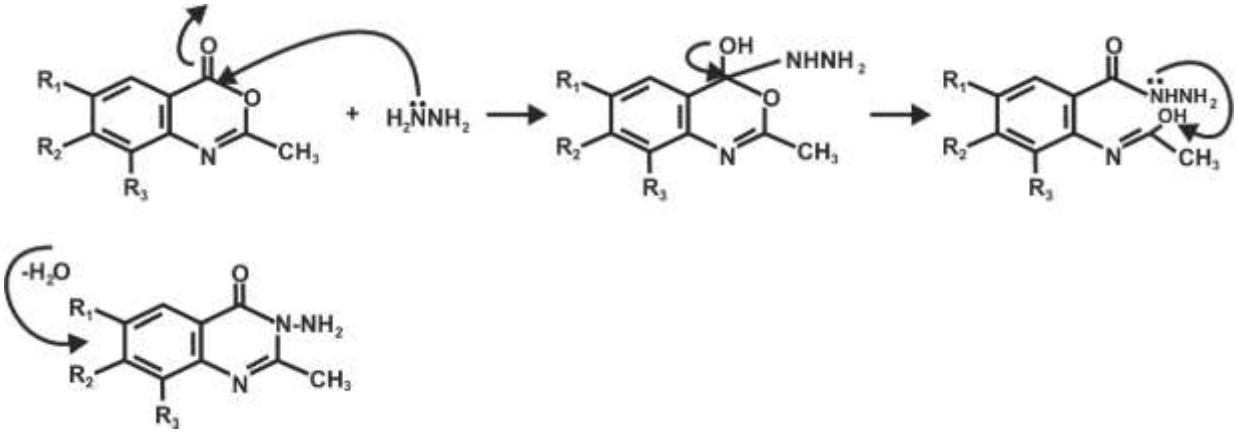
Figure 4. The effect of the complex (C_3) toward studied bacteria. SA=Staphylococcus aureus, BS=Bacillus species, EF=Enterococcus feacalis, EC=Escherichia coli, KP= Klebsiella pneumonia, PA= pseudomonas aeruginosa and CA=candida albicans
Significantly different From Ligand at P<O.05, Values are in mm.

RESULT AND DISCUSSION

The reaction of the 4-chloroanthranilate or methyl-2amino-4-chlorobenizoate with acetic anhydride yield the cyclic compound 2-methyl-7-chloro-4H-benzo[1,3]-oxzin-4-one as shown in the mechanism. The reaction of this compound with hydrazine yield the novel Ligand 3-amino-2-methyl-7-chloro-quinazolin4(3H)-one as shown in the mechanism.



POSSIBLE-MECHANISM



SYNTHESIS OF COMPLEXES

The complexes were synthesized by the reaction of the ligand with the metal ions in 1:2 molar rations in ethanolic medium. The ligand behaves as bidentate coordinate through oxygen and nitrogen donor atoms. All the complexes one fairly stable and can be stored for long periods at room temperature.

Elemental analysis

The composition of the complexes are summarized in Table 2. The C,H and m contents (both theoretically calculated values and actual values) are in accordance with the formula ML_2Cl_2 indicating that the ligand ia neutral. This can be explained by the absence of any deprotonating agent during the synthesis. The complexes are generally soluble in common organic solvents.

TABLE 2: Elemental Chemical Analysis Data of the Complexes

Elemental Analysis							
No	**Complexes**	**Theoretical Calculated Values**			**Actual Calculated Values**		
		C%	**H%**	**M%**	**C%**	**H%**	**M%**
C_1	CoL_2U_2	46.63	3.83	12.21	46.53	3.81	12.27
C_2	CuL_2U_2	45.63	4.83	12.21	45.33	4.81	12.30
C_3	ZnL_2U_2	47.92	3.45	13.14	47.82	3.40	13.00

Infra-red spectra

The study and comparison of the infrared spectra of the ligand and its complexes imply that ligand is bidentate, with the carbon oxygen and nitrogen as the 2 coordination sites. The presence of the ring vibrations and C-H absorption makes the spectra fairly complicated for complete assignment of individual bonds. The partial infrared data are presented in Table 3. In the infrared spectra of the complexes a considerable negative spectra of the complexes a considerable negative shift in V(C=0) is observed, indicating a decrease in the stretching force-constant of the C=O bond as a consequence of co-ordination through the carbonyl-oxygen atom of the free base. Another important band, which occurs at 1503cm^{-1} is attributed to V(C=N) azomethine mode (Radhakrishnan *et al.*, 1984, Agarual and Prakash, 1991) and remain unaffected after complexation. The bond due to NH^- stretching in free ligand occur in the 3340cm^{-1} region, in the spectra of all the complexes, this band is shifted to lower frequency and spears in the 3200 – 3270cm^{-1} region indicating the involvement of the N-atom of the ligand in coordination.

TABLE 3: Infrared Absorption Frequencies (cm^{-1}) of Ligand and its Complexes

No	Complexes	V(NH)	V(C=N)	V(C=O)	M-O	M-N
L		3340	1569	1690	-	-
C_1	CoL_2U_2	3270	1501	1630	448	442
C_2	CuL_2U_2	3210	1503	1635	450	440
C_3	ZnL_2U_2	3215	1499	1640	452	450

UV-Vis Spectra

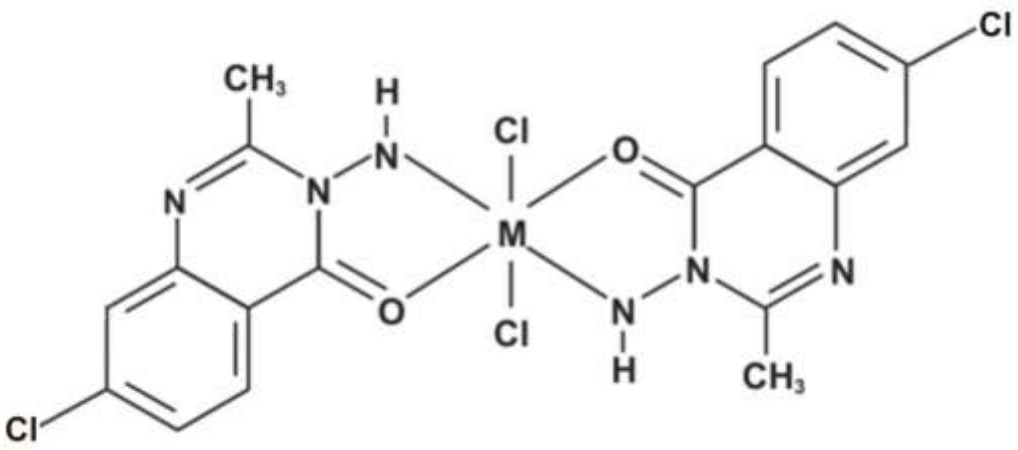
The ultraviolet spectrum of the synthesized ligand in DMF showed three absorption bands, the position of the first band at 235mm which represent the ($\overline{\Lambda}-\overline{\Lambda}^*$) transition while the position of the second and the third bands (which has higher intensity than the first band due to conjugated system) appeared at 360 and 370mm which represents the (n $-\overline{\Lambda}$) transition. Generally, the bands of the nearly synthesized complexes are shifted to shorter or longer wavelengths then that of ligands, but the high intensity of the bands is an indication of complex formation. The origin of the band observed at about 700mm in the electron spectra of complexes has been identified as d-d transition. In these complexes the bands observed at 300 – 400mm could be assigned to nitrogen-metal charge transfer absorption. The electronic absorption bands for the ligand and complexes are classified into two distinct groups those that belong to liquid transitions appeared in the uv region while d-d transitions are in the visible region. These transitions are assigned in relevance to the structures of complexes.

Bactericidal screening

The antimicrobial screening data show that the compounds exhibit antimicrobial properties and it is important to note that the metal chelates exhibit more inhibitory effects than the parent ligands. The increased activity of the metal chelates can be explained on the basis of chelation theory. It is known that chelation tends to make the complexes act as more powerful and potent bactericidal agents, thus killing more of the bacteria than the ligand. It is observed that, in a complex, the positive charge of the metal is partially shared with the donor atoms present in the ligands, and there may be $\overline{\Lambda}-$ electron delocalization over the whole chelation (Om Prakash *et al.,* 2002). This increases the lipophilic character of the metals chelate and favours its permeation through the lipoid layer of the bacterial membranes. The increased lipophilic character of these complexes seems to be responsible for their enhanced potent antibacterial activity. It may be suggested that these complexes deactivates various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms. It has also been proposed that the ultimate action of the toxicant is the denaturation of one or more proteins of the cell, which as a results impairs normal cellular processes. There are other factors which also increase the activity, which are solubility conductivity and bond length between the metal and the ligand.

Activity of the ligand and metal complexes against some human pathogenic microbes including Guam positive (*staphylococcus aureus, Bacillus species* and *Enterococcus aureus*), Guam negative (*Escherichia coli, Klebsiella pneumonia, Pseudomonas ariginosa*) and fungi candida albicans by the Agar wall diffusion method (see figures 1, 2, 3 and 5). From the result obtained from the method, it was found highly active even at low concentrations.

Figure 6: Proposed Structure of the Complexes



Conclusion

All the complexes have higher activity against the microorganisms compared to the ligand. Looking at reported results, it may be concluded that ligand acts as bidendate uni-negative ligand, coordination through one of the nitrogen atom and the oxygen. In the present investigations, all the complexes are found to be mononuclear, based on the IR spectra data. Based on the physicochemical and the spectra studies the tentative structures proposed for the complexes are shown in Figure 6.

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