
Synthesis, Characterization and In vitro Antifungal Activity Studies of Copper(II) Complexes
Derived from 6-hydroxyflavone Schiff bases

By

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ABSTRACT

This study reported the synthesis of the new ligands (E) - 4 - ((2-hydroxyphenyl)imino)-2-phenyl-4H-chromen-6-ol (L^1), (E) - 2 - ((6-hydroxy-2-phenyl-4H-chromen-4-ylidene)amino)benzaldehyde (L^2) and (E) - 2 - (6-hydroxy-2-phenyl-4H-chromen-4-ylidene)hydrazinacarbothidamide (L^3). These three Schiff base ligands were synthesized by refluxing equimolar amount of 6-hydroxyflavone with 2-aminophenol, 2-aminobenzoic acid and thiosemicarbazide as ligands L^1 , L^2 and L^3 respectively. The synthesized Schiff bases were subsequently reacted with copper(II) acetate to form the corresponding metal complexes C_1 , C_2 and C_3 . The compounds were characterized using FT-IR, Molar conductance, Melting point/decomposition temperature studies, Solubility test and Gravimetric analysis. The purity of the compounds was determined using TLC. The solubility test of the compounds indicated that they are soluble in most organic solvents except acetone, water and tetrachloromethane in which they are completely insoluble. The Schiff base ligands have melting point between 166 - 185 °C, whereas the copper(II) complexes decomposes in the range 161 - 202 °C. Molar conductance values of the complexes measured are low, indicating their non-electrolytic nature. The spectroscopic data indicated that the Schiff base ligands acted as tridentate and coordinated to the metal via imine nitrogen and deprotonated phenolic oxygen atoms. The in vitro antifungal activity of the synthesized ligands and their metal complexes were studied against two fungal species (*Aspergillus niger* and *Candida albicans*) and the results indicated that the complexes were more active than the ligands but less active compared to reference drug (fluconazole).

Keywords: 6-hydroxyflavone, 2-aminophenol, 2-aminobenzoic acid, Schiff bases and antifungal activity.

INTRODUCTION

A number of Infectious diseases such as cancer, diarrhea, malaria, tuberculosis and AIDS are global threat and their incidences are increasing significantly day by day. Although, some chemotherapeutic agents are

available in market places, the pathogenic organisms are developing resistance to these agents. So, it is imperative to find out safer, more effective and inexpensive chemotherapeutic agents. An extensive literature has developed in recent years in the field of chelating compounds with special

reference to their antimicrobial activities. Thus, synthesis and study of coordination compounds containing biologically important ligands have been an area of interest because of their numerous applications in biology, environment and other systems (Ahmed and Ibrahim, 2015). Transition metal complexes derived from Schiff base ligands are among the most widely studied coordination compounds (Refat *et al.*, 2013). Copper(II) complexes are particularly attractive since the metal is known to play a significant role in naturally occurring biological systems as well as a pharmacological agent (Miura *et al.*, 1999). Flavones constitute one of the major classes of naturally occurring products. Synthesis of flavones and their derivatives has attracted considerable attention owing to their significant biocidal (Kumar *et al.*, 2008), pharmaceutical (Patroniak *et al.*, 2005) and antioxidant activities (Yang *et al.*, 2008). They are naturally occurring heterocyclic compounds, widely distributed among higher plants in the form glycosides. The 6-hydroxyflavone is a part of the important class of compounds of flavones, which occur naturally in plants and has potential beneficial effects for human health. Pharmacological and spectral studies of synthetic biomimetic copper(II) complexes derived from 3-hydroxyflavone derivatives as anti-inflammatory agents was carried out Nagashri *et al* (2011). Diana-carolina *et al.*, (2014), reported synthesis, characterization, crystal structure and antimicrobial activity of copper(II) complexes with thiosemicarbazone derived from 3-formyl-6-methylchromone. This paper discusses the synthesis, characterization and *in vitro* antifungal activity studies of copper(II)

complexes derived 6-hydroxyflavone Schiff bases due to paucity of information.

MATERIALS AND METHOD

All glass wares were well washed with detergent, rinsed with distilled water and dried in an oven before use. All chemicals and solvents used were of analytical grade and purchased from BDH and Sigma-Aldrich and used without further purification. All weighing was carried out on a digital weighing balance (Sartorius ED 2245), melting points were determined using Gallenkamp melting point apparatus. Molar conductivity was determined using Jen way 4010 model, FT-IR Spectral analysis was recorded on a Fourier Transformed spectrophotometer Cary 630 Agilent Technologies model within wavenumber range of 4000–650 cm^{-1} .

Synthesis of Schiff base ligands

The Schiff base ligands (L^1 - L^3) were synthesized according to the procedure reported by Nagashri *et al.* (2011). This involves the condensation of 6-hydroxyflavone (0.1 g, 0.42 mmol) with 2-aminophenol (0.05 g, 0.42 mmol) as L^1 , 2-aminobenzoic acid (0.06 g, 0.42 mmol) as L^2 and thiosemicarbazides (0.04 g, 0.42 mmol) as L^3 respectively and were dissolved in ethanol (40 mL). Acetic acid (1.0 mL) was also added to each of the solutions and refluxed for 3 hours on a water bath with stirring. The solution was cooled at room temperature and placed in ice to allow the formation of precipitate. The precipitate formed was filtered, washed with acetone, dried at room temperature and kept in a desiccator for further analysis.

Synthesis of the Schiff base metal complexes

The metal complexes were synthesized by dissolution of copper(II) acetate monohydrate (0.012 g, 0.06 mmoles) in acetone (20 mL) and (0.04 g, 0.12 mmoles) of the ligand L¹ was also added to the solution in metal to ligand ratio of 1:2. The solution was swirled and triethylamine was added drop-wise. The solution was stirred for 2 hours at room temperature and placed in ice which resulted in the formation of a precipitate. The precipitate was filtered, purified by washing several times with acetone and dried in a desiccator. The same procedure was applied to the other ligands L² and L³ (Nagashri *et al.*, 2011).

In Vitro antifungal activity

The antifungal activity of the ligands and their metal complexes were carried out in the Microbiology Department, ABU, Zaria. The compounds were screened using the agar well diffusion method (Chohan and Kausar, 2000). The antifungal activity was tested against two fungal species (*Aspergillus niger* and *Candida albican*), whose actions were determined at varying concentrations using dimethylsulphoxide as a solvent. The fungal strains were sub cultured in potatoes dextrose medium. The medium was then sterilized by autoclaving at 120 °C for 15 min. After cooling to 45 °C, the medium was poured into 90 mm diameter Petri dishes and incubated at 28 °C. After solidification, Petri dishes were stored at 4 °C. Microorganisms were spread over each dish by using sterile bent Loop rod. The test was carried out by placing filter paper disks (3 mm diameter) with a known concentration of the compounds (ligands and the complexes) on the surface of agar plates inoculated with a test organism. Standard

antifungal drug (fluconazole), and solution of the compounds (ligands and complexes) were also screened under similar conditions for comparison. Plates were allowed to stand in a refrigerator for two hours before incubation to allow the tested compounds to diffuse through the agar. The Petri dishes were incubated for 48 h at 28 °C. The growth inhibition zones around the holes were observed, indicating that the examined compound inhibits the growth of microorganism. The inhibition zone was measured in millimeters carefully. All determinations were made in triplicate for each of the compounds. An average of the two independent readings for each compound was recorded. The minimum inhibitory concentration (Table 5) was considered to be the lowest concentration that has completely inhibited the growth of the test organisms on agar plates.

RESULTS AND DISCUSSION

The physical characteristics of both the Schiff base ligands and their metal complexes are presented in Table 1. All the compounds are coloured and were found to be stable at room temperature. The percentage yield of the Schiff base ligands and their copper(II) complexes are obtained in a yield ranged from 58 – 72 % for the Schiff base ligands and 56 – 60 % for the copper(II) complexes as presented in Table 1, and these are in close agreement with the findings reported (60 – 76 %) by Nagashri *et al.*, (2011). The moderately higher decomposition temperature suggested a good thermal stability of the complexes Aliyu and Ado, (2010). The characteristic frequencies of the expected functional groups are depicted in Table 2. The IR spectra of the Schiff base ligands (L¹, L² and L³) showed strong

absorption bands at 1651, 1651 and 1651 cm^{-1} respectively which were due to azomethine $\nu\text{C}=\text{N}$. In all the complexes, the absorption bands for $\nu\text{C}=\text{N}$ have slightly shifted to lower frequencies of 1602, 1602 and 1561 cm^{-1} in the metal complexes C_1 , C_2 and C_3 respectively and this might be due to the participation of azomethine nitrogen in coordination with the copper(II) ion and these are in agreement with the work of some researchers (Raman *et al.*, 2006; Nagashri *et al.*, 2011). Bands observed around 1575-1400 cm^{-1} in the spectra of all the ligands and the metal complexes were due to $\nu(\text{C}=\text{C})$ stretching of the aromatic ring system. Similar results were obtained in the studies conducted by Heba (2011) on similar compounds. The compounds (ligands and complexes) showed bands in the region 2800-2700 cm^{-1} and 3400-3100 cm^{-1} , which were assigned to $\nu(\text{C}-\text{H})$ asymmetric and symmetric vibration modes and $\nu(\text{O}-\text{H})$ stretching vibrations for coordinated water respectively (Aliyu and Abdullahi, 2009). These data are in close agreement with those reported by Prabhakara *et al.* (2016) for similar Schiff base ligands and their metal complexes. The IR spectra of ligand L^3 showed bands in the region 3304 cm^{-1} and 950 cm^{-1} which were due to $\nu(\text{N}-\text{H})$ of the azide moiety and $\nu(\text{C}=\text{S})$ respectively. The $\nu(\text{N}-\text{H})$ stretch was observed to have shifted to a lower frequency (3268 cm^{-1}) in the spectra of complex C_3 and this was due to the involvement of nitrogen atom in complexation. Bands around 1300-1200 cm^{-1} in the spectra of the ligands were due to phenolic $\nu\text{C}-\text{O}$ (Prabhakara *et al.*, 2016) and were observed to shift upward (1350-1400 cm^{-1}) in the spectra of the complexes and this confirms the participation of phenolic oxygens in complexation with the copper metal and this observation suggest that $-\text{OH}$

groups have been replaced by the metal ion forming M-O bonds via deprotonation. Most of the bands observed around 1250-980 cm^{-1} are consistent with C-C stretching of aromatic system and are also in agreement with the structural changes observed in the molecular carbon-carbon skeleton after complexation. The measured molar conductance of the complexes in dimethylsulphoxide is in the range of 20.4 - 33.5 $\text{ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ (Table 3), which are relatively low indicating that they are non-electrolytic in nature (Raman *et al.*, 2006). The solubility test (Table 4) showed that the compounds are insoluble in acetone, water and tetrachloromethane, but soluble in dimethylsulphoxid, methanol and ethanol. The water analysis of the complexes suggested three water molecules per complex compound.

The *in vitro* antifungal activity of the compounds (ligands, metal complexes and reference drug) was carried out against two fungal strains namely; *Candida albicans* and *Aspergillus niger*. From the MIC values (Table 5), it was observed that the complexes have the same activity of 25 mg/mL against *C. albicans*. This indicates that they both have the same potency against the organism. However, the ligands have higher MIC value (35 mg/mL) than the complexes and the reference drugs against *C. albicans* which indicates lesser activity. Therefore, the complexes have higher activity than the ligands but lesser compared to the reference drug and such increased activity of the complexes over the ligands can be explained on the basis of Overtone's concept (Anjaneyula *et al.*, 1986) and Tweedy's Chelation theory (Dharamaraj *et al.*, 2001). However, no activity was indicated against *A. niger* on all the compounds (ligands and complexes) compared to the reference drugs

which indicated an activity of 20 mg/mL against the same organism. It is evident that the reference drug demonstrated better inhibitory action against *A. niger* than the

compounds and this may be to the non-pathogenic nature of the *A. niger* and the resistant of the organism to all the compounds.

From the analysis of the complexes, the following general molecular structures are proposed;

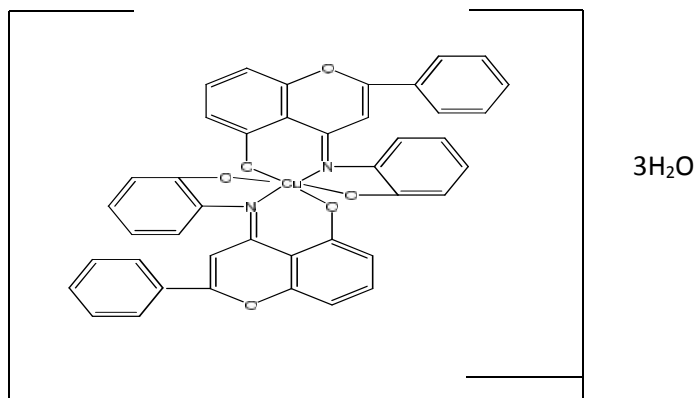


Figure 1: Proposed structure of the metal complex (C₁) [Cu(L¹)₂] · 3H₂O

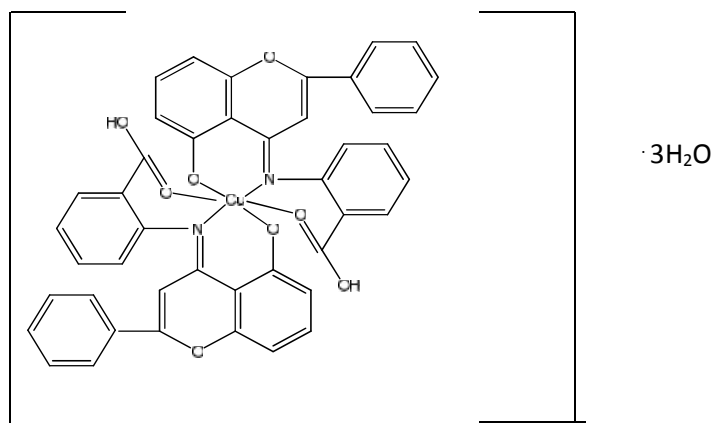


Figure 2: Proposed structure of the metal complex (C₂) [Cu(L²)₂] · 3H₂O

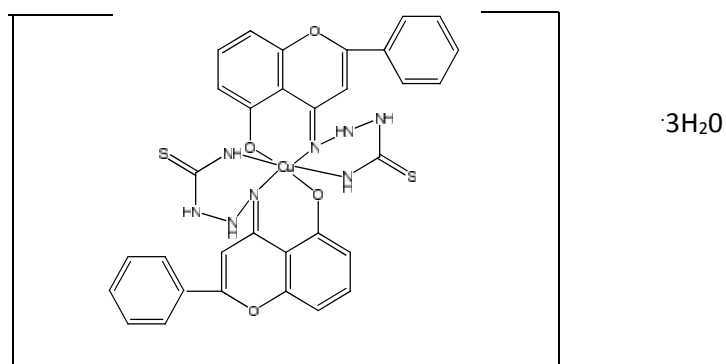


Figure 3: Proposed structure of the metal complex (C₃) [Cu(L³)₂] · 3H₂O

Table 1: Physical Properties of the compounds

| Ligand | M.F | M.P (°C) | D.T (°C) | Colour | % Yield |
|-----------------------------|--|----------|----------|-------------|---------|
| L ¹ | C ₂₁ H ₁₇ O ₂ N | 180-181 | - | Brown | 60 |
| L ² | C ₂₂ H ₁₇ NO ₃ | 183-184 | - | Milky | 72 |
| L ³ | C ₁₆ H ₁₅ N ₃ OS | 181-182 | - | Milky | 58 |
| Copper(II) Complexes | | | | | |
| C ₁ | C ₄₂ H ₂₈ CuN ₂ O ₈ | - | 197-200 | Light brown | 58.3 |
| C ₂ | C ₄₄ H ₂₆ CuN ₂ O ₈ | - | 200-202 | Pale blue | 60.1 |
| C ₃ | C ₃₂ H ₂₂ CuN ₆ O ₄ S ₂ | - | 198-200 | Pale blue | 55.7 |

M.F = Molecular formula, M.P = Melting point (°C), D.T = Decomposition temperature (°C)

Table 2: The IR spectra of the Schiff base ligands and the complexes

| Compound | Absorption bands (cm ⁻¹) | | | | | |
|-----------------------------|--------------------------------------|--------|---------------------|--------------------|--------|--------|
| | v(C=N) | v(C=C) | Phenoli c v(C-O) | Phenoli c v(OH) | v(N-H) | v(C=S) |
| Ligands | | | | | | |
| L ¹ | 1651 | 1572 | 1285 | 3172 | - | - |
| L ₂ | 1651 | 1570 | 1285 | 3168 | - | - |
| L ³ | 1651 | 1520 | 1289 | 3354 | 3304 | 950 |
| Copper(II) complexes | | | | | | |
| C ₁ | 1602 | 1572 | 1356 | 3175 | - | - |
| C ₂ | 1602 | 1572 | 1356 | 3172 | - | - |
| C ₃ | 1561 | 1438 | 1397 | - | 3268 | 931 |

Table 3: Molar conductance of the copper(II) complexes in 10⁻³ DMSO

| Compound | Specific conductance (ohm ⁻¹ cm ²) | Molar conductance (ohm ⁻¹ cm ² mol ⁻¹) |
|----------------|--|--|
| C ₁ | 20.4 x10 ⁻⁶ | 20.4 |
| C ₂ | 33.5 x10 ⁻⁶ | 33.5 |
| C ₃ | 32.7 x10 ⁻⁶ | 32.7 |

Table 4: The Solubility of the compounds in some common organic solvents

| Compound | Tetrachloro- methane | Acetone | DMSO | Ethanol | Methanol | Water |
|----------------|-------------------------|---------|------|---------|----------|-------|
| L ¹ | IS | IS | S | S | S | IS |
| L ² | IS | IS | S | S | S | IS |
| L ³ | IS | IS | S | SS | SS | IS |
| C ₁ | IS | IS | S | S | S | IS |
| C ₂ | IS | IS | S | S | S | IS |
| C ₃ | IS | IS | S | SS | SS | IS |

Key: IS = Insoluble, S = Soluble, SS= Slightly soluble, DMSO = dimethylsulphoxide

Table 5: Minimum inhibitory concentration (MIC) of the compounds and reference drug against the test organisms.

| Compound and Control | Fungi | |
|----------------------|--------------------|-----------------|
| | <i>C. albicans</i> | <i>A. niger</i> |
| L ¹ | 35 | ND |
| L ² | 35 | ND |
| L ³ | 35 | ND |
| C ₁ | 25 | ND |
| C ₂ | 25 | ND |
| C ₃ | 25 | ND |
| fluconazole | 22 | 20 |

ND= Not detected

CONCLUSION

In this paper the synthesis of Schiff base ligands derived from 6-hydroxyflavone with 2-aminophenol, 2-aminobenzoic acid, thiosemicarbazide and its copper(II) complexes have been described. The Schiff base ligands coordinated through its azomethine nitrogen and oxygen atom after deprotonation and this was supported by infrared spectral data. The molar conductivity data of the complexes indicated that they are non-electrolytes. The compounds are stable in air and soluble in polar solvents like methanol, ethanol, dimethylsulphoxid but insoluble in acetone, water and tetrachloromethane. The molar conductivity studies indicated that the complexes are non-

electrolytic. The *in vitro* antimicrobial study shows that the complexes have higher activities compared to the free ligand and the copper(II) acetate but less active compared to the standard drug.

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