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SYNTHESIS, CHARACTERIZATION AND ANTICANDIDA ACTIVITY OF SCHIFF BASES DERIVED FROM 7-AMINO-4-METHYLQUINOLIN-2(1H)-ONE AND SUBSTITUTED SALISALDEHYDES WITH THEIR Cu(II) COMPLEXES

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ABSTRACT
Schiff bases (SB^1-SB^3) were synthesized from equimolar condensation reaction of 7-amino-4-methyl-quinolin-2(1H)-one with substituted salisaldehydes. The Schiff base ligands were then reacted with Cu(II) acetate in a 1:2 metal-to-ligand ratio to form Cu(II) Complexes (A^1 - A^3). The synthesized Schiff bases and their complexes were characterized based on the Solubility test, Molar Conductance, Melting /decomposition temperature, Magnetic susceptibility, Flourier Transform Infrared Spectral Analysis (FT-IR), Proton Nuclear Magnetic Resonance (H^1-NMR), ESI-Mass spectrometry, and powder XRD analysis. The result of the solubility test shows that the Schiff bases and their complexes have poor solubility in water and other solvents except for hot DMSO. The Molar conductance values ranged from 22.5 - 25.5 $ohm^{-1}cm^2mol^{-1}$ showing that the complexes were non-electrolytes. The magnetic susceptibility revealed that all the Cu(II)complexes were paramagnetic and ranged from 1.70 to 1.84 BM. The spectral analysis shows that the Schiff bases coordinate to the copper ion through its imine nitrogen and phenolic oxygen. The Schiff bases and their complexes were screened for their inhibitory activity against four Candida albican isolates and Candida albican ACCT 2876, the result indicated moderate activity when compared with a standard drug (Terbinafine).
Keywords: Schiff base, Spectral analysis, Copper complexes

INTRODUCTION

Transition metals play a significant role in pharmaceutical chemistry. They can interact with several negatively charged compounds and show many oxidation states. Due to the action of transition metals, medicines based on metals have recently been developed and are being investigated as prospective candidates for pharmacological and therapeutic purposes (Selvaganapathy and Raman,2016: Ravichandran *et al.*, 2022). Quinoline alkaloid family is an important class of nitrogen heterocyclic compounds which are known for their different biological activity (Shang *et al.*, 2018). Quinolin-2(1H)-one derivatives have particularly demonstrated various therapeutic abilities as basis of many medicinal drugs used in the treatment of cancer, heart failure and inflammatory disease (Heravi *et al.*, 2014: Khusnutdinov *et al.*, 2014: Ryabukhin and Vasilyev, 2016).

The ability of metals to lose electrons to form positively charged ions allow them to play important roles in biological systems. Whereas metal ions are electrophilic, most biological molecules such as protein and (DNA) are electron rich. The attraction of these opposing forces leads to interaction between metal ions and biological molecules (Moustakas, 2021). According to some reports, metal ions can increase or trigger the activity of biologically significant molecules as well as several well-known medications including aspirin, paracetamol, and sulfamethoxazole (Osowale *et al.*, 2014). The synthesis of Schiff base ligands and their metal complexes have been extensively studied because of their interesting biological activities (Divya *et al.*, 2017).

In healthy people, Candida is a non-pathogenic component of the normal flora that lives on the mucous membranes of the upper respiratory tract, female genital tracts, and gastrointestinal tract. However, in immunocompromised people, it can occasionally transform into pathogenic yeast, invade the mucous membrane, and cause candidiasis (opportunistic infection) (Singh, 2013).

Pathogens like *Candida albican* can lead to both minor and severe systemic illness. It is the most prevalent yeast fungus pathogen that colonizes people by more than 80% of the human population (Van Dijck *et al*., 2018). Normally, these fungi coexist with their hosts in a commensal relationship, but in some cases, such as those caused by immune system deficiencies, extended antibiotic use, chemotherapy, starvation, and other factors, *Candida albican* may transform from commensal to pathogenic states (Mayer *et al*., 2013). The most prevalent type of candidiasis is oral commonly known as thrush candidiasis (Patil *et al*., 2015) diagnosed in humans and is typically treated by use of anti-fungal drugs such as nystatin or fluconazole in severe case. The second most common is vulvovaginal candidiasis or vaginal thrush (Gonçalves *et al.,* 2016), caused by excessive growth of *Candida albicans* in the vagina, which is also the second most common vaginal infection (after bacterial infections) (Zeng *et al*., 2018), with about 5-8% developing the recurrent form of the disease. The typical treatment is based on the topical application of cream or suppositories of clotrimazole or nystatin (Wu *et al*., 2019).

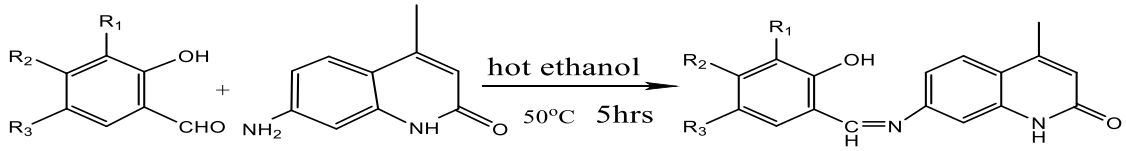
In this work we report a synthesis, characterization and anticandida activity of Schiff bases (SB^1-SB^3) derived from 7-amino-4-methylquinolin-2(1H)-one and substituted salisaldehydes with their Cu(II) complexes.

MATERIALS AND METHODS

All the reagents and solvents utilized in this work were of analytical grade and were used without further purification. They were purchased from Sigma-Aldrich, Germany. Fourier Transform Infrared (FT-IR) analysis were recorded on Agilent Technology Model Carry 630 FT-IR, Nuclear Magnetic Resonance (^{1}H-NMR) were carried out using Agilent Spectrophotometer. Melting point and decomposition temperature were determined using Stuart automatic melting point /SMT40/ Apparatus. Molar conductivity of the complexes was determined in DMSO using Jenway conductivity meter. *Candida albican* isolates were obtained from Ahmadu Bello University Teaching hospital, Zaria (ABUTH) and *Candida albican* ATCC 2876 from National Institute for Pharmaceutical Research and Development, Abuja (NIPRD). Sabouraud Dextrose Agar (SDA) was used as media for *Candida albican.*

Synthesis of the Schiff bases (SB^1-SB^3)

Schiff base ligands (SB^1-SB^3) were synthesized by an equimolar condensation reaction between substituted aromatic aldehyde and 7-amino-4-methylquinolin-2(1H)-one [7AMQ] in hot ethanol. A stirred suspension of quinolinone [7AMQ] (2mmol, 0.348g) and the appropriate aromatic aldehyde (2mmol) in hot ethanol 40ml was refluxed for 5 hours. On cooling to room temperature coloured precipitate formed in good yield which were filtered off and dried in desiccator for two weeks.



Compound	R1	R2	R3
SB^1	Cl	H	Cl
SB^2	Br	H	Br
SB^3	I	H	I

Figure 1: Reaction of substituted aromatic aldehyde with 7-amino-4-methylquinolin-2(1H)-one to give Schiff base ligands 1-3

Synthesis of Metal Complexes (1-3)

Copper (II) acetate (0.125mmol ,0.227g) was dissolved in methanol (30ml) and added drop wise with stirring to a hot methanolic solution of the appropriate Schiff base ligand (0.25mmol) to a final volume of 60ml, the resulting mixture was refluxed with stirring for 5 hours until a precipitate formed. All the complexes were re -crystallized from ethanol.

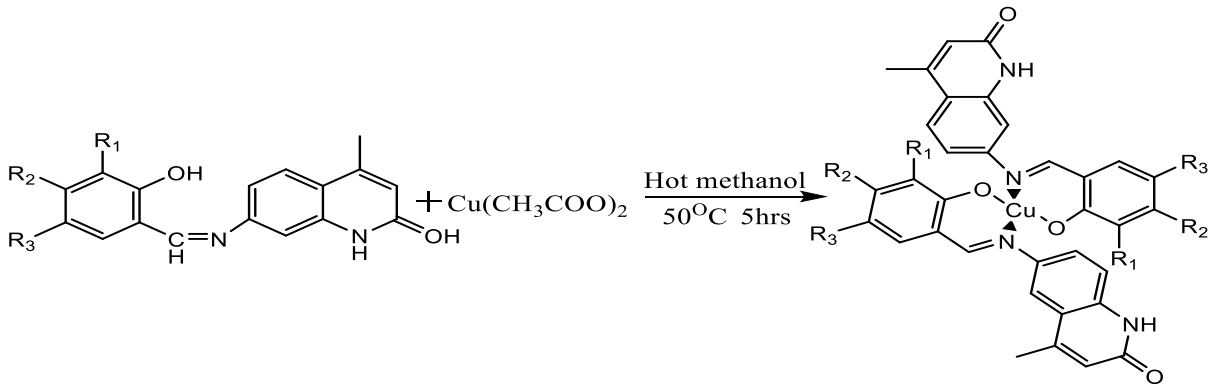


Figure 2: Reaction of Schiff base 1 - 3 with Copper (II) acetate to give Cu(II)complexes

RESULTS AND DISCUSSIONS

Physical properties of synthesized compounds

The interaction between 7AMQ with substituted aromatic aldehyde yield the Schiff base ligands SB^1-SB^3 which were all coloured with percentage yield in the range of 75-79 % and melting point between 264-277^{O}C. The reaction of the Schiff base ligands with Cu(II)acetate formed coloured complexes 1-3 with percentage yield in the range 64-69 % and decomposition temperature between 307-320^{O}C. The high and sharp melting and decomposition temperatures of the synthesized compounds indicate that they are thermally stable and obtained in pure form (Table1).

The result is in concomitance with the findings of (Creaven *et al*., 2009) for similar compounds.

Table 1: Physical properties of Schiff bases and their Cu(II) complexes

Compound	Molecular Formula	Melting/Decomposition temp (^{0}C)	Colour	Yield (%)
SB^1	$C_{17}H_{12}Cl_2N_2O_2$	264	Light orange	75.00
SB^2	$C_{17}H_{12}Br_2N_2O_2$	274	Pale orange	79.10
SB^3	$C_{17}H_{12}I_2N_2O_2$	277	Light orange	75.93
$[Cu(SB^1)_2]$	$C_{34}H_{22}Cl_4CuN_4O_4$	320	light blue	64.16
$[Cu(SB^2)_2]$	$C_{34}H_{22}Br_4CuN_4O_4$	318	Dark blue	67.20
$[Cu(SB^3)_2]$	$C_{34}H_{22}I_4CuN_4O_4$	307	Grey blue	69.80

SB^1 = [(Z)-7-((3,5-dichloro-2-hydroxybenzylidene) amino)-4-methylquinolin-2(1H)-one]
SB^2 = [(Z)-7-((3,5-dibromo-2-hydroxybenzylidene) amino)-4-methylquinolin-2(1H)-one]
SB^3 = [(Z)-7-((2-hydroxy-3,5-diiodobenzylidene) amino)-4-methylquinolin-2(1H)-one]

The solubility of the synthesized Schiff bases and their complexes were determined in water and other solvent (Table 2). The compounds were slightly soluble in ethanol, methanol and acetone, insoluble in water and hexane and completely dissolved in Dimethylsulphoxide (DMSO), hence all solvent bound analysis like molar conductance, antifungal activity was carried out in DMSO.

Table 2: Solubility of the synthesized compounds

Compounds	Water	Methanol	Ethanol	Acetone	Acetonitrile	Hexane	DMSO
SB^1	IS	SS	SS	SS	SS	IS	S
SB^2	IS	SS	SS	SS	SS	IS	S
SB^3	IS	SS	SS	SS	SS	IS	S
$[Cu(SB^1)_2]$	IS	SS	SS	SS	SS	IS	S
$[Cu(SB^2)_2]$	IS	SS	SS	SS	SS	IS	S
$[Cu(SB^3)_2]$	IS	SS	SS	SS	SS	IS	S

SB^1 - SB4 = Schiff base ligands1 - 4, IS=Insoluble, SS=sparingly soluble, S=Soluble, DMSO = Dimethylsulphoxide

In all the FT-IR spectra of the Schiff bases SB^1, SB^2 and SB^3, there is appearance of the characteristics strong υ(C=N) stretching vibration at 1599, 1548 and 1543 which shifted to wave

number, 1550, 1599 and 1594 (Figure 1-6) respectively, an indication of complexation of the imine group with the Copper (II) ion. The ligands show moderately strong bands in the range of 2903-2974cm^{-1} assigned as υ(O-H), on complexation the phenolic O-H group shows band in the lower wavelength 2914-2881cm^{-1} this supports the deprotonation and linkage of O atom to the Copper (II) ion, Similarly, the stretching of ν(Cu-O) and ν(Cu-N) bands of the complexes appeared in the lower wavelength region in the range of 532-536 and 440-484 cm^{-1} also signifying the complexation through nitrogen and oxygen atoms from the ligand (Shebl, 2009).

Table 3: IR frequencies of Schiff base ligands and their Cu(II)complexes

Compound ID	υ(C=O) cm^{-1}	υ(C=N) cm^{-1}	υ(Cu-O) cm^{-1}	υ(Cu-N) cm^{-1}	υ(O-H) cm^{-1}
SB^1	1664	1599	-	-	2903
SB^2	1654	1548	-	-	2922
SB^3	1654	1543	-	-	2974
[Cu(SB^1)$_2$]	1651	1550	536	469	2881
[Cu(SB^2)$_2$]	1654	1599	536	484	2918
[Cu(SB^3)$_2$]	1654	1594	532	440	2914

The H-NMR data (Table 4) indicated a similar pattern for the Schiff bases SB^1, SB^2 and SB^3.the data shows the presence of NH signal at δ values 5.72, 5.72 and 5.95ppm for the respective three Schiff bases(SB^1, SB^2 and SB^3). Their fused aromatic ring was evident based on the protons at position H-6, H-7 and H-9 resonating at δ values of 6.46, 7.34 and 7.81ppm. Another aromatic ring that was tetra substituted shows a proton signals at δ 6.37 and 7.63ppm assignable to H-4 and H-6 protons for the compounds. A methine proton associated with imine group was observed around 9.07, 9.04 and 8.95ppm, therefore imine group linking a tetra substituted aromatic ring and the benzo lactam fused ring confirming the formation of the Schiff bases SB^1, SB^2 and SB^3 (Figure 7).

Table 4: ^{1}H-NMR of the Schiff base ligands SB^1 – SB^3

Carbon position	SB^1	SB^2	SB^3
1	5.72(s)	5.72(s)	5.72(s)
3	5.95(s)	5.95(s)	5.95(s)
6	6.46	6.46 (dd,J=8.65,2.18)	6.46 (dd, J = 8.63, 2.19)
7	7.34 (dd, J = 8.64, 2.71)	7.35	7.34 (dd, J = 8.73,1.62)
9	7.81	7.27 (d, J = 2.09)	7.25 (d, J = 2.06)
11	2.28(s)	2.28(s)	2.28(s)
4′	6.37 (d, J = 2.26)	6.37 (d, J = 2.17)	6.37 (d, J = 2.14)
6′	7.63 (d, J = 2.66)	8.06 (d, J = 2.52)	8.19 (d, J = 2.07)
7′	9.07(s)	9.04(s)	8.95(s)

The molar conductance of the Cu(II)complexes 1, 2 and 3 in 10^{-3}moldm^{-3} DMSO were 22.5, 24.0, and 25.5 ohm^{-1}cm^2 mol^{-1} which is quite low indicating that the complexes were non electrolyte (Table 5). The values obtained fall within the range of 10-50 ohm^{-1}cm^2 mol^{-1} reported by Dhivya *et al.*, (2012) for non-electrolytic complexes.

Table 5: Conductivity measurement of the complexes

Complexes	Concentration mol dm^{-3}	Electrical conductivity Ohm^{-1}cm^{-1}	Molar conductivity Ohm^{-1}cm^2 mol^{-1}
[Cu(SB^1)$_2$]	1 ×10^{-3}	22.5 × 10^{-6}	22.5
[Cu(SB^2)$_2$]	1 ×10^{-3}	24.0 × 10^{-6}	24.0
[Cu(SB^3)$_2$]	1 ×10^{-3}	25.5 × 10^{-6}	25.5

Table 6 shows magnetic moment of the Cu(II)complexes 1, 2 and 3 as 1.70, 1.74 and 1.84 respectively, which shows that they are paramagnetic in nature and fall within the range of square planar geometry values.

Table 6: Magnetic moment measurement of the Cu(II) complexes

Complexes	Mass susceptibility (Xg) (emu g^{-1})	Molar susceptibility (Xm) (emu mol^{-1})	Magnetic moment μeff (B.M)
$[Cu(SB1)_2]$	1.0933×10^{-6}	8.2647×10^{-4}	1.70
$[Cu(SB2)_2]$	5.1219×10^{-6}	4.7825×10^{-4}	1.74
$[Cu(SB3)_2]$	1.4876×10^{-6}	1.0647×10^{-4}	1.84

B.M=Bohr magnetons

The result of elemental analysis of the Schiff bases and metal complexes were presented in Table 7.

Table 7: Elemental Analysis of the Schiff base ligands and complexes

Ligands	% Elemental Analyses Observed (Calculated)		
	C	H	N
SB^1	58.78 (58.81)	3.45 (3.48)	8.04 (8.07)
SB^2	46.78 (46.82)	2.72 (2.77)	6.38 (6.42)
SB^3	38.47 (38.52)	2.25 (2.28)	5.24 (5.28)
$[Cu(SB^1)_2]$	53.98 (54.02)	2.89 (2.93)	7.38 (7.41)
$[Cu(SB^2)_2]$	43.69 (43.73)	2.35 (2.37)	5.97 (6.00)
$[Cu(SB^3)_2]$	36.37 (36.40)	1.95 (1.98)	4.97 (4.99)

Table 8 displays the data from the powder XRD analysis of the complexes. The Scherer equation was used to determine the average crystal size, which demonstrated the complexes crystallinity and similarity in bonding modes across the board (Fatima *et al.*,2022).

Table 8: Powder X-ray diffraction analysis

Complexes	D_{xrd} (nm)
$[Cu(SB^1)_2]$	38.48
$[Cu(SB^2)_2]$	39.55
$[Cu(SB^3)_2]$	40.12

Dxrd (nm) = Average crystal size of the complexes

The electrospray ionisation mass spectrometry (ESI-MS) data of all the Schiff base ligands and their Cu(II)complexes were presented in Table 10. Each mass spectrum represents molecular ion peak (M+1) corresponding to the molecular weight of the respective compound. The molecular ion peak in most of the spectrum is the most abundant peak

Table 9: ESI- Mass Spectral data for Schiff bases ligands and their Cu (II) Complexes

Compounds	Found (m/z)	Expected (m/z)	Fragments
SB^1	346.98	347.20	$[L + H]^+$
SB^2	435.72	436.10	$[L + H]^+$
SB^3	529.50	530.10	$[L + H]^+$
$[Cu(SB^1)_2]$	754.70	755.92	$[Cu(SB^1)_2]^+$
$[Cu(SB^2)_2]$	932.62	933.72	$[Cu(SB^2)_2]^+$
$[Cu(SB^3)_2]$	1120.50	1120.71	$[Cu(SB^3)_2]^+$

Table 10: Zone of inhibition (mm) for Schiff base and their complexes

Anticandida agents	Test *Candida albican* 1	2	3	4	ATCC 2876
SB^1	20.0±0.4	20.0±0.3	20.0±0.4	20.0±0.3	20.0±0.5
SB^2	20.0±0.3	19.0±0.7	19.0±0.6	19.0±0.6	19.0±0.6
SB^3	22.0±0.5	21.5±0.3	21.3±0.7	21.4±0.6	21.0±0.5
$[Cu(SB^1)_2]$	28.0±0.3	27.5±0.4	26.9±0.5	26.9±0.5	28.0±0.5
$[Cu(SB^2)_2]$	24.0±0.3	24.0±0.3	24.0±0.3	24.0±0.3	24.0±0.3
$[Cu(SB^3)_2]$	27.8±0.4	27.6±0.6	27.5±0.5	27.6±0.5	27.8±0.6
Terbinafine	26.5±0.5	26.0±0.9	26.4±0.5	26.1±0.3	29.0±0.5

The Minimum Inhibitory Concentration (MIC) values were presented in Table 12. The MIC for the Schiff Bases, complexes and standard drug were determined against the test organisms. The result proved that the complex A1, A2 and A3 have lower MIC values against *Candida albican* (1) than the standard drug but in the other Candida isolates the standard drug have the lowest MIC values (higher activity).

Table 11: Minimum Inhibitory Concentration of Schiff bases and Cu (II) complexes against Candida albican isolates in (mM)

Anticandida agents	Formular weight($gmol^{-1}$)	Test Candida albican 1	2	3	4	ATCC2876
SB^1	347.20	125.01	125.01	125.01	125.01	125.01
SB^2	436.10	250.04	125.07	125.07	125.07	500.08
SB^3	530.10	125.07	2000.00	2000.00	2000.00	2000.00
$[Cu(SB^1)_2]$	755.92	25.00	25.00	25.00	25.00	100.00
$[Cu(SB^2)_2]$	933.73	25.03	100.14	50.06	100.14	100.14
$[Cu(SB^3)_2]$	1120.71	25.06	50.01	50.01	50.01	50.01
Terbinafine	291.43	86.08	11.04	21.44	11.04	17.57

CONCLUSION

Three Schiff base and their Cu(II)complexes were synthesized and characterized by IR, ^{1}H-NMR, ^{13}C-NMR, ESI-MS, Molar conductivity measurement, elemental analysis, magnetic susceptibility measurements and Powder XRD analysis. The IR spectra showed that the quinolin-2(1H)-one Schiff bases behave as bidentate ligands which coordinate to the Copper (II) ion through the deprotonated phenolic oxygen and the azomethine nitrogen of the ligands. Molar conductivity measurements showed that the complexes are non-electrolytes and their composition correspond to 1:2 metal-ligand ratio. Magnetic susceptibility measurements suggested a four-coordinate local symmetry around the Cu (square planar), and also revealed that the complexes are paramagnetic. The anticandida screening revealed that the quinolin-2(1H)-one Shiff bases are biologically active and their Cu(II)complexes showed significantly enhanced activity against the *Candida albican* strains in comparison to the free ligands.

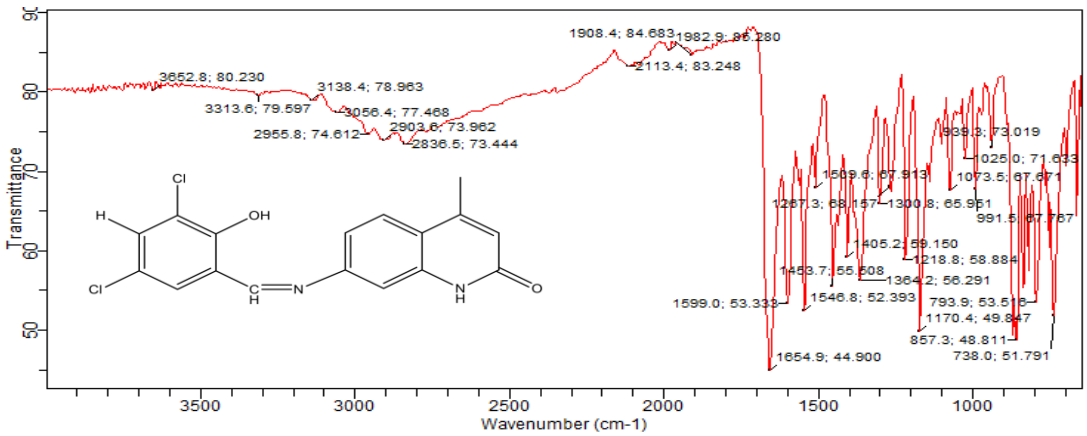


Figure 1: IR spectrum of Schiff base 1

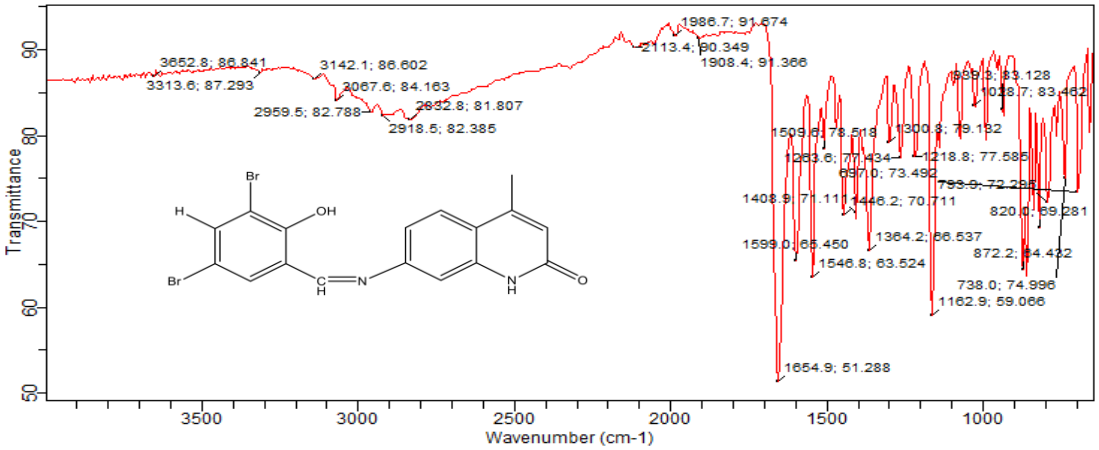


Figure 2: IR spectrum of Schiff base 2

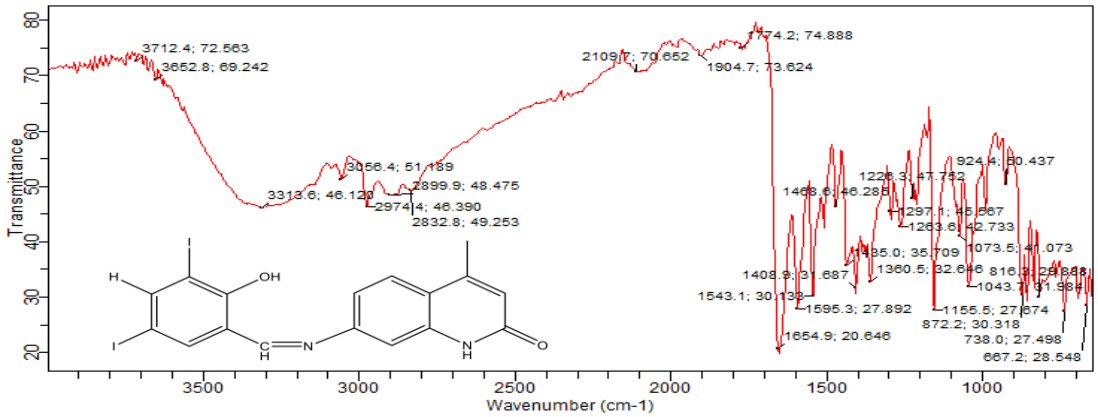


Figure 3: IR spectrum of Schiff base 3

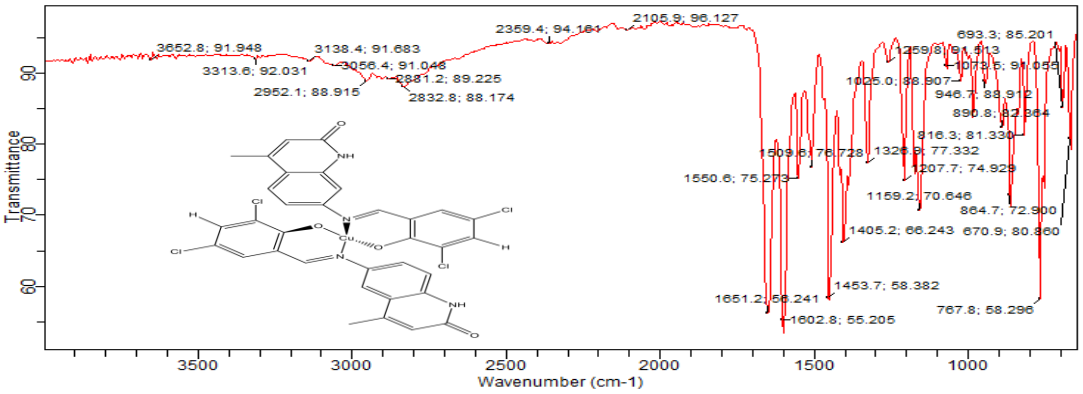


Figure 4: IR spectrum of complex 1

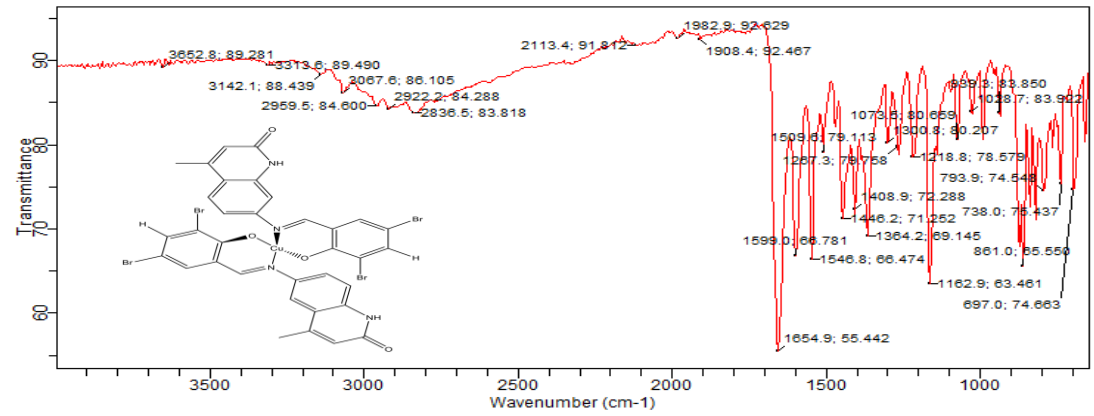


Figure 5: IR spectrum of complex 2

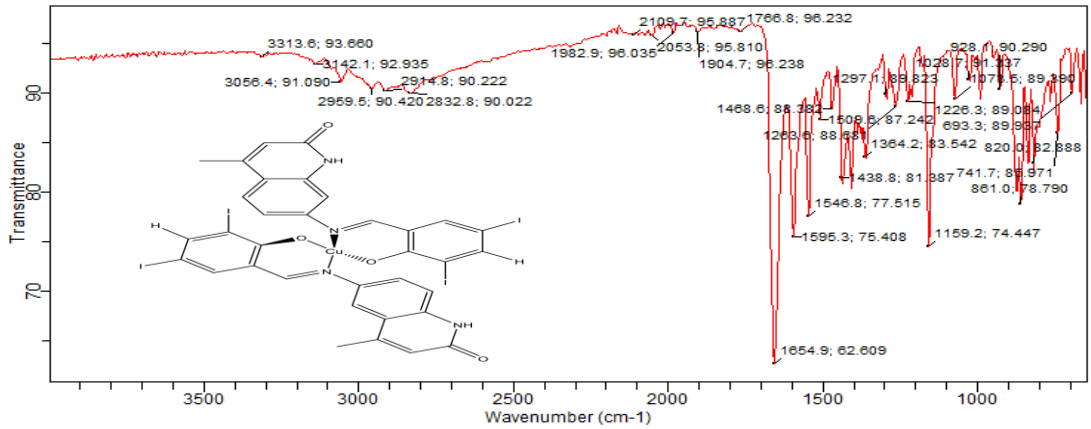


Figure 6: IR spectrum of complex 3

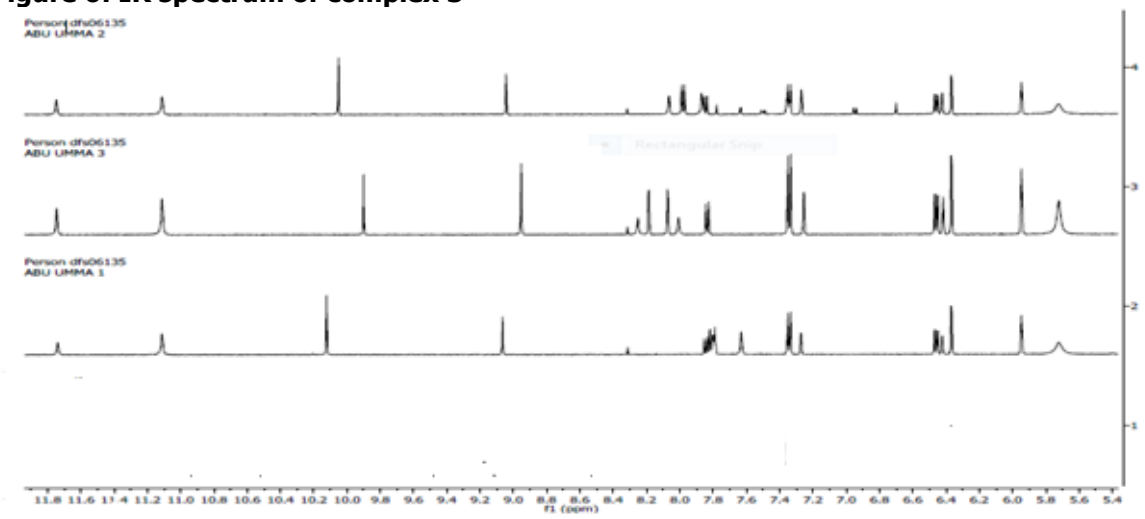


Figure 7: Stacked H-NMR of Schiff bases 1-3

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