Dutse Journal of Pure and Applied Sciences (DUJOPAS) Vol. 4 No. 1 June 2018

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF SCHIFF BASE DERIVED FROM SULFAMETHOXAZOLE AND ISATIN AND ITS Co(II), Cu(II) AND Zn(II) COMPLEXES

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Abstract

Schiff base from the condensation of sulfamethoxazole andisatin and its complexes with Co(II) Cu(II) and Zn(II) have been synthesized and characterized by elemental analysis, magnetic measurement and spectroscopic analysis. Infrared spectra of the Schiff base and the complexes agree with the formation and chelation of the Schiff base through the azomethine nitrogen and ketonic oxygen. Conductance measurement (1.64 – 30.50 Ohm^{-1} cm^2 mol^{-1}) suggest the non-electrolytic nature of all the complexes. Magnetic susceptibility analysis results were found to be 1.94 and2.67BM for Cu (II) and Co (II) complexes respectively. Elemental analysis suggested the ligand-metal ratio to be 2:1. Solubility test showed that all complexes and Schiff base are soluble in DMF and DMSO but insoluble in ether and other non-polar solvents. Decomposition temperature (165^{O}C – 215^{O}C) of the complexes were high which implied their stability. The Schiff base and its metal chelates have been screened for their antimicrobial activity against six pathogenic microbes(Staphyloccus aureus, Escherichiacoli, Salmonella typhi, Aspergillus flavus, Aspergillus niger and Mucorindicus). The Schiff baseshowed mild activity, whereas the complexes show higher antimicrobial activity against the tested isolates.

Keywords: Schiff base, sulfamethoxazole,synthesis,characterization, antimicrbial activity

INTRODUCTION

Schiff bases are condensation products of ketones or aldehydes with primary amines and have a great zeal of forming stable complexes with most of the transition metals(Lee, 1996).

Their great versatility, ease of formation and wide range of application make its chemistry still very relevant to the present researchers. In bioinorganic chemistry, the interest in the Schiff base complexes is as resultsofits enormous contribution to the development of medicinal chemistry (Kumble*et al*., 2017). The azomethine (-C=N-) linkage is essential for biological activity and several compounds containing the azomethine functional group have been reported to possess remarkable antibacterial, antifungal, anticancer, antiviral, antimalarial activity (Arulmurugan*et al*., 2010;Annapoorani and Krishman, 2013). In addition, some of the Schiff base complexes containing N and O donor atoms are effective as stereospecific catalysts for oxidation, reduction, hydrolysis, and other transformations of organic and inorganic compounds(Gupta and Sutar, 2008).

A large number of sulfa drugs derivatives were synthesized, characterized and tested for antimicrobial activity (Fatima, 2015;Abdulbaset and Ahmad 2014; Zahid *et al*., 2012). This might be due to their remarkable usage in the treatment of a wide range of bacterial infections (Brumfit and Hamilton, 1993). Incooperation of another group into the sulfa drug chain may enhance its performance and improves its resistance towards many bacterial strains. Furthermore, a lot of research have showed that addition of metal through chelation substantially improves the activity of the drugs towards pathogens (Jurca et al., 2017;Weaver *et al*., 2016). In this paper we presented the synthesis and characterization of a Schiff base of sulfamethoxazole and isatin and its Co (II), Cu (II) and Zn (II) complexes. In addition, we also report the *in vitro* antibacterial and antifungal screening activities of the Schiff base and the complexes obtained.

EXPERIMENTAL

All reagents and solvents used were of analytical grade and were used without further purification. Glasswares used were washed with detergent, rinsed with distilled water and dried in an oven at 110^{o}C before use. All weighing was carried out on an electric Metler balance model H3OAR, melting points/decomposition temperature were determined using Galenkempmelting point apparatus. Molar conductance measurements were carried out in DMSO using Jenwayconductivity meter (4010 model). Magnetic Susceptibility Measurement was conducted using Magnetic Susceptibility Balance (MK1 model), infrared spectral analyses were recorded using a fourier transform IR Genesis series model in the range of 650-4000cm^{-1}. Elemental analysis was carried out using D5201 test method at Petroleum Industry Laboratories Khartoom, Sudan. Antimicrobial activity test was conducted at the Department of Microbiology, Bayero University Kano.

Synthesis of Schiff base

Isatin (3.83 g, 0.01mol) and Sulfurmethoxazole (2.53g, 0.01mol) solution in 50cm^{3} ethanol was refluxed for three hours. The reaction mixture was cooled for 24 hours. Precipitate was obtained which was filtered, washed with distilled water and diethyl ether and dried in desiccator over $CaCl_2$ for 5 days (Suraj*et al*., 2012).

Preparation of Metal (II) Complexes

Metal(II) salt (0.005mol) were mixed with the ligand (0.01mol)in ethanol (50cm^3) and refluxed for five hours. The reaction mixture was cooled for 24 hours and the solid obtained was filtered, washed thoroughly with ether and dried in a desiccator over $CaCl_2$ for 7 days (Suraj *et al*., 2012).

Antibacterial Test

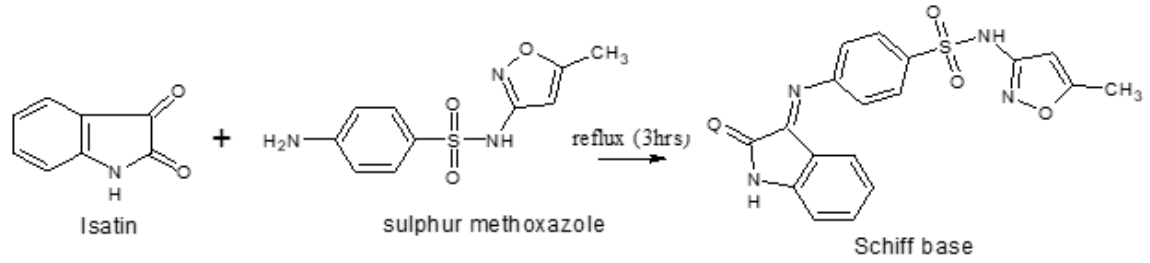
Clinical isolates of *Staphylococcus aureus, Escherichia coli,*and *Salmonella typhi*bacteria were obtainedfrom the cultures collection of Microbiology Laboratory, BayeroUniversity Kano, Nigeria and were identified using standard microbiological procedures described by Cheesbrough, 2002. The *in vitro* antibacterial activity was determine using Kirby-Bauer disc diffusion assay (CLSI, 2006).The inoculum was prepared by suspending overnight bacterial culture in saline solution (0.85%NaCl) and diluted to match the 0.5(10^8cells/mL) McFarland turbidity standard. The prepared inoculum was streaked with sterile cotton swab on to the surface of the nutrient agar (Yusha'u and Sadisu, 2011). The Schiff base ligand and its complexes were dissolved separately in DMSO to have three different concentrations of 200µg/ml, 300µg/ml and 400µg/ml. They were each transferred onto sterile paper disks (6.0 mm diameter). Commercial antibiotic (Amoxicillin) was used as a reference standard. The discs were placed onto the bacterial culture and growth inhibition zones (in mm) around the discs were observed and measured after 24 hours of incubation at 37^{o}C.The diameter of the zone of inhibition produced by the ligands and the complexes were compared with the standard Amoxillin drug (Yusha'u and Sadisu, 2011).

Antifungal Test

Clinical isolates of *Aspergillus flavus, Aspergillus niger and Mucorinducus*fungi were obtainedfrom the cultures collection of Microbiology Laboratory, BayeroUniversity Kano, Nigeria. The isolates were identified using standard microbiological procedures described by Cheesbrough, 2002. The *in vitro* antifungal activity was determined using Kirby-Bauer disc diffusion assay (CLSI, 2006).The inoculation method was as described by Hassan *et al*., (2006). The prepared inoculum was rubbed onto the surface of solidified Potato Dextrose Agar (PDA) already poured into Petri dishes. The ligand and its complexes were dissolved separately in DMSO to have three different concentrations of200µg/ml, 300µg and 400µg/ml. They were placed on the surface of the culture media (potatoes dextrose agar) and incubated at room temperature for 48hrs. The diameter of zone of inhibition produced by the ligand and the complexes were compared with the standard antifungal Ketoconazole as reference (Hassan *et al*., 2006).

RESULTS AND DISCUSSION

Refluxingsulfamethoxazole and isatinafforded the targeted Schiff base in good yield (Scheme 1). It was found to be yellowcoloured which is clearly different from the colours of the sulfamethoxazole and the isatin starting material.This gave initial hope for the formation of the Schiff base. Reacting the prepared Schiff base with the respective Co(II), Cu(II) and Zn(II) salts gave the corresponding metal (II) complexes. They showed different variety of colours which also differ from that of the Schiff base (Table 1).



Scheme 1: Preparation of the Schiff base

The complexes were found to be of high yield and good thermal stability (186-215^{o}C), they were air and thermally stable, soluble in DMSO, DMF and acetone but insoluble in diethyl ether and the other non-polar solvents. Though the Schiff base is insoluble in CCl_4, the complexes were found to be slightly soluble indicating the polarity of the complexes. Generally, the compounds solubility improves with complexation, as the complexes are more soluble in polar solvents than the Schiff base.

Table 2 present electrical conductance measurement results of the complexes conducted in DMSO at 10^{-3}M using a procedure reported by Geary,(1971).The values are within the range of 1.64 to 30.50 Ohm^{-1} cm^2 mol^{-1} which are very low for the complexes to be conducting as such they are allnon-electrolytic(Geary,1971). The results therefore indicated that no ions are present outside the coordination sphere. However, two chloride ions were observed inside the coordination sphere in addition to the Schiff base ligand. The results are in good agreement with the elemental analysis data were chlorides ions were detected after degradation of these complexes using nitric acid and then precipitation using silver nitrate reagent. Similar observation and conclusion was reported by Fatima, (2015).

Table1: Physical Properties of Ligand and its Metal (II) Complexes

Compound	Colour	Decomposition temp (^{o}C)	Melting Point (^{o}C)	PercentageYield(%)
L	Yellow	-	121	90.70
$[CoL_2Cl_2]$	pink	215		66.23
$[CuL_2Cl_2]$	Bole	198		69.58
$[ZnL_2Cl_2]$	Off white	186		71.40

L = ($C_{18}N_4H_{14}O_4S$)

Table2: Molar Conductivity test of Metal (II) Complexes

Complex	Concentration(Mol dm^{-3})	Specific conductance (Ohm^{-1} cm^{-1})	Molar conductance Ohm^{-1} cm^2 mol^{-1}
$[CoL_2Cl_2]$	1 $X10^{-3}$	30.50 x 10^{-6}	30.50
$[CuL_2Cl_2]$	1 $X10^{-3}$	25.20 x 10^{-6}	25.20
$[ZnL_2Cl_2]$	1 $X10^{-3}$	1.64 x 10^{-6}	1.64

L = $(C_{18}N_4H_{14}O_4S)$

Room temperature magnetic susceptibility measurement results is presented in Table 3. The data revealed that Co (II) and Cu (II) complexes were paramagnetic while Zn(II) complexes was diamagnetic.The magnetic moment values of the Co (II) complex (2.67 BM) and Cu (II) complex (1.94 BM) were found to be within their corresponding range of values reported in the literature for octahedral complexes(Angelici, 1977).

Table 3: Magnetic Susceptibility value of the Metal (II) Complexes

Compound	Magnetic Susceptibility (cm^3 g^{-1})	Molar Magnetic Susceptibility (cm^3 mol^{-1})	B.M(μ_{eff})
$[CuL_2Cl_2]$	2.88 x 10^{-6}	2.65 x 10^{-3}	1.94
$[CoL_2Cl_2]$	3.40 x 10^{-6}	3.10 x 10^{-3}	2.67
$[ZnL_2Cl_2]$	Diamagnetic	Diamagnetic	Diamagnetic

L = $(C_{18}N_4H_{14}O_4S)$

The IR spectral data of the Schiff base showed vibrational peak at 1639 cm^{-1} which was absent in the spectra of both sulfamethoxazole and isatin, further the spectral peak attributed to the amine of the sulfamethoxazole was observed to disappear in the spectra of the Schiff base.This new peak in the spectra of the Schiff base at 1639 cm^{-1} can be attributed to the newly formed azomethine ν(C=N) vibrational peak. In addition to the azomethine peak there is a peak at 1730 cm^{-1} which correspond to carbonyl peak in the Schiff base, However, these bands (azomethine and carbonyls) had been shifted in the complexes to 1648 – 1655 cm^{-1} and 1704– 1737cm^{-1} respectively, indicating their participation in the formation of the complexes. Absorption bands in the range 750 to 761 cm^{-1}observed in the complexes were attributed to metal – nitrogen (M-N) bond confirming metal – nitrogen bond formation (Table 4).

Table 4: IR Spectral Data for Ligand and its Metal (II) Complexes

Compound	ט(C=N) cm^{-1}	ט(C=O) cm^{-1}	ט(M-N) cm^{-1}
L	1639	1730	-
$[CoL_2Cl_2]$	1650	1704	756
$[CuL_2Cl_2]$	1655	1732	750
$[ZnL_2Cl_2]$	1648	1737	761

L = $(C_{18}N_4H_{14}O_4S)$

Table 5: Elemental analysis (CHN) of the Ligand and it's metal (II) complexes.

Compound	C Observed (calculated)	H Observed (calculated)	N Observed (calculated)
L	56.23 (56.50)	4.007 (3.66)	14.53 (14.62)
$[CoL_2Cl_2]$	47.25 (47.27)	3.252 (3.28)	12.35 (12.25)
$[CuL_2Cl_2]$	47.93 (47.03)	3.243 (3.66)	12.18 (12.19)
$[ZnL_2Cl_2]$	46.84 (46.94)	3.243 (3.26)	12.15 (12.19)

L = $(C_{18}N_4H_{14}O_4S)$

The elemental analysis(CHN) of the Schiff base and its metal(II) complexes were determined to find the appropriate metal to ligand ratio of the compounds.The values obtained showed an excellent agreement with the calculated values for the corresponding elements and the result (Table 5) suggested 1:2 metal – Schiff base ratio in all the complexes which matched the expected formulation of the Schiff base and the complexes (Figure 1).

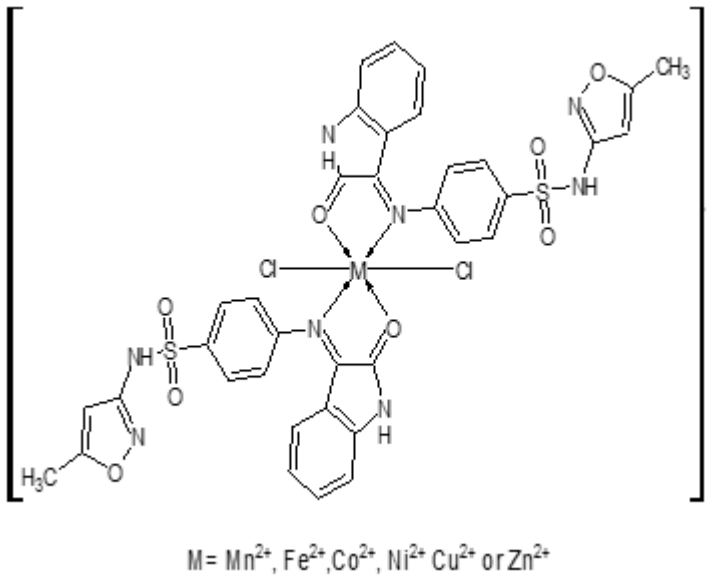


Figure 1. The proposed molecular structure of the metal(II) Schiff base complexes

The antibacterial activity of the Schiff base and the metal (II) complexes have been determined and the results presented in Table 6. The diameter of inhibition zone(mm) was measured for each treatment. Inhibition zone of Amoxicillin was also determined as positive control. *Staphylococcus aureus* was found to be resistant to the Schiff base at all concentrations, Cu (II) complex recorded the highest activity against the isolates which improves with increase in concentration.*Escherichia coli*was found to be resistive to Co(II) complex at all concentration while the other complexes Cu (II) and Zn (II) showed mild activity.*Salmonella typhi*was found to be prone to attack by all the compounds including the Schiff base. All the compounds were found to have activity lower than that of the amoxicillin even at higher concentration.

Table 6: Antibacterial Activity of Ligand and its Metal (II) Complexes

	Staphylococcus aureus			*Salmonella typhi*			*Escherichia coli*		
	Zone of Inhibition (mm)			**Zone of Inhibition (mm)**			**Zone of inhibition (mm)**		
Compounds	**200 (µg/ml)**	**300 (µg/ml)**	**400 (µg/ml)**	**200 (µg/ml)**	**300 (µg/ml)**	**400 (µg/ml)**	**200 µg/ml**	**300 µg/ml**	**400 µg/ml**
L	06	06	06	06	07	09	07	08	10
$[CoL_2Cl_2]$	10	11	13	09	10	12	06	06	06
$[CuL_2Cl_2]$	10	13	16	10	11	13	06	10	12
$[ZnL_2Cl_2]$	10	12	14	07	09	11	08	10	13
Ampicillin	-	30	-	-	30	-	-	34	-

L = $(C_{18}N_4H_{14}O_4S)$

The Schiff base showed strong activity on *Mucorinducus* and *Aspergillusflavus,* but no activity was observed against *Aspergillus niger*. Cobalt(II) complex showed good activity against all the fungal speciestested replicating its strong antibacterial activity. Cu(II) and Zn (II) complexes were found to be inactive against *Aspergillusflavus*but mildly active against *Aspergillus niger* and *Mucorindicus* at higher concentrations. Though, some of the compound were active, none matched the activity of the ketoconazole antifungal used as positive control (Table 7).

Table 7: Antifungal activity of ligand and its metal(II) complexes

	Aspergillus flavus			*Aspergillusniger*			*Mucorindicus*		
	Zone of Inhibition (mm)			**Zone of Inhibition(mm)**			**Zone of inhibition (mm)**		
Compounds	**200 (µg/ml)**	**300 (µg/ml)**	**400 (µg/ml)**	**200 (µg/ml)**	**300 (µg/ml)**	**400 (µg/ml)**	**200 µg/ml**	**300 µg/ml**	**400 µg/ml**
L	7	9	11	6	6	6	10	12	14
$[CoL_2Cl_2]$	9	10	12	7	8	10	9	10	12
$[CuL_2Cl_2]$	6	6	6	6	6	10	6	6	10
$[ZnL_2Cl_2]$	6	6	6	7	9	10	6	8	9
Ketoconazole		28			31			28	

L = $(C_{18}N_4H_{14}O_4S)$

CONCLUSION

Schiff base derived from the reaction of sulfamethoxazole and isatin and its metal complexes with Co (II), Cu(II) and Zn (II) ions have been prepared and characterized. Infrared, conductivity, magnetic moment and other studies indicated that the metal (II) ions are coordinated to the azomethine-nitrogen and carbonyl oxygen. Elemental analysis data revealed 2:1 ligand to metal ration and in good agreement with the expected empirical formula of the compounds. The antimicrobial data revealed that both Schiff base and the

complexes were active against the isolates tested which improves upon complexation. Furthermore, the activity of both Schiff base and the complexes increase with increase in concentration, but they were all lower than that of the reference antibacterial, amoxicillin and antifungal ketoconazole used as positive control.

REFERENCES

Abdulbaset A.E., Ahmad M.A. (2014) Preparation, characterization and biological evaluation of Schiff base of some drug substances. *Journal of medical and Bioengineering*.3(3),185-189

Angelici, R.J. (1977): "*Synthesis and Techniques in Inorganic Chemistry*",2nd edition, W.B Savders Company, pp 115-125

Annapoorani S., Krishnan C., (2013); Synthesis and spectroscopic studies of trinuclear N4 Schiff base complexes. International Journal of Chemistry and Technical Research, 5(1): 180-185

Arulmurugan S., Helen P. K., Venkatraman B.R., (2010); Biological activities of Schiff base and itscomplexes: A Review. *Rasayan Journal of Chemistry* 3(3) 385-410

Brumfit W. and Hamilton M.(1993), reassessment of the rationale for the combination of sulphonamides with diaminopyrimides, *journal of chemotherapy*, 5(6): 465-470

Cheesbrough, M. (2002). "*District Laboratory Practice in Tropical Countries*". Part 2. Cambridge University Press, UK. 132-143. Debnath

Clinical and Laboratory Standards Institute (CLSI). (2006). *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Ninth Edition*; CLSI document M2-A9; CLSI: Wayne, PA, USA,

Fatima A. J. A., (2015); Ca (II) and Au (II) sulfamethoxazole sulfa-drug complexes: Synthesis, spectroscopic and anticancer evaluation studies. *Oriental Journal of Chemistry* 31(3);1277-1285

Geary WJ (1971). The use of conductivity measurements in organic solvents for characterization of coordination compounds, *Coordination Chemistry Review.* 7(1):81-122

Gupta, K. C. and Sutar, A. K. (2008); Catalytic activities of Schiff base transition metal complexes. *Coordination Chemistry Reviews* 252 (12), 1420-1450

Hassan, S. W., Umar, R. A., Lawal, M., Bilbis, L. S. and Muhammad, B. Y. (2006); Evaluation of antifungal activity of *Ficussycomorus* (moraceae). *Biological and Environmental Science Journal for the Tropics* 3(2):18-25.

Jurca T., Eleonora M., Gratiela L. V., Eugenia M. M., Luminata F. (2017); *Metal Complexes of Pharmaceutical Substances*, Spectroscopic Analyses – Developments and applications, DrEramSharmin (Ed), Intech 123-139

Kumble D., Geetha M. P., and Asha F. P. (2017): application of metal complexes of Schiff base as an antimicrobial drug: A review of recent works. *International Journal of current pharmaceutical Research* 9(3); 27-30

Lee J. D. (1996), *Concise Inorganic Chemistry* Oxford University Press, 5th edition, Pp 800-834

Suraj B.A. Deshpande M.N. and Deshmukh J.H. (2012), Synthesis and Characterization of transition metal complexes of Schiff base derived Isatin and 2-amino, 4-chlorobenzoic acid. *Rayasan J. Chem* 4(13): 671 – 673.

Weaver G. W., Elsegood M. R., Tariq M., Amina M. (2016); synthesis and characterization of new Schiff base transition metal complexes derived from drug together with biological potential. *Journal of Nuclear Medicine and Radiation Therapy*7(6):1-4.

Yusha'u M. and Sadisu F.U (2011): Inhibition activity of deuterium microcarpum extracts on some clinical isolates. *Biological and Environment Science Journal for the Tropics* 8(4): 113-117.

Zahid H. C., Hazoor A. S., Claudiu T. S (2012) Synthesis, characterization and biological studies of sulfonamide Schiff bases and some of their metal derivatives, *Journal of Enzyme Inhibition and Medicinal Chemistry*, 27(1):58-68