SYNTHESIS, CHARACTERIZATION AND *IN VITRO* BIOLOGICAL STUDIES OF METAL SCHIFF BASES

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January 2016

DECLARATION BY CANDIDATE ON PLAGIARISM

I Ikechukwu Peter Ejidike, declare that:

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This is to certify that this research is a record of original work carried out by Ikechukwu Peter Ejidike under my supervision in the Inorganic Materials Research laboratory of the Department of Chemistry, University of Fort Hare in fulfilments of the requirements for the award of Doctor of Philosophy in Chemistry.

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DEDICATION

This thesis is dedicated to the King of Kings, Lord of Lords and the Rose of Sharon.

And to my most precious Jewels– Chief & Mrs. Nelson Edna EJIDIKE, My Siblings.

All that've been, and in all I'll ever be

You've been my guide and my first teacher

In all, I owe you for giving me the best

You are my most precious jewels

I love you Dad and Mum

The best parents in the world

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ABBREVIATIONS AND SYMBOLS

SBs	Schiff bases
NAMI-A	Imidazolium trans-[tetrachloro-(*S*-dimethylsulfoxide)(1*H*-imidazole)ruthenate(III)]
KP-1019	Indazolium trans-[tetrachlorobis(1*H*-indazole)ruthenate(III)]
ROS	Reactive Oxygen Species
H_2O_2	Hydrogen peroxide
O^{2-}	Superoxide radical anion
HO^{-}	Hydroxyl radical
H_2L	*N*-(5-chloro-2-hydroxyphenyl)-3-methoxy-salicylaldimine
BPMC	Benzofuran[phenylmethine]carbohydrazone
BDMeOPMC	Benzofuran[3,4-dimethoxyphenylmethine]carbohydrazone
HMAE	2-hydroxy-5-methylacetophenone-*N,N′*-ethylenediamine
TCNH	(*E*)-*N′*-(thiophen-2-ylmethylene)nicotinohydrazide
DHA	Dehydroacetic acid (3-acetyl-6-methyl-(2*H*) pyran-2,4(3*H*)-dione
BHMePC	Benzofuran-2 carbohydrazide-5-methylsalicylaldehyde
BHClPC	Benzofuran-2 carbohydrazide-5-chlorosalicylaldehyde
NLO	Nonlinear optical properties
CDHBPC	3-(4-5-(4-chlorophenyl)diazenyl)-2-hydroxybenzylideneamino) phenylimino)methyl)-4*H*-chromen-4-one
CPDA2HB	5-(4-chloro-phenylazo)-2-hydroxybenzaldehyde
4APF	4-[(Furan-2-ylmethylene)-amino]-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one

APMOC	3-[(4-Aminophenylimino) methyl]-4-oxo-4*H*-chromene
CDHBAP	4-((*E*)-4-((*E*)-(4-chlorophenyl)diazenyl)-2-hydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one
NMO	N-methylmorpholine-N-oxide
BPPD	Bis(2-(pyridin-2-ylimino)phenyl)-4,4'-(diazene-1,2-diyl) dibenzoate
XRD	X-ray diffraction
FRAP	Ferric reducing antioxidant power
HL	*N*-(2-hydroxylacetophenone)-3-oxapentane-1,5-diamine
MIC	Minimum Inhibitory Concentration
$(HS)_2T$	*N,N'*-bis(salicylidene)thiourea
HL_1	4,4'-{(2-hydroxy-5-isopropylbenzene-1,3-diyl) bis[methylidenenitrilo]}-bis(2,6-di-tert-butylphenol)
HL_2	4,4'-{(2-hydroxy-5-tertbutylbenzene-1,3-diyl) bis[methylidenenitrilo]}bis(2,6-di-tert-butylphenol)
H_2LL	(4*E*)-4-[(2-{(*E*)-[1-(2,4-dihydroxyphenyl)ethylidene] amino}ethyl)imino]pentan-2-one
H_5L	Naringenin-2-hydroxybenzoyl hydrazine
DNA	Deoxyribonucleic acid
CT-DNA	Calf thymus-Deoxyribonucleic acid
BSA	Bovine serum albumin
FBS	Fetal bovine serum

CD	Circular dichroism
PDN	*N*-(1-phenyl-2-hydroxy-2-phenylethylidine)-2',4'-dinitrophenyl hydrazine
PHP	*N*-(1-phenyl-2-hydroxy-2-phenylethylidine)-2'-hydroxyphenyl imine
HHP	*N*-(2-hydroxybenzylidine)-2'-hydroxy phenyl imine
Dpmp	2,6-di((phenazonyl-4-imino)methyl)-4-methylphenol
Caov-3	Ovarian cancer cell line
HL-60	Leukemic cell line
MCF-7	Human breast carcinoma cell line
HT-29/ HCT116	Colon carcinoma
HEPG2	Liver carcinoma
Hep-G2	Human hepatic carcinoma cell line
TK-10	Human renal cancer cell line
UACC-62	Human melanoma cancer cell line
HeLa	Human cervical cancer cell line
SRB	Sulforhodamine B
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
IC_{50}	Inhibitory Concentration 50%
DPPH	1,1-Diphenyl-2-picrylhydrazyl
ABTS	2,2'-azino-bis-(-3-ethylbenzothiazoline-6-sulfonic acid)
BHT	Butylated hydroxyl toluene
BHBDAE	*N,N′*-bis(2–hydroxybenzylidene)–1,1–diaminoethane

MSOPD	*N,N′*-bis(3-methylsalicylidene)-ortho-phenylenediamine ligand
DMF	*N,N′*-dimethylformamide
DMSO	Dimethylsulfoxide

RESEARCH OUTPUTS

1. **Ejidike, I. P**.; Ajibade, P. A. Synthesis, characterization, *in vitro* antioxidant and anticancer studies of ruthenium(III) complexes of symmetric and asymmetric tetradentate Schiff bases, *Journal of Coordination Chemistry* **2015**, *68*, 2552-2564.

2. **Ejidike, I. P**.; Ajibade, P. A. Transition metal complexes of symmetrical and asymmetrical Schiff bases as antibacterial, antifungal, antioxidant, and anticancer agents: progress and prospects. *Reviews in Inorganic Chemistry* **2015**, *35*, 191-224.

3. **Ejidike, I. P**.; Ajibade, P. A. Synthesis, characterization, antioxidant, and antibacterial studies of Some metal(II) complexes of tetradentate Schiff base ligand: (4*E*)-4-[(2-{(*E*)-[1-(2,4-Dihydroxyphenyl)ethylidene]amino}ethyl)imino]pentan-2-one. *Bioinorganic Chemistry and Applications* **2015**, *2015*, 890734, 1-9.

4. **Ejidike, I. P**.; Ajibade, P. A. Synthesis, characterization and biological studies of metal(II) complexes of (3*E*)-3-[(2-{(*E*)-[1-(2,4-dihydroxyphenyl)ethylidene]amino} ethyl)imino]-1-phenylbutan-1-one Schiff base. *Molecules* **2015**, *20*, 9788-9802.

5. **Ejidike, I. P**.; Ajibade, P. A. Ruthenium(III) complexes of heterocyclic tridentate (ONN) Schiff base: Synthesis, characterization and its biological properties as an antiradical and antiproliferative agent. *International Journal of Molecular Science* **2016**, *17*, 60; doi:10.3390/ijms17010060.

6. **Ejidike, I. P**.; Ajibade, P. A. Synthesis and *in vitro* anticancer, antibacterial, and antioxidant studies of unsymmetrical Schiff base derivatives of 4-[(1*E*)-*N*-(2-aminoethyl)ethanimidoyl]benzene-1,3-diol. *Research on Chemical Intermediates* **2016**, pp 1-13, doi: 10.1007/s11164-016-2479-x.

MANUSCRIPTS SUBMITTED FOR PUBLICATION

1. **Ejidike, I. P**.; Ajibade, P. A. Synthesis, Spectroscopic, Antibacterial and Free radical scavenging studies of Cu(II), Ni(II), Zn(II) and Co(II) complexes of 4,4'-{ethane-1,2-diylbis[nitrilo(1*E*)eth-1-yl-1-ylidene]}dibenzene-1,3-diol Schiff base. (Journal of Chemistry, Submitted)

2. **Ejidike, I. P**.; Ajibade, P. A. Synthesis, Characterization, Anticancer and Antioxidant studies of Ru(III) complexes of monobasic Tridentate Schiff bases (Bioinorganic Chemistry and Applications, Submitted)

PRESENTATION IN CONFERENCE

Ejidike, I. P.; Ajibade, P. A. Zn(II), Cu(II), Co(II) and Ni(II) Complexes of Unsymmetrical Tetradentate Schiff Base as possible Antioxidant Agents. 17th South African Chemical Institute Inorganic Chemistry Conference, Rhodes University, Grahamstown, South Africa. 28 June – 2 July 2015.

ABSTRACT

Transition metal complexes of symmetrical and asymmetrical Schiff bases have played a significant role in the field of coordination, inorganic and bioinorganic chemistry as models for biological, analytical and industrial applications. The thesis deals with the synthesis, characterization and *in vitro* biological studies of metal Schiff bases.

Three symmetrical and unsymmetrical tetradentate Schiff bases: [HLL^1, ehopd], [HLL^2, ehata], [HLL^3, ehbta] with the N_2O_2 chromophore, eight unsymmetrical tridentate Schiff bases: [HLL^4, ehmta] [HLL^5, ehben], [HLL^6, ehmez], [HLL^7, ehacp], [HLL^8, ehacn], [HLL^9, ehvan], [HLL^{10}, ehvet], [HLL^{11}, ehbzc] with the N_2O chromophore alongside their corresponding Ru(III), Zn(II), Cu(II), Ni(II) and Co(II) complexes were synthesized and characterized by elemental analyses, melting point/decomposition temperature FTIR and UV-Vis spectroscopy. The results of the spectroscopic studies revealed that the Schiff base ligands coordinated to metal ions through the (>C=N) nitrogen and phenolic oxygen atoms with evidence of new bands due to the ν(M-N) and ν(M-O) vibrations respectively in the spectra of metal complexes.

The antibacterial activity of the metal complexes, tetradentate and tridentate Schiff bases was screened against three Gram-positive bacteria, viz. *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus cereus* and three Gram-negative bacteria viz. *Pseudomonas aeruginosa*, *Escherichia coli*, and *Shigella flexineri*. The evaluation results revealed that the metal complexes exhibited higher antibacterial activity than the free Schiff base ligands. In addition, *in vitro* antioxidant activities of the compounds were also investigated through their scavenging effect on 1,1-Diphenyl-2-picrylhydrazyl (DPPH)

and 2,2′-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals. It was observed that Ru(III) and metal(II) complexes exhibited strong scavenging activities against DPPH radical and moderate activities against ABTS radical. The compounds were screened *in vitro* against three cancer cell lines: human renal cancer cell (TK-10), human melanoma cancer cell (UACC-62) and human breast cancer cell (MCF-7) using the SRB assay. The results demonstrated that treatment with the synthesized compounds affected cell viability efficiently toward MCF-7 cells.

Chapter One

Introduction

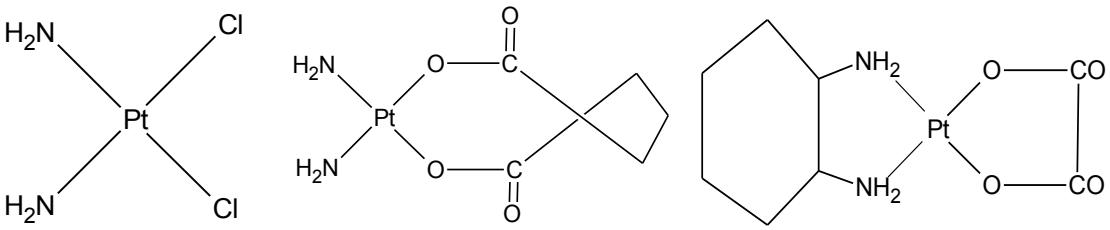
CHAPTER ONE

INTRODUCTION

1.1 Introduction to bioactive metal complexes

Recent advancement in inorganic chemistry has brought about the formation of a diverse metal complexes derived from various organic donor ligands for therapeutic uses. The earliest accounts on the usage of transition metal complexes as anti-cancer agents and leukaemia started in the 16th century [1]. Developing active transition metal anticancer complexes has attracted significant interests among the bio-inorganic chemists over the world, particularly, after the success of cisplatin **(1)**, cis-[$PtCl_2(NH_3)_2$] and carboplatin **(2)**, [$Pt(cbca)(NH_3)_2$] where cbda = 1,1-cyclobutanedicarboxylate (Figure 1.1), that have been more than 30 years in clinical use. In recent times, oxaliplatin **(3)**, [Pt(dach)(ox)] where dach = R,R-1,2-diaminocyclohexane and ox = oxalate (Figure 1.1), a third generation of Pt complex have been approved for clinical use [1, 2].

To date, more than 3000 platinum compounds have been prepared and tested in an attempt to reduce the toxicity of the cisplatin [3] and these interesting interaction associated to this type of complex, have motivated several researches [4-6]. The development of metal based drugs with pharmacological potential and unique therapeutic opportunities has been possible due to the fact that metals possess various oxidation states and the ability to interact with negatively charged molecules [7].



Cisplatin **(1)** Carboplatin **(2)** Oxaliplatin **(3)**

Figure 1.1: Structure of common Pt based anticancer compounds

1.2 Schiff bases

Schiff bases (SBs), named after Hugo Schiff (1834-1915), possess functional group containing a carbon-nitrogen double bond with the nitrogen atom linked to an aryl or alkyl group, with the exception of hydrogen [8]. Schiff base compounds are condensation products of primary amines and carbonyl compounds (aldehydes and ketones) and they were discovered by a German chemist and Nobel Prize winner, Hugo Schiff in 1864 [9, 10]. Schiff bases in a broad sense are compounds containing azomethine group (>C=N) and have the general structure $R^1R^2C=NR^3$ **(4)** (Figure 1.2) where R^1, R^2 and R^3 are aryl, alkyl, cycloalkyl or heterocyclic groups that are of different substitutes. Present day chemists still prepare diverse Schiff base ligands referred to as ‘‘fortunate ligands’’ [11].

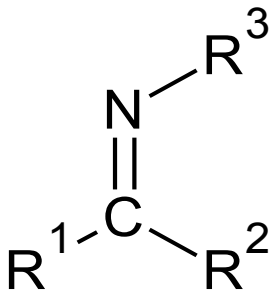


Figure 1.2: The general structure of Schiff base **(4)**

Presence of aryl substituents in Schiff bases usually ease the synthesis and stability of Schiff bases while Schiff bases containing alkyl are relatively unstable. The reactivity of aldehydes are generally faster than those of the ketones in condensation reaction, thereby resulting in the formation of Schiff bases with a centre that are less steric than the ketones, relatively unstable and freely polymerizable [12]. Schiff bases bonding ability depends on the nature of atoms that act as coordination site, such as N, O, and S, the electronegativity and steric factors. Schiff base acts as active ligands due to the presence of low electronegativity of nitrogen, N of the azomethine group (>C=N), lone pair of electrons on the nitrogen atom, electron donating character of the double bond [8], and thus bring about stability in metals several oxidation states, regulating metal activities for variety of useful biological, catalytic conversions.

1.3 Metal complexes of Schiff bases as model of bioactive compounds

The identification of novel bioactive metal complexes that play significant role in nature as illustrated by the operation of the giant molecules including haemoglobin, chlorophyll etc., have always been a challenge to the inorganic chemists since the nineteenth century. In biological and medical processes, inorganic elements contribute immensely, as organic compounds used in medicine are activated or bio-transformed by metal ions metabolism [13], because they do not have an estimable or identifiable mode of action. Hence, in natural biological systems, essential activities of metalloproteins and metalloenzymes are closely related to the hydrolysis of esters, formation of Schiff bases, carboxylation or decarboxylation, and catalytic activity in reactions like hydrogen exchange [14-16]. The recognition of Schiff base complexes as models for biologically active

compounds has brought rapid advancement within the field of coordination and bio-inorganic chemistry and spawned extensive research on their synthesis and applications [17-21].

Schiff's bases and their complexes continue to attract many researchers because of their wide applications in food industry, dye industry, analytical chemistry, catalysis, antimicrobial activity and pharmacological application like antitumor, antifungal, antibacterial, antimicrobial [8-11]. Schiff bases (SBs) are important intermediates for the synthesis of some bioactive compounds such as ß-lactams [22] and employed as ligands for the complexation of metal ions [23]. The strong attraction for the bonding of Schiff bases to inner and non-inner transition metal ions is essential in metal complex synthesis [24]. It can be juxtaposed that, the unsymmetrical Schiff base ligands have exhibited several benefits over the symmetrical equivalents within the area of configuration elucidation together with geometry prediction of the metal ions sites of binding in the metal-biological system and the choice of natural structures with synthetic constituents [25-27]. It has been confirmed that some Schiff bases show increased bio-activity when given out as metal complexes [28] and a number of metal chelates with anticancer activity have also been reported [29].

Aliphatic diamine compounds, such as ethylenediamine are useful intermediates for the syntheses of bi-, tri-, tetra-, and multidentate ligands chelating different metal centres [30-33]. The N_2O_2-, NNO-type ligand, usually obtained by the condensation reactions of diamine with various aldehydes, can coordinate to either one metal centre or several metal centres depending on the binding sites available or structural arrangement of the amino groups in the precursor [34-36]. Many symmetrical and unsymmetrical tetradentate Schiff bases of both aliphatic and aromatic diamines and dissimilar aldehydes/ ketones have been investigated and

reported [37-39]. Also, significant biological and physical behaviours of Schiff bases are linked to the hydrogen transfer equilibria and intermolecular hydrogen bonding [40].

1.4 Development of metal complexes as anticancer agents

Cancer is a range of disease characterized by out-of-control cell growth or tumour of cells coupled with malignant behaviour. This leads to a mass of eccentric cells that grow out of regulation. Predicated in the GLOBOCAN 2008 assessments, about 12.7 million cancer cases and 7.6 million cancer deaths are predicted to have happened in 2008; from these figures, 56% of the cases and 64% of the bereavements occurred in the developing world [41]. Most cancers have characteristics that reflect their origin, and those emanating from diverse cell types vary with different syndromes [42]. German chemist Paul Ehrlich, in the early 1900s, first documented the effectiveness of animal screens; a series of chemicals for their potentials against diseases, which in turn bring about cancer drug development [43].

The practise of chemotherapy for cancer treatment began in the twentieth century and aims at killing malignant tumor cells more or less selectively. Since then, there has been an important progress in the model development of cancer chemotherapy as shown in Figure 1.3. The use of chemotherapy had made a remarkable permissive effect in earlier stages of tumor treatment. They were aimed at killing malignant tumor cells more or less selectively, but close to the focal point of some strong tumors; cell division has successfully stopped, making them callous to chemotherapy [43].

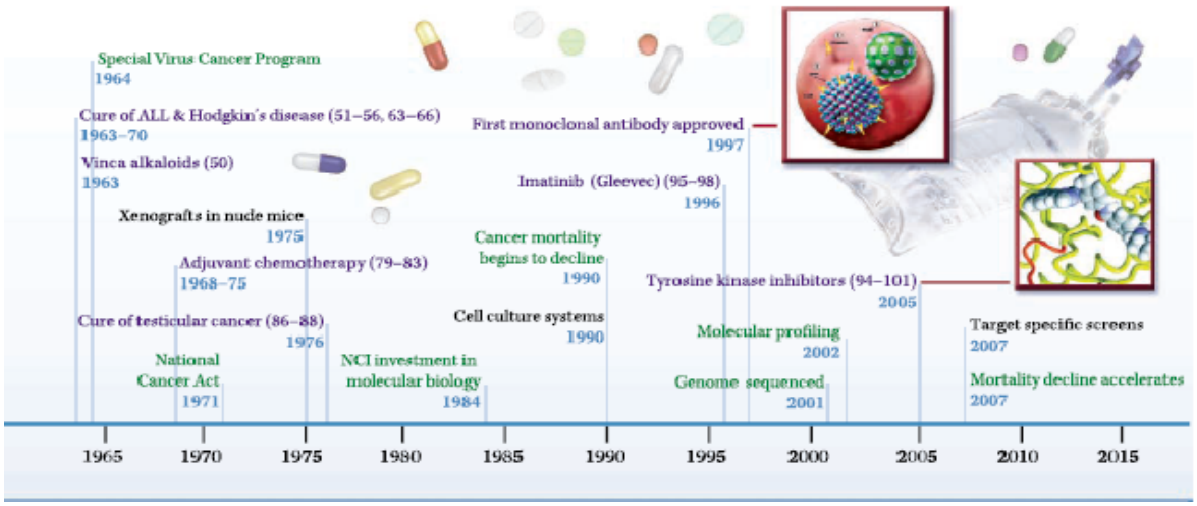


Figure 1.3: Key advances in the cancer chemotherapy in the years 1960-2015 [43].

Development of therapeutic agent with wider spectrum of activity and ability to overcome cancer resistance is the core focusing area of researchers. Alkylating agents are the most commonly used chemotherapeutic agents in the treatment of antineoplastic ailment because they possess a very wide spectrum of activity. Among this class are the cisplatin and its analogues: carboplatin and oxaliplatin which are in current clinical use and have been the widely studied class of chemotherapy due to their severe effect on human fertility [44].

With respect to the success of cisplatin as an important class of metal-based chemotherapeutic agent, alongside with the tremendous research efforts that have been invested into the development of analogous compounds over the past half-century, other metal-based anticancer drugs have been developed and approved for clinical use as antitumor agents [45]. Their mechanism of action was observed to be strongly related to their ability to bind to DNA and modify its structure, which leads finally to induction of apoptosis or necrosis [46].

Efforts have been directed towards designing compounds that are capable of displaying particular components of resistance and the configuration of whimsical Pt compounds with profoundly distinctive methods of activity [47]. Recent investigation has led to the discovery and development of the ruthenium-based anti-tumor complexes known as imidazolium trans-[tetrachloro-(*S*-dimethyl sulfoxide)(1*H*-imidazole) ruthenate(III)] (NAMI-A) and indazolium trans-[tetrachlorobis(1*H*-indazole)ruthenate(III)] (KP-1019), which are currently in clinical trials [45].

Bioactive iridium and rhodium complexes containing polyaromatic ligands have recently been reported as fascinating potential alternatives to the existing platinum and ruthenium metallo-drugs and highly active against a series of cancer cell lines. The polyaromatic and modifications of the ancillary ligands facilitate interactions with DNA and also confer increased hydrophobicity and altered redox properties to the resulting metal complexes [45].

1.5 Discovery of antibacterial agents, its resistance and mechanisms

In 1670's, Antoni van Leeuwenhoek identified bacteria for the first time with the aid of microscope he invented for the observations of muscle fibres, bacteria, spermatozoa, and blood flow in capillaries. But the link of these bacteria with disease was not known until nineteenth century. Robert Koch, a scientist in 1880's was able to elucidate the microorganisms that are responsible for diseases such as tuberculosis, cholera, and typhoid during the latter half of nineteenth century [48-49]. Paul Ehrlich, a Nobel Prize winner for his contributions to immunology, developed a field which he defined as chemotherapy— the use

of chemicals against infection in 1904 meaning chemicals at concentrations permitted by the host could interfere with the proliferation of microorganisms [48].

Purely synthetic antimicrobial drug, known as salvarsan, arsenic-containing compound demonstrated to be viable against the protozoal malady dozing disorder (*trypanosomiasis*), and the spirochaete ailment of *syphilis* was developed by Ehrlich in 1910 [50]. Until the introduction of penicillin in the 1940s by Florey and Chain, an outstanding agent in terms of safety and efficacy, saved the lives of many wounded soldiers during World War II [48, 51, 52], salvarsan and it's derivative neosalvarsan served as the frequently prescribed drug despite the adverse side effects [53]. Microorganisms, disease-causing types, have been able to develop resistance in order to fight back man's hostility which sought to deprive them of their territory using antimicrobial agents (Figure 1.4) [54].

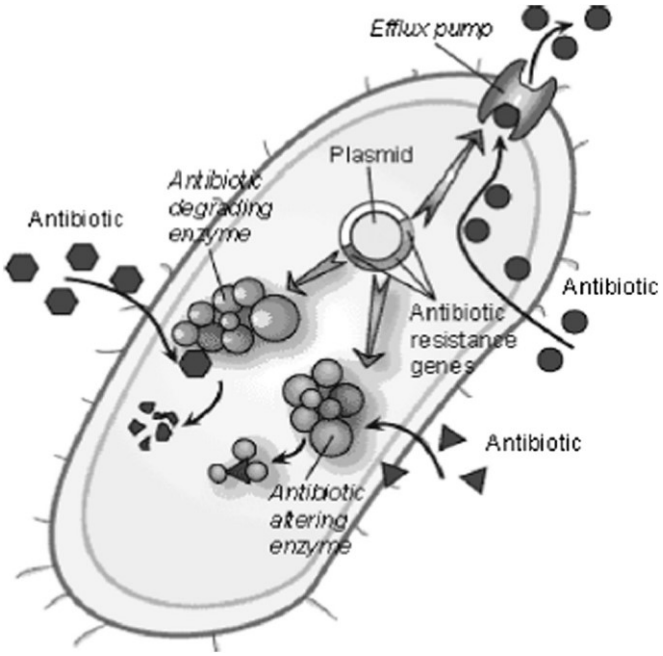


Figure 1.4: Diagram showing how some antimicrobial agents are rendered ineffective [54].

Many antibacterial agents have been developed and made available to control the spread of bacterial diseases such as syphilis, tuberculosis, bubonic epidemic, diphtheria, typhoid, gas gangrene, tetanus, gonorrhoea, leprosy [48]. At present, resistance to antimicrobial agents among bacteria, parasites, viruses and other disease-causing organisms has become a public health problem worldwide [55]. A continuous improvement towards antimicrobial agents in several facets with respect to the antimicrobial spectrum and activity has emerged towards achieving better pharmacodynamics in areas such as the absorption of oral drugs, concentration in the blood, and distribution to the inflammatory focus [52].

A major setback that affected the development of antibiotics and their application to clinical medicine has been the increase in bacterial resistance towards antibacterial drugs. This can be due to the use of antibiotics which in turn increases selective pressure in the bacteria population, thereby permitting the survival of the resistant bacteria and passing away of the susceptible ones [53]. The mechanisms as well as the chemical nature of the antimicrobial agents as a way to knowing how resistance against them develops [54]. Mechanisms by which antibacterial agents act can be categorized based on the bacterial structure or the function that is affected by the chemotherapeutic agents [54, 56] includes:

- Inhibition of cell metabolism
- Inhibition of nucleic acid transcription and replication.
- Inhibition of the bacterial cell divider amalgamation.
- Inhibition of ribosome function.
- Inhibition of folate metabolism.

1.6 Free radicals, metals and cancer prevention agents in oxidative anxiety organic frameworks

Therapeutic agents with antioxidant potentials have been studied for their ability to protect organism and cell from harm that is affected by oxidative anxiety. Free radicals interact with proteins in other normal metabolic processes, leading to the formation of many types of pathologic changes [57]. Free radicals refer to particles or atomic sections that contain more than one or its equivalent unpaired electrons. The existence of unpaired electrons typically brings about a level of reactivity upon a radical species [58]. Generation of reactive oxygen species (ROS) by ordinary cell digestion system and exogenous agents have been established [59]. ROS are produced under pathological conditions, thereby resulting in oxidative stress in living organism when endogenous antioxidant defences are inadequate [60-61].

Such radicals include: Superoxide radical anion (O^{2-}*); hydrogen peroxide (H_2O_2) framed in natural molecules by the halfway diminishing of sub-atomic oxygen and hydroxyl radical (HO*) believed to happen through the one-electron decrease of hydrogen peroxide (H_2O_2) [8]. The steady increase in the production of reactive oxygen species through either endogenous or exogenous reactions have been termed oxidative stress and this type of reaction is common to many types of cancer cell. They also take part in major physiological activities of intracellular signalling and regulation pathways (Figure 1.5) [62] and partly linked with the participation of redox-active metals [63].

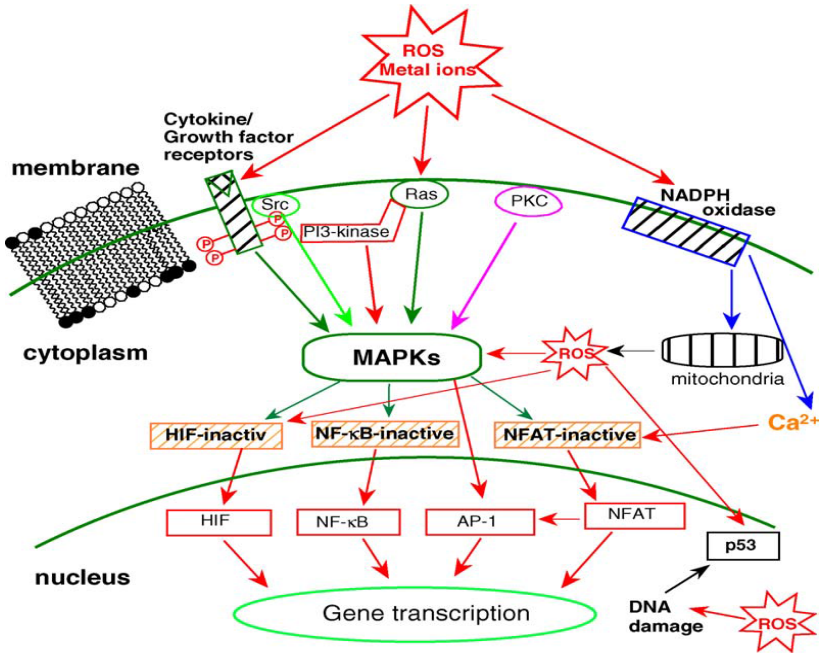


Figure 1.5: ROS and metal ions-induced signalling pathways [64].

Four groups to which free radicals found in biological systems may be assigned are:

- Small bioactive compounds radicals, like quinones and flavins
- Oxygen or dioxygen radicals: O^{*-} ; HO* ; O_2^{*-} ; HO_2*
- Analogs of oxygen or dioxygen radicals: RO_2*; where R is an alkyl group
- Radicals of amino acid residue, such as the tymsyl radical and the cysteinyl radical present in proteins
- In substrates, the radical intermediates, such as methane or an alcohol [65]

In order to protect the biological molecules against attacks of free radicals and/ or reduce the impact of oxidative stress on the cell, scientists in various disciplines have developed new compounds like antioxidants, natural and synthetic free radical scavengers [66].

Schiff base compounds and their metal complexes have been developed and studied as successful scavengers of ROS, acting as cancer prevention agents. Therefore, metal based

antioxidants (free radical scavengers) have received late consideration for their capacity to shield life forms and cells from harm actuated by oxidative anxiety [67]. This can be identified with the way that Schiff bases (SBs) give open doors for actuating substrate chirality, tuning of metal centre electronic variable, and improving of solubility and stability of homogeneous or heterogeneous promoters [68].

1.7 Rational and motivation

Problem of cell resistance to drugs has increased world's mortality rate due to various infectious diseases that are directly related to cancer cells resistance to chemotherapeutic agents, bacteria multiple resistances to antibiotics. Although cisplatin and its derivatives are currently used as anti-cancer drugs, they are not universal to all kinds of cancers [69]. There is need to develop novel chemotherapeutic agents for the treatment of diseases with excellent mechanisms of action and structural-activity relationship. Hence, efforts are being intensified towards the preparation of metal based chemotherapeutic agents with higher effectiveness, increased selectivity for tumor tissue, disease-causing organisms, lowered toxicity, wider spectrum of activity.

Schiff base ligands and their metal complexes have been established as potential biochemically active chemotherapeutic agents with antimicrobial, antiviral, antifungal, antiradical and anticancer properties [39]. Considerable attention is given to the study of Schiff bases with functionalization and modification in the chemical structure of the compound, changing the pharmacological activity of the agent, and in-turn improves the chemotherapeutic properties for pharmacological target in antitumor, bacteriological, anti-inflammatory, anthelmintic activities and free radical activities. Example of such include:

Co(II), Ni(II) and Cu(II) complexes of Schiff bases derived from 4,4-diaminodiphenyl sulfone and 8-formyl-7-hydroxy-4-methylcoumarin/5-formyl-6-hydroxycoumarin [70], Schiff base-ruthenium(II)/(III) complexes [71-77], Ru(III) mixed ligand complexes of 2-hydroxy-1-naphthylideneimines [73], f-(*Z*)-2-(pyrrolidin-2-ylidene) hydrazinecarbothioamide with Ni(II), Co(II) and Cu(II) [67], bivalent metal complexes incorporating tetradentate dinitrogen–dioxygen ligand [78], lanthanide complexes of 1-phenyl-3-methyl-5-hydroxypyrazole-4-carbaldehyde-(4'-hydroxybenzoyl) hydrazine [59]. The use of Schiff bases in therapeutic applications as promising drug agents have been reported by several researchers but there are few reports in terms of unsymmetrical Schiff base derived from 1,2-diamines and different aldehydes/ketones giving rise to N_2O_2 and N_2O donors with antimicrobial, antioxidant and antiproliferative properties, the rational for this research work.

1.8 Aims and objectives

This research is aimed at the synthesis and characterisation of symmetrical and unsymmetrical tetradentate Schiff base; unsymmetrical tridentate Schiff base and their ruthenium(III), zinc(II), cobalt(II), nickel(II), and copper(II) complexes.

The research objectives are:

(1) To synthesize and characterize symmetrical tetradentate Schiff bases of the N_2O_2 donor ligands.

(2) To synthesize and characterize unsymmetrical tetradentate Schiff bases of the N_2O_2 donor ligands.

(3) To synthesize and characterize unsymmetrical tridentate Schiff bases of the N_2O donor ligands.

(4) To synthesize the Zn(II), Co(II), Ni(II), Cu(II) and Ru(III) complexes of the Schiff base ligands.

(5) To characterize the synthesised metal complexes using analytical and spectroscopic techniques.

(6) To evaluate the *in vitro* antibacterial, antioxidant and anticancer potentials of Schiff base ligands and their corresponding metal complexes.

Chapter Two

Literature Review

Part of Chapter Two has been published as:

Ejidike, I.P.; Ajibade, P.A. Transition metal complexes of symmetrical and asymmetrical Schiff bases as antibacterial, antifungal, antioxidant, and anticancer agents: Progress and prospects. *Reviews in Inorganic Chemistry*, **2015**, *35*, 191-224.

CHAPTER TWO

LITERATURE REVIEW

2.0 Background to the Chapter

The chemistry of Schiff base-metal complexes has fascinated several chemists in different parts of the globe in search of promising chemotherapeutic agents for disease control. The ease with which the Schiff base ligands are designed and synthesized have made them to be referred to as 'fortunate ligands', possessing azomethine derivatives, the C=N linkage that is essential for biological activity, including: antibacterial, antifungal, antioxidant, anticancer and diuretic activities. A variety of Schiff's base and its complexes studied as model molecules for biological, analytical and industrial applications have made this part of inorganic chemistry highly interesting [83-90].

Application of Schiff bases and their transition metal complexes have shown a broad biological activity in the area of DNA binding studies, bacterial and fungal inhibition, herbicides, insecticides, nematocides and rodenticides. Metal complexes of Schiff bases application can also be found in the areas like free radical chains termination, as antileishmanial agents, as radiotracers in nuclear medicine, anti–inflammatory, plant growth hormone regulator/ booster, cytotoxic activity [91-95]. The review summarises the use of Schiff base ligands and their corresponding metal complexes in therapeutic applications as promising antifungal, antimicrobial, free radical scavengers and anticancer agents; analytical tools for metal analysis.

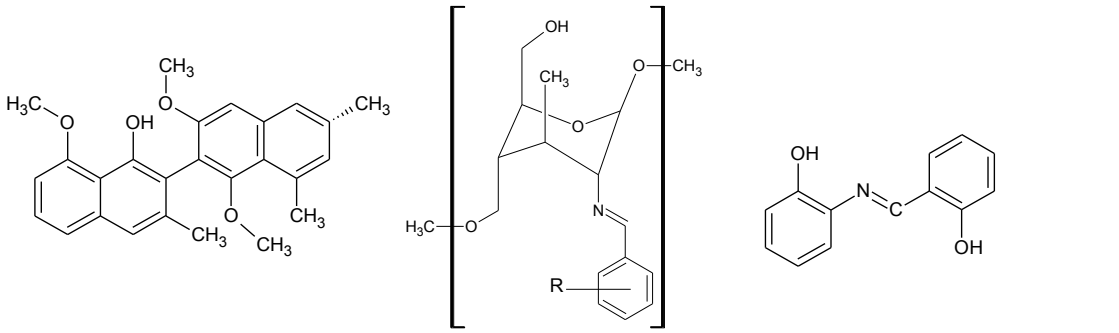
2.1 Introduction to Schiff bases and their complexes as biologically active agents

Schiff bases are widely studied ligands which coordinate to metal ions via azomethine nitrogen. In azomethine subordinates, the >C=N assembly is crucial for biological activity. The presence of this assemblage have made its complexes to be considered as imperative stereo-chemical models in main group and transition metal coordination chemistry because of straightforwardness in preparation and assortment in structure [83]. Interaction of metal ions with N, O and S atoms from Schiff bases organic compounds have gained much attention in recent years and have produced an enthusiastic arrangement of ligands, possessing characteristic features that can be adjusted by introducing unusual organic substituents, which bring about variety in the fundamental donor properties [8].

The use of Schiff bases in biological or therapeutic applications as promising drug agents or biological probes and analytical tools have been reported by several researchers. Also, the bioactivity of Schiff base compounds as antibacterial [39, 84-86], antiradical [87-89], anticancer [90-92], antifungal [93-94], antiviral agents [95-97] have been reported. There has been increasing focus on binding of small molecules such as Schiff base compounds to DNA [78, 98]. Their utilization in cancer prevention, sustenance bundles and as an O_2 sensor/ identifier has been reported [78].

Furthermore, Schiff bases are found in diverse natural, semi-synthetic, and synthetic compounds (Figure 2.1) and have been established to be significant for their biological activities [91]. In the past few decades, Schiff base ligands and their metal complexes have been amongst the most widely examined coordination compounds. This can be attributed to their inexorably essential as biochemical, and scientific reagents. Schiff base complexes have

been reported to offer ease and flexibility in their synthetic technique, diverse properties, and use as biologically active compounds [8].



Ancistrocladidine(5) (Antimalarial activity)

Chitosan-derived Schiff base(6) R =H or OH (Antifungal activity)

***N*-(Salicyliden)-2-hydroxyaniline)(7)** (Antibacterial activity)

Figure 2.1: Some examples of biologically active Schiff base compounds.

2.2 Schiff bases and their metal complexes as antifungal and antimicrobial agents

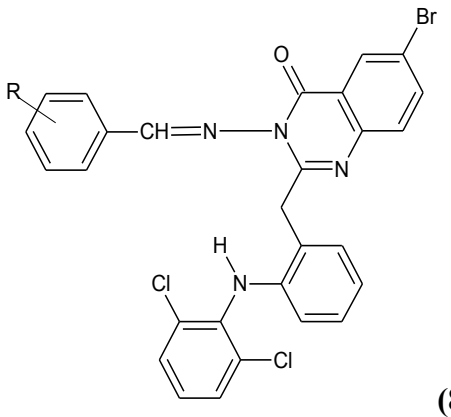
Infectious diseases that are directly related to bacteria exhibiting multiple resistances to antibiotics have increased world's mortality rate. Therefore, the need to develop novel antibacterial drugs with excellent mechanisms of action and structural-activity relationship has become an urgent biomedical necessity [10, 99]. Schiff base ligands and their biologically active complexes which provide potential sites for biochemically active compounds have been concentrated broadly in the course of the last few decade [100] for their antimicrobial [101] and anticancer [102] properties. Schiff base metal complexes are among the versatile models used as organic oxygen transporter [103-105].

Transition metals existing in various naturally occurring biomolecules are key to life process; hence, they can coordinate with O- or N-terminals from proteins in mixed models. They have an important role in the compliance and utility of living macromolecules [106-107], acting as an antimicrobial agent [108]. Phosphate Schiff base ligands of tetra- and hexa-

coordinate metal chelate have been reported to possess remarkable bacterial properties. However, this interesting biological activity could be enhanced upon complexation with the metal ions [109].

2.2.1 Antimicrobial and antifungal activities of non-heterocyclic Schiff bases

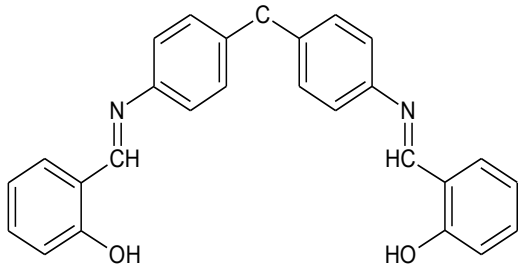
Patel and Patel [79] synthesized a series of novel Schiff bases, known as 6-bromo-2-[2-(2,6-dichlorophenyl)amino]benzyl-3-(substitutedbenzylideneamino)-quinazolin-4(3*H*)one **(8)**. This compounds were subjected to *in vitro* antimicrobial activity using cup plate method while, *S. aureus, P. aeruginosa, B. subtilis,* and *C. albicans* strains were employed. *Penicillin G* and *Amphotericin B* were set as standard drugs. Results obtained revealed that all compounds possess moderate to poor antifungal activity and good antibacterial activity [79].



(8)

Essa *et al*. [110] have reported the synthesis of new open and macrocyclic Schiff bases and its evaluation for antimicrobial activity. Schiff bases with open conformation were prepared by the condensation of salicylaldehyde and *o*-vanillin with 4,4'-diaminodiphenylmethane **(9)**, 4,4'-diamino diphenyl sulphide **(10)**, and diethyl ester of terephthalic acid, respectively. According to the authors, macrocyclic Schiff bases were obtained as the product of the condensation reaction of 1,6-bis(2-formylphenyl)hexane with

thiocarbohydrazide. Biological activity of the synthesized compounds against four microorganism strains namely: *K. pneumoniae, E. coli, S. aureus,* and *S. typhimurium* and all the compounds were found to be moderate to strongly active [110].



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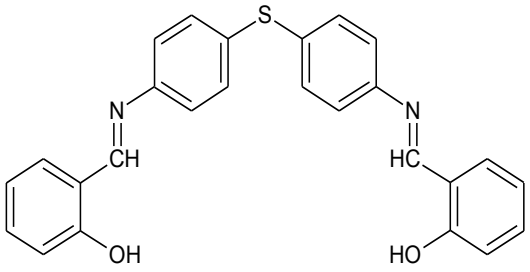
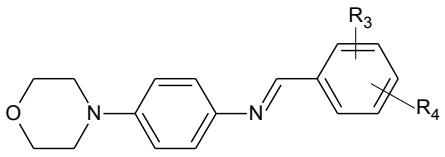


(10)

Panneerselvam *et al.* [111] depicted the synthesis and *in vitro* antimicrobial action of a series of 4-(4-aminophenyl)-morpholine-derived Schiff bases (compounds I-III) **(11)**. The authors revealed that micro-organisms: *S. aureus* and *Micrococcus luteus* were the utmost delicate strains to the morpholine-determined Schiff base I (MIC = 20 and 32 µg/ mL, individually). Streptococcus epidermidis was more delicate to the morpholine-determined Schiff base II (MIC = 17 µg/mL) while *Bacillus cereus* and *E. coli* were more sensitive bacteria strains to compound III (MIC = 21 and 16 µg/mL, respectively) [111].



R_3 = o-Cl and R_4 = H (I)

R_3 = o-OH and R_4 = H (II)

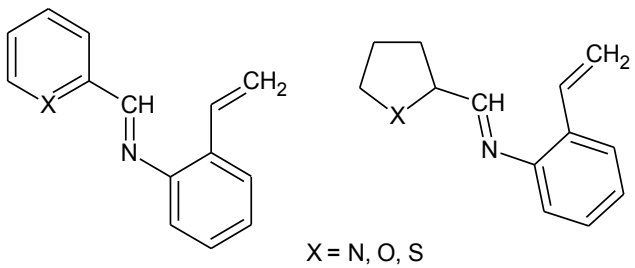
R_3 = p-OH and R_4 = H (III)

(11)

2.2.2 Heterocyclic Schiff base complexes

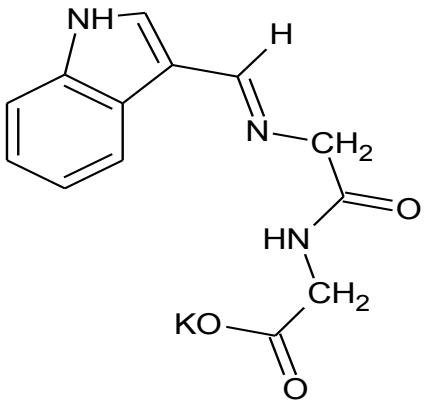
Gürbüz *et al.* [112] conveyed the preparation of Cr(III), Fe(II), Ni(II), Fe(III), Cu(II), Zn(II), Co(II) complexes of *N*-(5-chloro-2-hydroxyphenyl)-3-methoxy-salicylaldimine (H_2L), an ONO type tridentate ligand, resulting from 4-chloro-2-aminophenol with 3-methoxy-salicylaldehyde. Octahedral geometry was proposed for Fe(II), Cr(III), Co(II), Ni(II) complexes, five coordination geometries for Zn(II) and Fe(III) while Cu(II) complex is four-coordinated having acetato bridged dimeric structure. Antimicrobial activities of the compounds were evaluated against six bacteria and *C. albicans* as fungi showed that the complexes exhibited a broad range of biological activity than the tridentate ligand [112].

Biologically active M(II) complexes of Schiff bases got from heterocyclic aldehydes and vinyl aniline of the type: $[M(LX)_2Y_2]$ wherever M = Cu(II), Mn(II), Ni(II), and Co(II), LX = bidentate ligand (obtained from furfuraldehyde, thiophene-2-carboxy aldehyde or pyridine-2 aldehyde with vinyl aniline); Y = Cl **(12)** have been found to exhibit an octahedral geometry [113]. The isolated compounds were screened for their antifungal and antibacterial activity against bacterial species like- *Escherichia coli, Klebsiella, Staphylococcus aureus* and *Psuedomonas* and fungal species like- *Candida krusei* and *Candida albicans* [113].



(12)

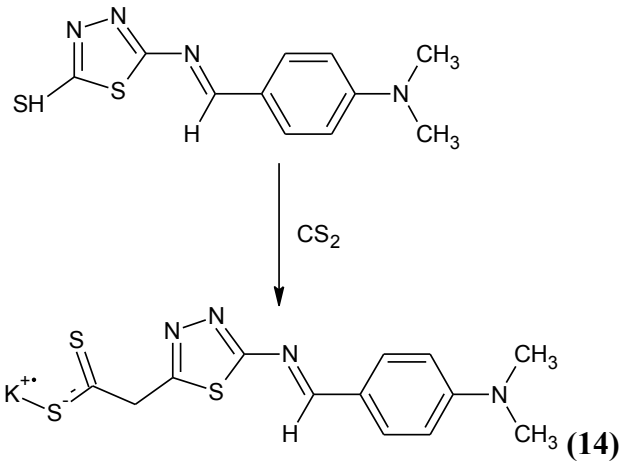
Joseyphus and Nair [114] reported biological studies of some Ni(II), Co(II) and Cu(II)-indal-glygly Schiff base complexes got from indole-3-carboxaldehyde and glycylglycine as Schiff base ligand **(13)**. XRD studies showed that the Ni(II), Co(II) and Cu(II) complexes possess the crystallite size of 31, 40 and 67 nm, respectively. The antimicrobial activity of the isolates were tried *in vitro* against the bacterial strains viz *S. aureus, K. pneumaniae, P. vulgaris, E. coli,* and *P. aeruginosa* and the fungal species *C. albicans, R. stolonifer, A. niger*, *A. flavus* and *R. bataicola* by Kirby Bayer Disc Diffusion method [114].



(13)

In 2014, Ni(II), Co(II), and Cu(II) complexes of Schiff base resulted from condensation reaction of potassium 2-*N*(4-*N,N*-dimethylaminobenzyliden)-4-trithiocarbonate-1,3,4-thiadiazole **(14)** (Scheme 2.1) synthesized and characterized by standard physico-chemical measures was reported [100]. Antimicrobial action of the Schiff base and its complexes were tested to assess their inhibiting potential against *Pseudomonas aeruginosa* and *Staphylococcus aureus* utilizing two different concentrations: 5 and 10 mM. The outcomes demonstrated that Ni(II) complex exhibited greater microbial inhibition action over Cu(II), Co(II) complexes and ligand as contrasted with ampicillin standard medication [100].

Tetradentate macrocyclic Schiff base ligand: 1,5,8,13-tetraaza-2,9-dimethyl-4,11-diphenylcyclotetradeca-2,4,9,11-tetraene (L) and its complexes of the sort $[M(L)]Cl_2$ and $[M'(L)Cl_2]Cl$, where M = Pd(II), Pt(II) and M' = Rh(III), Ir(III) has been reported to exhibit higher antimicrobial action against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and antifungal activity against *Aspergillus-niger, Fusarium odum, Aspergillus-glaucus,* when contrasted with the free ligand [115].

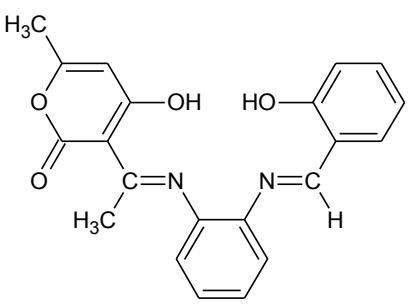


(14)

Scheme 2.1: Structure of Potassium 2-*N*-(4-*N,N*-dimethylaminobenzyliden)-4-trithiocarbonate-1,3,4-thiadiazole

Synthesis and XRD studies of Schiff base: 4-hydroxy-3-(1-{2-(2-hydroxybenzylidene)-amino-phenylimino}-ethyl)-6-methyl-pyran-2-ones **(15)**, and some rare earth metal complexes **(16)**: La(III), Sm(III), Pr(III), Nd(III), Ce(III) and Gd(III), was reported [116]. The ligand and their metal complexes were tested for the antimicrobial action against *Escherichia coli, Staphylococcus aureus,* and *Bacillus sp.* by paper disc plate method and antifungal activity against *Aspergillus niger, Trichoderma* and *Fusarium oxysporum* by Mycelia dry weight strategy. Results revealed that the complexes were biologically active and showed improved antimicrobial action than the free ligand (Schiff base) [116]. Co(II),

Ni(II), Cu(II), Mn(II) and Fe(III) complexes of the same ligand was synthesised and tested *in vitro* against bacteria like *Staphylococcus aureus* and *Escherichia coli* by paper disc plate method and reported that the restraint by metal chelates was greater than that of the free ligand [117].



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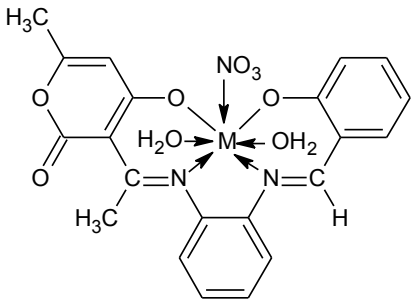
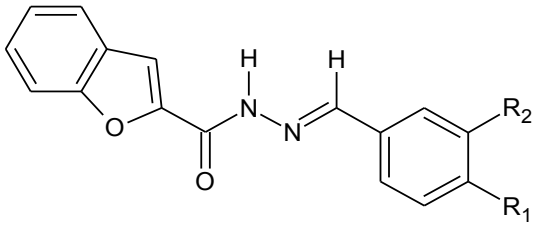


(16)

Shivakumar *et al.* [118] have reported the preparation of benzofuran[phenylmethine] carbohydrazone [BPMC] and benzofuran[3,4-dimethoxyphenylmethine]carbohydrazone [BDMeOPMC] **(17)**, its metal complexes of the sort MLX_2, where M = Co(II), Ni(II), Cd(II), Cu(II), Hg(II) and Zn(II), L = BPMC or BDMeOPMC and X = Cl and their biological activities. Co(II) and Cd(II) complexes of [BPMC] were establish to be moderately active toward *E.coli* whereas Cu(II), Zn(II) and Ni(II) complexes of [BPMC] and Cu(II) and Zn(II) complexes of [BDMeOPMC] were more active against *S. aurious* as compared to the free ligands. It was reported that none of the complexes are active against *A. niger*, but in the event of *A. fumigatus*, Cu(II),Co(II), Ni(II) and Cd(II) complexes of [BDMeOPMC] were more active than the parent ligands [118].

Al-Shaalan [119] reported the synthesis, characterization and biological activities of mononuclear and binuclear complexes of Cu(II), Mn(II), Ni(II), Co(II), Fe(II) and UO_2 (VI) with ligand (HL) obtained by the condensation reaction of *o*-hydroxyacetophenone with 7-

chloro-4-quinoline. High antibacterial activities was observed when ligand (HL) and metal complexes were screened against a strain of Gram +ve bacteria (*Staphylococcus aureus*), Gram -ve bacteria (*Escherichia coli*), and fungi (*Candida albicans*) [119].



$R_1 = R_2 = H$, [BPMC]

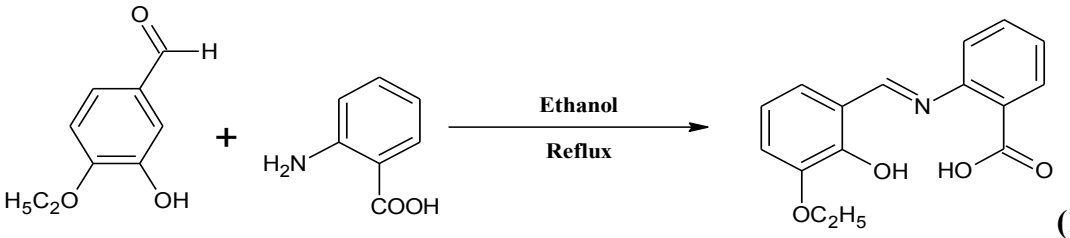
$R_1 = R_2 = OCH_3$, [BDMeOPMC] **(17)**

Kaushal and Thakur [23] reported the syntheses and biological screening of Schiff base complexes of Titanium(IV) of composition $[TiCl_2(SB)_2]$ synthesized by reacting $TiCl_4$ and Schiff bases (SBs) where (SBs = A1(Tetracycline hydrochloride Schiff base); B1(Streptomycin Schiff base); C1(Ceffixime Schiff base); D1(Ampicillin Schiff base) in fixed molar ratio 1:2. The synthesized compounds were screened for antimicrobial action against pathogenic bacteriological strains i.e. *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Aeromonas hydrophila*, *Aclaligenes faecalis*, *Shigella sonnei*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. It was reported that metal complexes possess higher antimicrobial activities than their corresponding Schiff bases [23].

Mounika *et al*. [120] have reported the Schiff base, 3-ethoxy salicylidene amino benzoic acid **(18)** synthesized from the condensation reaction of 3-ethoxy salicylaldehyde and 2-amino benzoic acid (Scheme 2.2) and its Ni(II), Co(II) Cu(II) and Zn(II) metal complexes. The spectra studies revealed that the ligand acts as neutral tridentate and binds to each metal

atom via >C=N nitrogen and oxygen molecules of hydroxyl group of the 3-ethoxy salicylaldehyde, besides the hydroxyl group of the carboxyl group of 2-amino benzoic acid. The compounds were tested for antibacterial and antifungal action using disc diffusion method (technique). The outcomes indicated that the metal complexes exhibited enhanced antibacterial action than the free ligand [120].

Antimicrobial studies of Cr(III), Fe(III) and Mn(III) complexes of Schiff base 2-hydroxy-5-methyl acetophenone-*N,N′*-ethylenediamine (HMAE) derived by the condensation of 2-hydroxy-5-methyl acetophenone and ethylenediamine showed that the ligand and its complexes showed considerable bacteriocidal action against *E. coli, S. aureus*, *B. subtilis, A. aerogenes* and are verging on latent against *B. megatherium, P. vulgaris* and *P. fluorescen* [121].



(18)

Scheme 2.2: Synthesis of Schiff base, 3-ethoxy salicylidene amino benzoic acid

In 2014, Chandrasekaran and co-workers [122] reported Mn(II), Zn(II), Cu(II) and Co(II) complexes of Schiff base (*E*)-*N′*-(thiophen-2-ylmethylene)nicotinohydrazide (TCNH), obtained by the condensation of nicotinic acid hydrazide and thiophene-2-carboxaldehyde, and were tested against three pathogenic micro-organism *Staphylococcus aureus, Aspergillus Niger* and *Escherichia coli*. All the metal complexes displayed higher antibacterial actions than the free ligand [122]. Spectroscopic and biological studies of a tetradentate macrocyclic

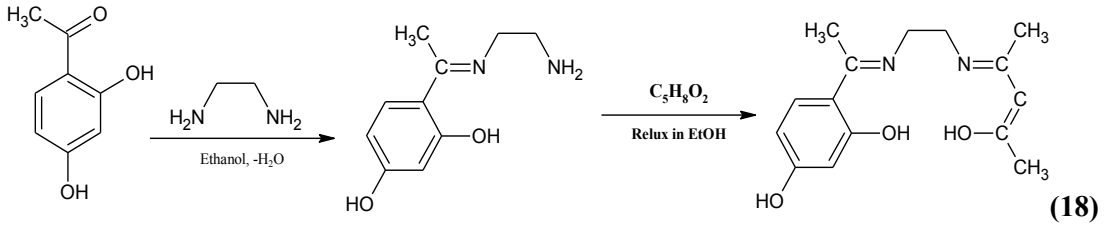
ligand: 2,3,9,10-tetraketo-1,4,8,11-tetraazacycoletradecane resulted from the condensation of diethyloxalate and 1,3-diamino propane and its Mn(II), Co(II), Ni(II), Pd(II) and Pt(II) complexes have been reported [123]. The antimicrobial screening information demonstrated that the metal chelates possess better inhibitory effects than the free ligand [123].

Jadhav *et al.* [80] reported the potentiometric study of Cu(II), Mn(II), Co(II), Fe(III) and Ni(II) complexes with dibasic tridentate ligand (ONN) derived from the water loss reaction of dehydroacetic acid (3-acetyl-6-methyl-(2*H*) pyran-2,4(3*H*)-dione or DHA), fluoro benzaldehyde and *o*-phenylenediamine. The antimicrobial and antifungal activities of the complexes were accounted for additional action when contrasted to that of the ligand [80].

Fleck *et al.* [124] reported the synthetic features, crystal structures and antimicrobial actions of manganese(III) and cobalt(III) complexes comprising of a tetradentate Schiff base: namely $[Mn(L)Cl].H_2O$, $[Co(L)Cl]_2 \cdot 2CH_3COCH_3$ and $[Co(L)NCS]_2$, 2,2'-[propane-1,2-diylbis(nitriloeth-1-yl-1-ylidene)]diphenol. Structural examinations indicated that the focal Mn(III) ion adopted a tetragonal pyramidal geometry while the Co(III) ions adopted distorted octahedral geometry with a binuclear phenoxo-bridged thiocyanato complex by frail intermolecular H-bonding or C–H--π communications in their solid state. *In vitro* screening to evaluate the development inhibitory action of the synthesised compounds against two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* and two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* indicated mild to moderate bactericidal activities which increased with dose [124]. The synthesis, characterization and antibacterial studies of Co(II), UO_2(II), Ni(II), Zn(II), Cd(II), Hg(II), Cu(II) and Th(IV) com, X = NO_3 or CI and Schiff base [L] derived from the water loss reaction of 5-methylsalicylaldehyde with benzofuran-2-carbohydrazide (BHMePC) or 5-

chlorosalicylaldehyde (BHCIPC). The outcomes demonstrated that the ligands' action was enhanced when evaluated in the form of metal complex [125].

Ejidike and Ajibade [126] reported the antibacterial, antioxidant studies of Co(LL), Ni(LL), Cu(LL), and Zn(LL) complexes of unsymmetrical Schiff base: (4*E*)-4-[(2-{(*E*)-[1-(2,4-dihydroxyphenyl)ethylidene]amino}ethyl)imino]pentan-2-one (H_2LL) **(18)** (Scheme 2.3) derived from 2',4'-dihydroxyacetophenone, ethylenediamine and acetylacetone. The antibacterial studies of the compounds investigated against *Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli,* and *Shigella flexneri,* revealed that the metal complexes displayed higher bioactivity than the free ligands. The antioxidant activity by DPPH and ABTS method revealed that synthesized compounds are equipped for giving electron or hydrogen atom which interacts with the free radicals or cause chain response termination in a dosage pattern [126].



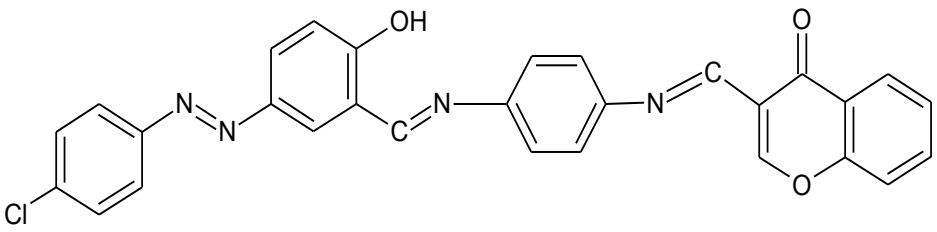
(18)

Scheme 2.3: Synthesis of Schiff base ligand (H_2LL)

Physicochemical and biological properties of Nickel(II), copper(II) and zinc(II) complexes of unsymmetrical Schiff base got from 2-hydroxy-1-naphthaldehyde, 2,4-pentanedione and *p*-phenylenediamine of the sort [$M(C_{10}H_6OCH{:}N(C_6H_4)N{:}C(CH_3)CH{:}C(CH_3)O)$], and their adducts with 2,2'-bipyridine (bipy) and 1,10'-phenanthroline (phen) have been recounted [127]. The magnetic moments and electronic spectra validate the octahedral geometry for Ni(II) and Cu(II) complexes and the adducts six-coordinate. The

bacteriological properties of the ligand and compounds against various microbes and a fungus strains was accounted for The compounds were found to display great activity against the selected organisms than the free ligand. [CuLphen] demonstrated equivalent action to gentamycin (standard), while the ligand, the zinc Schiff base complexes and the bipy adduct of the zinc complex had no activity against the tested organisms [127]. Minimum inhibitory concentrations (MICs) of the sensitive compounds stretched from 1.0–12.0 mg/mL [127].

Antibacterial and nonlinear optical properties (NLO) studies of Co(II), Ni(II), VO(II), Cu(II) and Zn(II) via Schiff base ligand: 3-(4-5-(4-chlorophenyl)diazenyl)-2-hydroxy benzylideneamino)phenylimino)methyl)-4*H*-chromen-4-one [CDHBPC] derived from 5-(4-chloro-phenylazo)-2-hydroxybenzaldehyde [CPDA2HB] and 3-[(4-Aminophenylimino) methyl]-4-oxo-4*H*-chromene [APMOC] have been reported [128]. The Schiff base and the metal complexes were found to be highly active against the antibacterial and antifungal species [128]. Metal(II) complexes of Co(II), Ni(II), VO(II), Cu(II) and Zn(II) have been prepared from the azo Schiff base 4-((*E*)-4-((*E*)-(4-chlorophenyl)diazenyl)-2-hydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (CDHBAP) **(19)** (Figure 2.2) and screened against microbial species viz: *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Shigella sonnie, Salmonella typhi* [129].



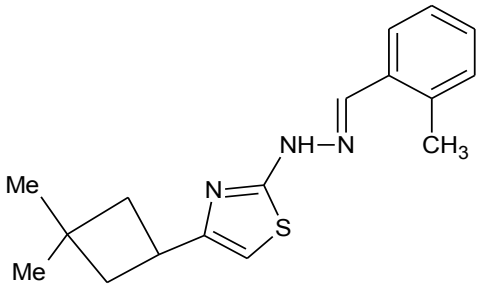
(19)

Figure 2.2: Schiff base ligand: 3-(4-5-(4-chlorophenyl)diazenyl)-2-hydroxy benzylideneamino) phenylimino)methyl)-4*H*-chromen-4-one [CDHBPC]

2.2.3 Antimicrobial activity of ruthenium Schiff base complexes

In 2009, Ru(II) complexes derived from the responses of $[RuCl_2(DMSO)_4]$ with naturally dynamic macrocyclic Schiff base ligands containing N_2O_2 and N_4 contributor group with a proposed octahedral geometry has been report by Shankera and co-workers [130]. All the macrocycles and macrocyclic Ru(II) complexes alongside existing antibacterial medications were screened for antimicrobial action against Gram (+ve): *Bacillus subtilis, Staphylococcus aureus* and Gram (−ve): *Escherichia coli, Klebsiella pneumonia* microorganisms. The compounds were observed to be more dynamic when contrasted with streptomycin and ampicillin [130]. According to Thangadurai and Ihm [94], ruthenium precursor: $[RuX_3(E)_3]$ or $[RuBr_3(PPh_3)_2(MeOH)]$ have been used in the preparation of Ru(III) complexes of the sort $[RuX(L)_2(E)]$ (X = Br or Cl; L = novel bidentate Schiff base ligand **(20)** derived from 4-(1-methyl-1-mesitylcyclobutane-3-yl)-2-aminothiazole; E = PPh_3 or $AsPh_3$).

The complexes manifest as effective impetuses for the oxidation of alcohols to carbonyl compounds but are unable to oxidise alkenes in the existence of N-methylmorpholine-N-oxide (NMO) as co-oxidant. The antimicrobial activity of the ligand and complexes has also been tested against six microorganisms [131]. Ruthenium(III) complexes of the type $[RuY(LL')(E_2)]$ (Y = Cl or Br; LL' = tridentate Schiff bases; E = PPh_3 or $AsPh_3$) with Schiff bases (O, N, X): salicyladehydethiosemicarbazone (X = S); salicyladehydesemicarbazone (X = O); *o*-hydroxyacetophenonethiosemicarbazole (X = S); *o*-hydroxyacetophenonesemicarbazole (X = O) and biocidal activity was reported [132].

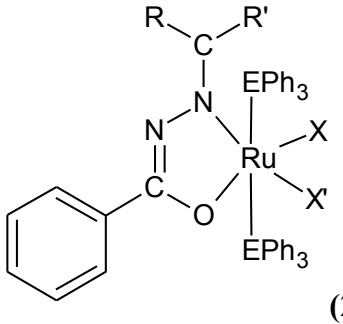


(20)

Binuclear ruthenium(III) Schiff base complexes configured with bis-salophen/bis-naphophen (N_4O_4) of the sort [(EPh_3)(X)Ru-L-Ru(X)(EPh_3)] (where X = Cl or Br; E = P or As; L = binucleating dianionic tetradentate ligands have been reported to act as potential catalyst for the oxidation of essential and auxiliary alcohols to aldehydes or ketones in the existence of N-methylmorpholine-N-oxide (NMO) [133], while stable low turn Ru(III) complexes of the sort [$RuX_2(EPh_3)_2(L)$] (where X = Cl or Br; E = P or As; L = mono fundamental bidentate Schiff bases) have shown development inhibitory activity against the microscopic organisms *Staphylococcus aureus* (209p) and *E. coli ESS* (2231), also possess catalytic properties [73].

Hexa-coordinated ruthenium(III) complexes **(21)** with monobasic bidentate Schiff base prepared from the condensation of benzhydrazide with furfuraldehyde, 2-acetylthiophene, 2-acetylfuran (Figure 2.3) have been synthesized from a prepared ruthenium precursors and Schiff bases in benzene with proposed octahedral structure have been reported [134]. The complexes were found to exhibit catalytic action for the oxidation of cinnamylalcohol, cyclohexanol and benzyl alcohol in the existence of N-methylmorpholine-N-oxide as co-oxidant. Biological activity of the complexes was screened against the microorganisms strains such as *Salmonella typhi*, *E. coli, Pseudomonas* and *Staphylococcus aureus*. The action was equated with/ to standard *Streptomycin* [134]. Antibacterial activities

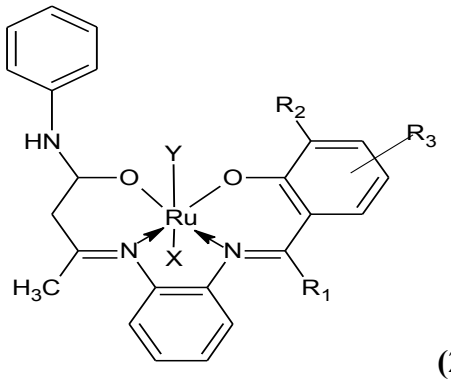
of heterocyclic Schiff base: bis(2-(pyridin-2-ylimino)phenyl)-4,4'-(diazene-1,2-diyl) dibenzoate (BPPD, L) and its Co(II), Zn(II), Cu(II) and Ni(II) have been tested against *Staphylococcus aureus* and *Escherichia coli* [135]. The complexes showed greater antimicrobial activities as compared to the ligand in the order: Cu(II)L > Zn(II)L > Ni(II)L > Co(II)L > L [135].



(21)

(X = O or S; X' = Cl or Br; E = P or As; R = H or CH_3; R' = C_4H_3S or C_4H_3O)
Figure 2.3: Hexa-coordinated ruthenium(III) complexes with bidentate Schiff base

Ruthenium(II) Schiff base complexes **(21)** of the sort [RuX(EPh_3)(L)] (where, E = As/ P; X= Cl/ Br; L = dianion of the Schiff bases derived from acetoacetanilide with *o*-phenylenediamine and *o*-vanillin/ salicylaldehyde/ *o*-hydroxyacetophenone/ 2-hydroxy-1-naphthaldehyde (Figure 2.4) were tested for their bacteriological action against *Salomonella typhi*, *Pseudomonas aeruginosa*, *Vibrio cholera* and *Staphylococcus aureaus* [76]. Powder X-ray diffraction, catalytic and electro-chemical studies were additionally reported. Biological activity of ruthenium(II) complexes of the sort [RuCl(CO)(L)(PPh_3)(B)] [B = PPh_3, pyridine (py) or piperidine (pip)], alongside bidentate Schiff base ligands obtained from the buildup response of salicylaldehyde with aniline, o-, m- or p-toluidine have been reported [20].



(21)

R_1 = H/CH_3; R_2 = H/ OCH_3; R_3 = H/ C_4H_4; Y = PPh_3/ $AsPh_3$; X = Cl/ Br

Figure 2.4: Ruthenium(II) Schiff base complexes of the type [$RuX(EPh_3)(L)$]

2.3 Free radicals scavenging properties of Schiff bases and their metal complexes

Free radicals have been involved in the causation of a few oxidative harms infections. For example, liver cirrhosis, atherosclerosis, malignancy, diabetes, maturing Cancer prevention agent alludes to an exacerbate that can repress the oxidation of lipids or different atoms by keeping the start or proliferation of oxidative chain responses and can in this manner counteract or repair the harm done to the body's cells by oxygen [136].

The productions of this free radical could bring about cell divider and DNA harm, prompting endless maladies, for example, malignancies and cardiovascular ailment when present in excessive concentrations in human system as the body might not be able to control its effect [137,138]. Metal based cancer prevention agents have increased recent consideration for their capacity to shield living creatures and cells from harm brought on by oxidative push or rummage free radicals [139]. The antioxidant (antiradical) activity of a synthetically prepared compound can be measured using the scavenging ability of that compound to trap free radicals [140].

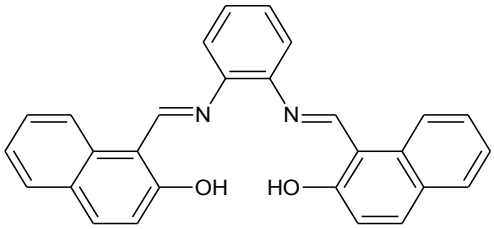
2.3.1 Antioxidants properties of Schiff base ligands

In 2012, Gwaram *et al.* [141] reported the synthesis, antioxidant activities and X-ray crystal structures of Schiff base ligands got from the build-up reaction of gallic hydrazide with pyridine and acetophenone derivatives, alongside with their acetylcholinesterase inhibition and antioxidant activity. The reaction of gallic hydrazide with selected hydroxyacetophenones and pyridine analogues resulted in the precipitation of the corresponding polyphenolic compounds: *N*-(1-(5-Chloro-2-hydroxyphenyl)-ethylidene)-3,4,5-trihydroxybenzohydrazide; *N*-(1-(5-Bromo-2-hydroxyphenyl)-ethylidene)-3,4,5-tri-hydroxybenzohydrazide; *N*-(1-(2-Hydroxy-5-methoxyphenyl)-ethylidene)-3,4,5-trihydroxy benzohydrazide; 3,4,5-Trihydroxy-benzoic acid [1-pyridylethylidene] hydrazide; 3,4,5-tri-hydroxybenzoic acid [1-(4-acetyl-pyridin-2-yl)-ethylidene] hydrazide. The DPPH free radical and ferric reducing antioxidant power (FRAP) assays revealed that the newly isolated ketone affiliates of gallic hydrazide-derived Schiff bases possess strong antioxidant activities [141].

2.3.2 Antioxidants activities of symmetrical Schiff base metal complexes

DPPH* free radical rummaging action of the Ln(III) complexes of the type: $[LnL(NO_3)2(H_2O)_x](NO_3)$, where Ln(III) = Nd, Pr, Dy, Sm, Gd, Er, La, Er and Tb; x = 0 for Nd, Sm; 1 for La, Pr, Nd, Gd, Dy, and 2 for Tb, arranged from the condensation of *o*-phenylenediamine with 2-hydroxy-1-naphthaldehyde in a 2:1 molar proportion **(22)** [142]. It was observed that the antioxidant action of Ln(III) complexes on DPPH* is dose-dependent and possess greater action than that of the free ligand L. Also, it was found that complexes of Tb(III) and Dy(III) proved the uppermost rummaging actions while the La(III) and Pr(III) complexes showed the least actions which could be because of the ionic size impact,

demonstrating that the DPPH* rummaging action of the Ln(III) complexes relies on upon the focal metal particle [142]. Schiff base ligand *N*-(2-hydroxylacetophenone)-3-oxapentane-1,5-diamine (HL), and its Ni complex of the type $[Ni_2(L)_2(NO_3)_2]$ have been synthesized and evaluated for antioxidation and DNA-binding properties. The suppression ratio of OH radical amplified with upsurge in complex concentration [89].

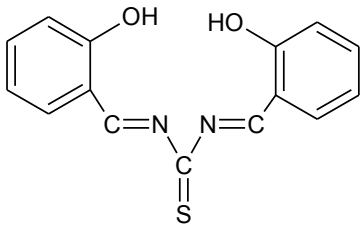


(22)

The antioxidant efficiencies of homo- and hetero-nuclear Ni(II) and Cu(II) complexes of new oxime-sort ligands were determined by DPPH* and superoxide radical scavenging activities. The obtained IC_{50} values, suggests the compounds to potential medications to wipe out the radicals [143]. Also, the antimicrobial activities against several pathogenic microorganisms and DNA binding properties by UV-VIS spectroscopy were investigated. Regarding the minimum inhibitory concentration (MIC; μg/μL), it was witnessed that the complexes have reasonable bacteriological features and could tie to DNA via an intercalative mode respectively [143].

Reddy *et al.* [57] reported the antioxidant activity of bimetallic Cu(II), Co(II), Ni(II), U(VI), Zn(II) complexes with common stoichiometry $[H_2L\ M(X_2)(H_2O)_2]$ (where X = chloride/ sulphate, H_2L = terephthaladehyde bis(thiosemicarbazone). *In vitro* activity of the compounds by the NO, hydroxyl radical rummaging, DPPH and reducing power methods was examined [57].

The Schiff base *N,N′*-bis(salicylidene) thiourea $(HS)_2T$ (Figure 2.5) **(23)** and complexes of copper(II), nickel(II) and zinc(II) was synthesized by Xinde and co-workers [144]. The characterization results revealed that the Schiff base works as quadridentate ligand appended to the metal ions through the phenol oxygen and imino nitrogen atoms, i.e. blue shifted and thus impact on the conjugation system because of the ligation amongst the ligands and the metal particles [144]. The biological activity: scavenging consequence on superoxide radicals by Pyrogallol-NBT method and Poisoning Oncomelania Hupensis by soaking and spray method tested on the synthesised compounds was reported. The tests revealed that the compounds have a strong poison effect on Oncomelania Hupensis as well as scavenging superoxide free radicals [144].

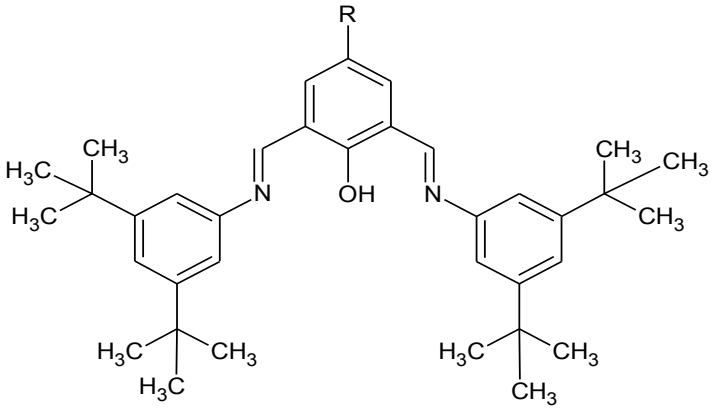


(23)

Figure 2.5: Structure of Schiff base *N,N′*-bis(salicylidene)thiourea $(HS)_2T$

Synthesis and antioxidant property of Quercetin-Tb(II) complex by DPPH, ABTS and FRAP methods, have revealed that the chelation of metal particles by Quercetin diminish the redox capability of Quercetin-metal complex while Tb(III) ion distort the chemical properties of the Quercetin [145]. Ceyhan *et al.* [146] have reported synthesized, characterization, antimicrobial and antioxidant activity of Schiff base ligands 4,4'-{(2-hydroxy-5-isopropylbenzene-1,3-diyl)bis[methylidenenitrilo]}-bis(2,6-di-tert-butylphenol) (HL_1), 4,4'-{(2-hydroxy-5-tertbutylbenzene-1,3-diyl)bis[methylidenenitrilo]}bis(2,6-di-tert-butylphenol) (HL_2) (Figure 2.6) **(24)** and their transition metal complexes Cu(II), Ru(III), Ni(II), Pd(II) and

Co(II). Antioxidant properties of the Schiff bases were appraised in a progression of *in vitro* investigations: DPPH* free radical rummaging and reducing power activity of superoxide anion radical created non-enzymatic systems [146]. The sterically hindered Schiff bases (HL_1 and HL_2) are free radical scroungers, and may reduce the effects of free radical damage occurring in the human body [146].



(24)

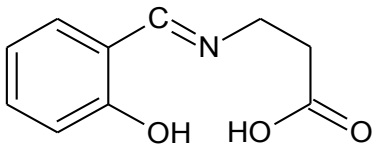
Figure 2.6: Structure of ligands (HL_{1-2}), R= 4-isopropyl (HL_1), 4-tert-butyl (HL_2)

DPPH radical scavenging activities of Cu(II), VO(II), Ni(II) complexes with Schiff base: 4,4′-Bis-({2-[(2-hydroxy-phenylimino)-methyl]-benzylidene}-amino)-biphenyl-3,3′-diol resulting from the abridgment of: 2-aminophenol, *o*-phthalaldehyde and 3,3′-dihydroxybenzidine have shown that free ligand negligible DPPH activity could be significantly enhanced upon complexation with metal ions [147].

2.3.3 Antioxidants activities of asymmetrical Schiff base metal complexes

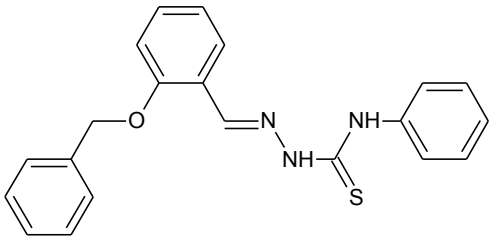
Zinc(II) and single crystal X-ray of copper(II) complexes comprising a tridentate O,N,O'-donor Schiff base: *N*-salicylidene-β-alanine(2-) **(25)** got from β-alanine and salicylaldehyde; have been reported by Vanco and co-worker [148]. All complexes were

assessed by the antiperoxynitrite action measure and alloxan-prompted diabetes model. A noteworthy cancer prevention agent and antidiabetic exercises were found for copper(II) complexes while no significant biological activity was revealed for zinc(II)-complex. It was established that copper(II) complexes from this study could act as nutritional supplements with biological activities relevant in prevention of diseases connected with progression of oxidative stress [148]



(25)

Complexes of benzyloxybenzaldehyde-4-phenyl-3-thiosemicarbazone ligand **(26)** derived from benzyloxybenzaldehyde and 4-phenyl-3-thiosemicarbazide with chlorides of Ni(II) and Cu(II) have been prepared and checked for DPPH* free radicals scavenging potentials and hindrance of iron(III) instigated lipid peroxidation at concentration of 100 µm [149]. The free ligand showed good action in DPPH* foraging (42%) and ferric particle affected lipid peroxidation (60%) as compared to the standard cancer prevention agent α-tocopherol, while Cu(II) and Ni(II) compounds possess no action against DPPH* rummaging and ferric ion impelled lipid peroxidation [149]. Antioxidant action of copper (II), cobalt (II) complexes of 4-alkyl-isocoumarin-3-carboxylic acid and 4-alkyl-3-aroyl-isocoumarin have exhibited higher biological activity than the corresponding ligands [150]. Panhwar and Memon [151] reported the characterization and antioxidant study of Cr(III)-Morin compound. The complex was establish to exhibit a higher cancer prevention agent potency than morin as assessed by DPPH* and FRAP schemes [151].



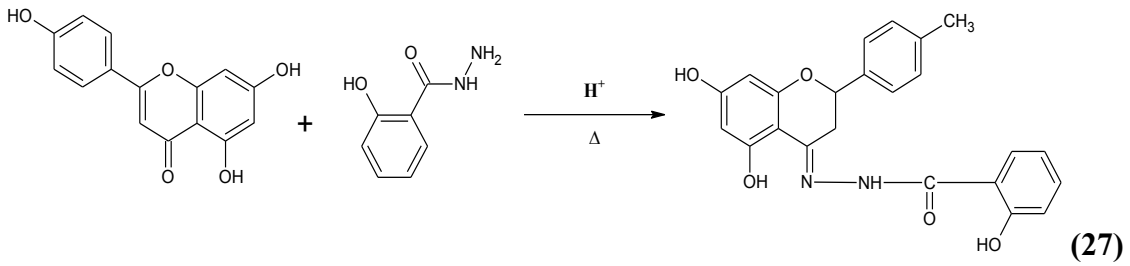
(26)

In 2012, (*E*)-2-(3-(2-imino-1-methylimidazolidin-4-ylidene)-1-methylguanidino) acetic acid and its novel Cu(II), Cr(III), Co(II), Ni(II) complexes derivate from Schiff base was reported [152]. Structural properties of the newly synthesized compounds were evaluated using different spectroscopic methods, all the metal complexes exhibited octahedral geometry with the exception of Ni(II) chelate with square planar pattern. The free-radical scavenging activities of ligand and metal chelates have been determined by DPPH approach and tested compounds exhibited free-radical DPPH inhibitory activities [152].

According to Al-Amiery *et al.* [67], the free-radical scavenging ability of the metal complexes of Ni(II), Co(II), and Cu(II) chlorides with f-(Z)-2-(pyrrolidin-2-ylidene) hydrazinecarbothioamide have been evaluated by their cooperation with the steady free radical 2,2-diphenyl-1-picrylhydrazyl, and all member of the compounds showed promising cancer preventing actions. The synthesized metal complexes were also tried against chose sorts of organisms and found to have noteworthy antifungal actions. Calculations from the molecular orbital gave a comprehensive depiction of the orbitals, comprising modal patterns, spatial attributes, and the donations of individual fragments [67].

A general formula of the type $M(H_3L)$ has been reported for the synthesis of M = Cu(II), Ni(II), Zn(II) complexes with a new ligand, naringenin-2-hydroxybenzoyl hydrazine (H_5L) **(27)**, by the condensation of naringenin with 2-hydroxy benzoyl hydrazine (Scheme

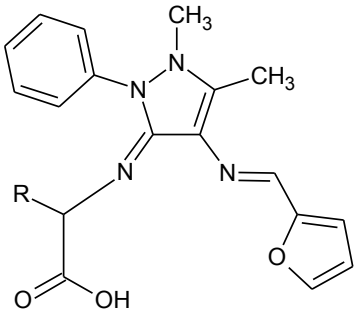
2.4) [153]. The *in vitro* antioxidant activity of the free ligand and metal complexes were achieved against hydroxyl radical and superoxide, and were found to retain potent action and displays a better cancer prevention agent action than the standards like vitamin C and mannitol. Order of the suppression ratio for HO* is H_5L > $Zn(H_3L)$ > $Ni(H_3L)$ at different concentrations. In particular, the Cu(II) complex demonstrated an excellent action on the superoxide radical [153].



(27)

Scheme 2.4: Synthesis of naringenin-2-hydroxybenzoyl hydrazine (H_5L)

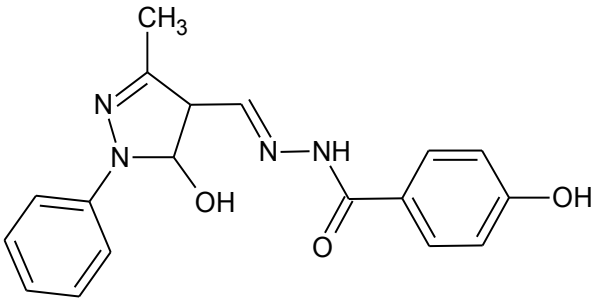
Raman *et al.* [154] reported the a sequence of novel Ni(II), Cu(II), Co(II) and Zn(II) complexes with Schiff bases, got by the abridgment of 4-aminoantipyrine with furfural and amino acid: glycine (L1); alanine (L2); valine (L3)] **(28)** and metal(II) salt. The inherent binding coefficients for the complexes were observed to be in the direction of 102 to 105 demonstrating that the complexes bind to DNA via intercalation and perform as effectual cleaving agents. The *in vitro* antimicrobial and antifungal measure demonstrated that these compounds were noble antimicrobial mediators against different bacterial pathogens. Antioxidant activity of $[Ni(L1)_2]$ and $[Zn(L1)_2]$ complexes with good IC_{50} values indicates the ability to scavenge hydroxyl radical [154].



Where R = H, CH_3- and $(CH_3)_2CH$- **(28)**

$Sm(NO_3)_3 \cdot 6H_2O$ and three anionic tetradentate Schiff-base ligands obtained from 8-hydroxyquinoline-2-carboxyaldehyde with benzoylhydrazine, 2-hydroxybenzoylhydrazine, and isonicotinylhydrazine, respectively have being utilized for the preparation of dinuclear Sm(III) complexes with 1:1 metal to ligand stoichiometry [155] have showed strong ability of antioxidation, but the complexes and ligands containing a dynamic phenolic hydroxyl cluster show robust rummaging effects on hydroxyl radical, while the Sm(III) complex comprising *N*-heteroaromatic substituent showed higher rummaging impacts for superoxide radical. Also, every one of the ligands and complexes binds strongly to calf thymus DNA via intercalative mode with the coupling constants ranging at 10^5-10^6 M^{-1}, but the complexes exhibited additional affinities to DNA than the ligands [155].

The antioxidant activity of lanthanide complexes of 1-phenyl-3-methyl-5-hydroxypyrazole-4-carbaldehyde-(4'-hydroxybenzoyl) hydrazone **(28)** has been studied by comparing their scavenging effects on hydroxyl radical (HO*) [59]. IC_{50} values for the ligand and complexes are 6.40 and 3.22–4.36 μM, respectively. Communication between the synthesised compounds and BSA/ DNA was studied by UV-Vis and fluorescence spectroscopy [59].



(28)

2.4 *In vitro* anticancer and DNA interaction studies of Schiff base metal complexes

Deoxyribonucleic acid (DNA) binding characteristics that are directed toward metal complexes with the drive towards the development of new compounds that can regulate hereditary data and/ or inhibit the development and replication of cancer cells through the hindrance of transcription has been the interest of many researchers over the past decades [156-158]. DNA is the primary pharmacological target of many antitumor compounds [159] and possible interaction of metal compounds with DNA have been a subject of importance for development of successful chemotherapeutic drugs through the following three non-covalent modes: Intercalation, groove binding, and external electrostatic effects [160].

Therefore, metal complexes for this interaction should possess air stability, ideal in natural environment and water-soluble (hydrophilic property), and among such metals with well-known biological properties, great attention has been made towards metals like ruthenium, copper, vanadium, iron, rhodium, titanium, gallium, cobalt, palladium and gold as a way of killing cancer cells.

The DNA-metal complexes interaction comprising of multi-dentate aliphatic and aromatic ligands, with square planar, square pyramidal, tetrahedral or octahedral N_4 or N_2O_2,

NNO, ONO (Schiff base) coordination, has been evaluated [161], and the reason being that such compounds have some constructive features [162-163]; attachment of the ligand to the metal is usually achieved in a well-ordered fashion. The coupling of these compounds to DNA is typically accomplished by noticeable absorbance variations in the UV–Vis frequency extent and fluorescence emission, as a result of the excitation of charge transfer transitions [164].

Moreover, the treatment of cancer diseases have been channelled towards the synthesis of novel metal complexes analogous to cisplatin [cis-$[PtCl_2(NH_3)_2]$, carboplatin [$Pt(cbca)(NH_3)_2$ with cbda = 1,1-cyclobutanedicarboxylate] and oxaliplatin, Pt(dach)(ox) where dach = R,R-1,2-diaminocyclohexane and ox = oxalate, [2,165-166], with this regards, divers number of non-platinum metal complexes which also exhibit remarkable anticancer activities [167-169] has received great attention. Thus, Schiff base ligands and their corresponding transition and inner-transition complexes has exhibited high antiproliferative and cytotoxic activity against different cancerous cell lines, and investigation using CT DNA indicate that the target of this complexes may be the guanine residues of the DNA helix [170-171].

DNA binding studies are very important for the advancement of novel therapeutic reagents and DNA investigations [172]. It has been well established that the geometry of the complex binding to DNA is a crucial factor in the study of the interactions of metallointercalators with nucleic acids [173] and hence, they can bind to DNA covalently and non-covalently [174].

2.4.1 DNA interaction studies of Schiff base ligands

Characterization of chiral Mn(III) salen complexes stemmed from respective chiral salen ligands, viz., (1*S*,2*S*)-*N,N′*-bis-[3-tert-butyl-5-chloromethyl-salicylidine]-1,2-cyclo-hexanediamine, (1*R*,2*R*)-*N,N′*-bis-[3-tert-butyl-5-chloromethyl-salicylidine]-1,2-cyclohexane-diamine, (1*S*,2*S*)-*N,N′*-bis-[3-tert-butyl-5-*N,N′N″*-triethylaminomethylsalicylidine]-1,2-cyclo-hexanediaminedichloride, (1*R*,2*R*)-*N,N′*-bis-[3-tert-butyl-5-*N,N′N″*-triethylaminomethyl-salicylidine]-1,2-cyclohexanediaminedichloride, (1*S*,2*S*)-*N,N′*-bis-[3,5-ditertbutylsalicylidene]-1,2-cyclohexanediamine and (1*R*,2*R*)-*N,N′*-bis-[3,5-di-tert-butyl-salicylidene]-1,2-cyclo hexanediamine, have been achieved using microanalysis, IR, LC-MS, UV-Vis [175]. Calf thymus DNA (CT-DNA) interaction properties of these complexes was premeditated using absorption spectroscopy, circular dichroism measurements, competitive binding study, thermal denaturation study, viscosity measurements [175].

Ozaslan *et al.* [176] conveyed that Schiff base ligands: [*N*-(1-phenyl-2-hydroxy-2-phenyl ethylidine)-2',4'-dinitrophenylhydrazine] (PDN), [*N*-(1-phenyl-2-hydroxy-2-phenyl ethylidine)-2'-hydroxyphenylimine] (PHP) and [*N*-(2-hydroxybenzylidine)-2'-hydroxy phenyl imine] (HHP) have bring about decrease in the average weight of tumor, i.e., increase in dose-concentration leads to increase in reduction of tumor growth and also cause a decrease in the cancer cells growth in mice EAC cells. Furthermore, it was revealed that they possess the capability to rebuild depleted haematological parameters, and show protective effect on hematopoietic system [176].

Schiff bases achieved from 4-aminoantipyrine and 3-methoxy/ 3-ethoxy/ 5-bromo/ 5-chloro/ 5-nitro salicylaldehyde as ONO donor ligands (L) [177] viz., L_1 =2,3–dimethyl-1-phenyl-4-(3-ethoxy-2-hydroxybenzylideneamino)-pyrazol-5-one, L_2 =2,3–dimethyl-1-phenyl

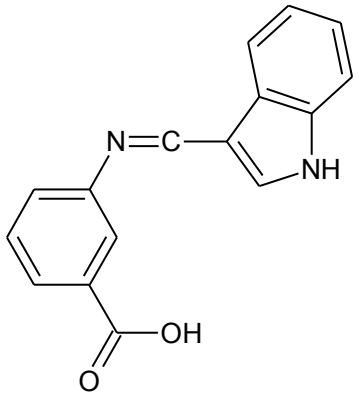
-4-(-2-hydroxy-3-methoxybenzylideneamino)-pyrazol-5-one, L_3= 2,3–dimethyl-1-phenyl-4-(-2-hydroxy-5-nitrobenzylideneamino)-pyrazol-5-one, L_4= 2,3–dimethyl-1-phenyl-4-(5-chloro-2-hydroxybenzylideneamino)-pyrazol-5-one, L_5= 2,3-dimethyl-1-phenyl-4-(5-chloro-3-hydroxybenzylideneamino)-pyrazol-5-one and A= 2,2'–bipyridine (bpy) as N,N-donor ligand have been used for the preparation of mixed–ligand complexes MLA of cobalt(II) complexes, and an intercalative mode of binding has been suggested from the interaction of the complex [Co^{II}-L_4-A] by means of calf thymus DNA (CT DNA) using absorption spectroscopic technique [177].

2.4.2 DNA interaction investigations of Schiff base metal complexes

Cu(II), Zn(II) and Ni(II) complexes of 3-carbaldehyde chromone thiosemicarbazone have been investigated for their interaction with DNA by spectra and viscosity technique [178]. The outcome revealed that the compounds bind to DNA by means of intercalation, while Zn(II) complex exhibited the most strongly binding mode. The metal complexes embed and stack between the DNA base pairs more effortlessly and profoundly than the free Schiff base ligand. This is can be related to the coordination impacts (metal particles to free ligand) that might boost the planar functionality of the metal compounds. Also, *in vitro* antioxidant screening showed the complexes have noteworthy antioxidant action against superoxide and hydroxyl radicals, while the cancer prevention impacts of Cu(II) complex is much more better than the complexes of Zn(II) and Ni(II) [178].

In 2012, Nair *et al.* [179] recounted the nuclease activity of the synthesized heterocyclic Schiff base **(29)** of Co(II), Cu(II), Zn(II) and Ni(II) metal complexes got from indole-3-carboxaldehyde and m-aminobenzoic acid assayed on CT DNA using gel

electrophoresis in 0.5 mM NaCl/ 5 mM Tris–HCl (pH 7) at a proportion of UV absorbance at 260 and 280 nm and H_2O_2. The reaction was modulated via the bounding of metallo-complexes to hydroxyl radical or species of peroxo produced from the co-reactant H_2O_2. The bacteriological action of the synthesized ligand and its complexes were screened by disc diffusion technique against the bacterial strains. The bacteriological and CT DNA cleavage activities signposted the complexes have better biological activity than the free ligand and in order of: Cu(II) >Co(II) > Ni(II) >Zn(II) > Ligand [179].



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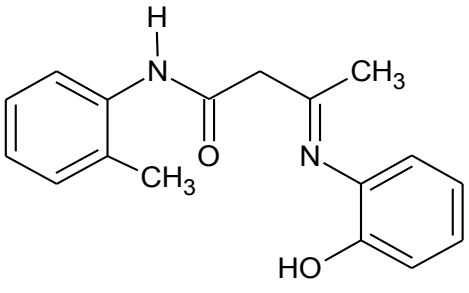
The DNA-binding properties of three lanthanide(III) complexes, of the type $M(H_2L)_3 \cdot 3NO_3$ [where M = Nd(III), Sm(III), Eu(III) and H_2L = 1-(4-Aminoantipyrine)-3-tosylurea] have been synthesized and investigated by UV-VIS absorption spectroscopy, fluorescence spectra at 441 nm, cyclic voltammetry, viscosity measurements, and circular dichroism (CD) spectroscopy [180]. Findings from this study propose that the synthesized complexes bind to DNA through “groove binding mode”. Moreover, the *in vitro* antioxidant action such as the superoxide and hydroxyl radical of the metal complexes was resolved spectrophotometrically [180].

In 2012, Pothiraj and co-workers [181] reported the mononuclear complexes of Cu(II) and Zn(II) having a Schiff-base ligand (HL) **(30)** and 1,10- phenanthroline/ 2,2'-bipyridine as anchoring ligands. From electronic spectra and magnetic susceptibility data, octahedral geometry was projected for the complexes. DNA-binding behaviours of these complexes by absorption titration, electrochemical, and viscosity methods, demonstrated that the complexes bind to calf thymus DNA in an intercalative manner, and the gel electrophoresis results reveal that [Cu(L)(phen)Cl] cleaves pBR322 DNA effectively.

The ligand and its complexes were also screened against some selected microorganisms for their antimicrobial activities. It was revealed that the compounds are better bactericidal agents than the ligand, while all present compounds synthesised are not as effective against the tested microorganisms as currently used drugs [181]. The cytotoxicity assays of 2-((2-((benzo[*d*]oxazol-2-yl)methoxy)phenoxy)methyl)benzoxazole (L) and alongside its transition metal complexes: Co-L, Ni-L, Cu-L, Zn-L, against four different tumor cell lines (A549, Hep G2, K562, K562/ADM) have been evaluated by MTT assay [182]. DNA binding studies indicated that both the ligand and the complexes bonded to DNA by intercalation manner, and antitumor activity of these compounds gave the order: Cu-L > Ni-L = Co-L > Zn-L >> L [182].



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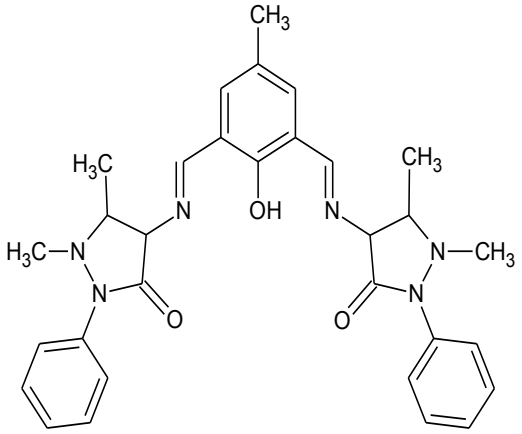
Liu *et al.* [183] have reported the single-crystal X-ray diffraction investigation and biological action of cobalt dinuclear complex: $(Co_2(Dpmp)_2(NO_3)_2(H_2O)_2 \cdot NO_3 \cdot EtOH$ and zinc mononuclear complex $Zn(Dpmp)(NO_3)_2$ where Dpmp = 2,6-di((phenazonyl-4-imino) methyl)-4-methylphenol (Figure 2.7) **(31)**. The compounds were evaluated for their antioxidant action and capability to bind to bovine serum albumin (BSA) and calf thymus DNA (CT DNA) using UV-VIS absorption, fluorescence at 280 nm, viscosity quantities and circular dichroism (CD) spectroscopies. The compounds were reported to display good binding penchant to BSA and CT DNA, and also able to stimulate cleavage of pUC19 DNA without any reducing agent. *In vitro* antioxidant investigations reveal the complexes to have noteworthy cancer prevention agent action against superoxide and hydroxyl radicals [183].



(31)

Figure 2.7: Structure of 2,6-di((phenazonyl-4-imino)methyl)-4-methylphenol ligand (Dpmp)

DNA binding properties of a unique Schiff-base ligand (H_5L), hesperetin-2-hydroxy benzoyl hydrazone, and its copper(II), zinc(II) and nickel(II) complexes ($M \cdot H_3L$) [M(II) = Cu, Zn, Ni] have been examined via electronic absorption spectroscopy, ethidium bromide displacement experiments, fluorescence spectra, salt effect viscosity measurements and iodide quenching experiments [184]. Findings from this study propose that all the compounds

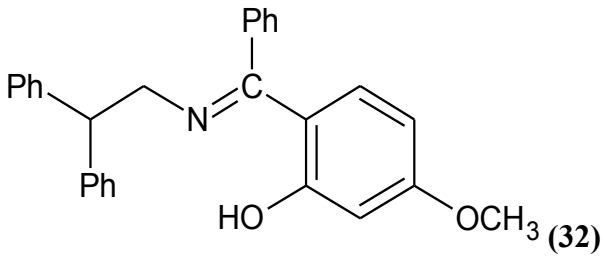
fix to DNA by means of intercalation binding method. Moreover, the cancer prevention agent activity of the ligand and its metal complexes was investigated by superoxide and hydroxyl radical rummaging procedures *in vitro*. The metal complexes were established to have effective antioxidant action and be better than the free ligand alone and some regular antioxidants like vitamin C and mannitol [184].

The Cu(II), Ni(II), Co(II), and Zn(II) complexes of symmetrical tetradentate Schiff base prepared by water loss from 5-nitro-*o*-vanillin and diaminoethane have been reported [36]. The DNA-binding behaviour of these complexes was investigated and it was suggested that they intercalate between DNA base pairs. The metal(II) ions are coordinated by two phenolic oxygen and two azomethine nitrogen (–CH=N) of the ligand with square-planar geometry around Cu(II) and Ni(II) complexes and tetrahedral geometry around Co(II) and Zn(II) complexes. Antibacterial action was greater against Gram(+) than Gram(-) microbes for Cu(II) complex and antifungal action was greater against *Candida albicans* and *Aspergillus niger* for the Cu(II) complex [36].

Saglam *et al.* [185] have reported the oxidative cleavage of DNA by homodinuclear Cu(II), heterodinuclear Cu(II)-Mn(II) and homotrinuclear Cu(II) complexes with an oxime-sort ligand. The results of the cleavage studies gotten electrophoretically indicated that even though the evaluated complexes prompts similar conformational variations on supercoiled DNA by translating supercoiled form to nicked form and then to linear form in a chronological mode as the complex concentration or response period is amplified, the homotrinuclear Cu(II) complex is observe to be less effective than the two other complexes. The metal complex that was able to induce DNA cleavage was additionally examined for its radical restraint by utilizing different radical scroungers like: superoxide dismutase (SOD),

potassium iodide, azide and thiourea [185]. It was reported that the nucleolytic system involving copper and/or manganese complex intervened reactive oxygen species like hydroxyl radicals being in charge of the oxidative DNA cleavage [185].

Biologically important Cu(II), Co(II), Mn(II) and Ni(II) complexes was synthesised [186] and confirmed as a neutral bidentate Schiff base derived from 2,2-diphenylethanamine and 2-hydroxy-4-methoxy benzophenone (Figure 2.8) **(32)**, and organizing via azomethine nitrogen and oxygen atom of hydroxyl assemblage. The interaction investigations of these complexes with CT-DNA using spectra and electrophoresis techniques have indicated an evidences for groove binding of the DNA with the synthesised metal complexes, and in addition, the complexes showed their efficient antimicrobial activities against bacteria (*Escherichia coli* and *Staphylococcus aureus*) [186].



(32)

Figure 2.8: Structure of 2-((2,2-diphenylethylimino)(phenyl)methyl-5-methoxyphenol)

Copper(II) complex of quinolin-4(3*H*)-one Schiff base ligand (Figure 2.9) **(33)** derived from the reaction of 3-amino-2-methyl-4(3*H*)-quinazolinone with diverse substituted aromatic aldehydes have been reported [187]. The DNA interaction investigations suggests that the copper(II) complex performs as avid binding and cleaving agent [187].

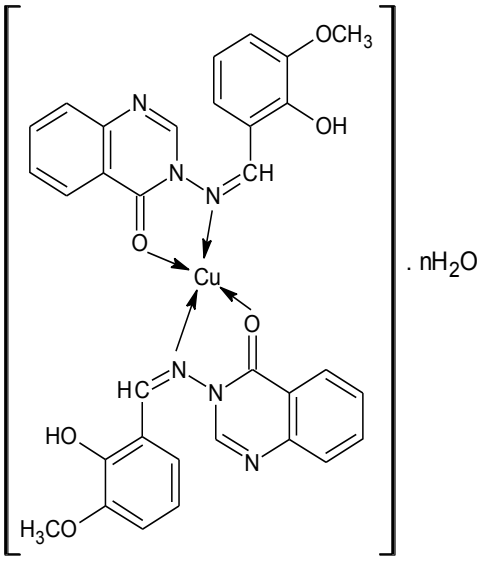


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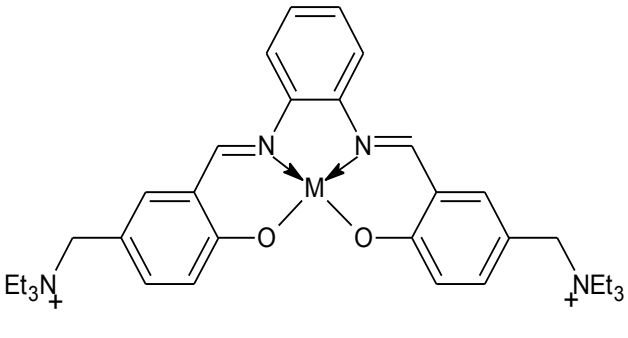
Figure 2.9: Structure of copper(II) complex of quinolin-4(3*H*)-one

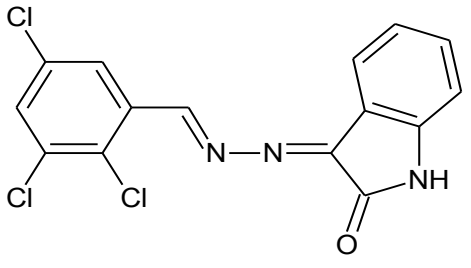
Silvestri *et al*. [188] reported the association of native calf thymus-DNA with the Zn(II) and Cu(II) complexes containing 5-triethyl ammonium methyl salicylidene orto-phenylendiimine Schiff base (Figure 2.10) as ZnL^{2+} and CuL^{2+}, **(34)** has been observed as a key player in the metal complex-DNA molar proportion utilizing 1 mM Tris–HCl aqueous solutions at unbiased pH with UV absorption spectrophotometry, fluorescence spectroscopy and circular dichroism (CD). The outcomes supported an intercalative interaction of both ZnL^{2+} and CuL^{2+} with DNA, demonstrating that CuL^{2+} has an proclivity of approximately 10 times greater than ZnL^{2+}. A close-fitting ZnL^{2+}–DNA and CuL^{2+}–DNA binding was likewise established by the surfacing, in the two metal complex-DNA solutions, an extensive impelled circular dichroism [CD] band in the region 350-450 nm. It was also hypothesized that CuL^{2+} impels the arrangement of supramolecular collections of DNA in aqueous solutions [188].



(34)

Figure 2.10: Structure of the ZnL^{2+}, CuL^{2+} complex, L^{2+} = 5-triethyl ammonium methyl salicylidene orto-phenylendiimine).

In 2011, Raman and co-workers [189] reported a Schiff base **(35)**, got by the condensation of isatin monohydrazone with 2,3,5-trichlorobenzaldehyde, and its Zn(II), Ni(II), Co(II), and Cu(II) complexes. Oxidative cleavage exercises of the complexes was examined utilizing supercoiled pBR322 DNA by gel electrophoresis and they displayed higher nuclease activity for the Cu(II) complex than other complexes, hence the cooperative interaction of metal ions is a factor to cleave DNA. The author also revealed that the antimicrobial activities showed that the metal complexes are more dynamic than the ligand; Cu(II) and Zn(II) complexes were observed to be more dynamic than the Ni(II) and Co(II) complexes [189].

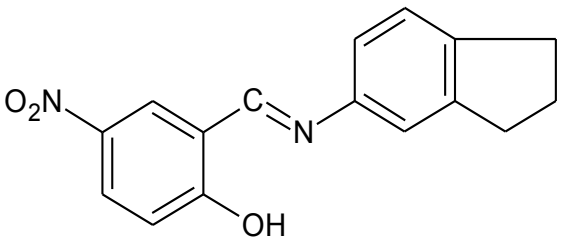


(35)

2.4.3 Anticancer activity of Schiff base metal complexes

Anticancer properties of copper(II) complexes containing Schiff bases resulting from *S*-benzylde thiocarbazate and saccharinate, has been investigated against the leukemic cell line (HL-60), and observed that only copper(II) complex of the type [Cu(NNS)(sac)] exhibited strong cytotoxicity; higher than the standard anticancer drug Doxorubicin against the ovarian cancer cell line (Caov-3) [190]. Schiff base, 2-[(2,3-dihydro-*1H*-inden-4-ylimino)methyl]-5-nitrophenol (Figure 2.11) **(36)** coordinating to Zn(II), Cu(II), Mn(II), and Pd(II) ions through the phenolic O and the azomethine imine N atoms have been reported [191]. The selection of a four-coordinate, tetrahedral geometry for the Mn(II) and Zn(II) complexes, and a four-coordinate, square planar geometry for the Pd(II) and Cu(II) complexes were proposed.

The *in vitro* anticancer investigation of the free ligand, Cu(II), Zn(II), and Pd(II) complexes against two (2) cell lines: MCF-7 (human breast adenocarcinoma) and HT-29 (colon carcinoma) revealed that the Pd(II) complex exhibited the greatest cytotoxic action against MCF-7 cells with an IC_{50} of 5.94 μM, and this is within similar order of action as cisplatin, while the ligand and Zn(II) complex exhibited wide-ranging band action against two gram-positive microbes, three gram-negative microorganisms, and a fungus [191].



(36)

Figure 2.11: Schiff base structure of 2-[(2,3-dihydro-*1H*-inden-4-ylimino)methyl]-5-nitrophenol

Chaviara *et al.* [192] synthesized a sequence of compounds with the preparatory materials [Cu(dienX_2Y_2)] and their adducts [Cu(dienXXY_2)(2a-5mt)] (then dien = diethylenetriamine, dienXX = Schiff bases of diethylenetriamine with 2-furaldehyde or 2-thiophene-carboxaldehyde, X = O, S; Y = Cl, Br, NO_3 and 2a-5mt = 2-amino-5-methylthiazole) by stepwise reactions, the pentadentate Schiff base (dienX_2) was bonded in a tridentate approach via the 3N atoms of the dien with heterocyclic aldehydes and the coordination circle of Cu was accomplished by the endocyclic N^3 from 2a-5mt and by two (2) Cl, Br or NO_3 groups in a distorted octahedral geometry, also the insertion of 2a-5mt affected the collection of electronic, physicochemical and steric properties of the subsequent compounds [192]. According to the authors, the antibacterial and antiproliferative studies of the compounds synthesized against a board of diverse normal and growth cell lines (MRC-5, OAW-42, MCF-7, HT-29, HeLa, T47D) and microscopic organisms (*E. coli, B. subtilis, B. cereus*) demonstrated that the adducts of the sort [Cu(dienXXY_2)(2a-5mt)] exhibited expanded action both in malignancy cells and in microbes, contrasted with the preparatory material of sort [Cu(dienXXY_2)] [192].

Recent report on the *in vitro* cytotoxic screening of organoantimony(V) and organobismuth(V) complexes of the type ML_2, with L = acetylsalicylic acid (HL_1) or 3-acetoxybenzoic acid (HL_2) and M = triphenylantimony(V) (M1) or triphenylbismuth(V) (M2), [M1$(L1)_2$] (1), [M1$(L2)_2$]·$CHCl_3$ (2), [M2$(L1)_2$], (3) and [M2$(L2)_2$] (4) complexes against murine macrophages showed that antimony(V) complexes were the least toxic [193]. Following the selectivity indexes, organoantimony(V) complexes was reported to emerge as the most promising antileishmanial agents and organobismuth(V) complex as the best antibacterial agent [193]. Antitumor and antimicrobial studies of copper (II) complexes of Schiff bases derived from 7*H*-2,6-diaminopurine and 4*H*-3,5-diamino-1,2,4-triazole with 2-

pyridinecarbaldehyde, salicylaldehyde, 2,4-dihydroxybenzaldehyde and 2-hydroxy-1-naphthaldehyde were reported [194].

In vitro antitumor activity of $[Cu_2L_2Cl_2(H_2O)_2]$ complex showed a moderate activity against the breast cancer (MCF7), colon carcinoma (HCT116) and liver carcinoma (HEPG2) cell lines; gave an activity order of MCF7 > HCT116 > HEPG2 [194]. Cytotoxic and antitumor activity of Pd(II), Pt(IV) and Au(II) complexes of Schiff bases [195]: 4-[(Furan-2-ylmethylene)-amino]-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one (4APF) derived from 2-furaldehyde and 4-amino antipyrine have been tested against breast carcinoma cells (MCF-7) cell line as cellular damage. The 4APF Schiff base ligand and the metal complexes were likewise evaluated for their antibacterial action [195]. Creaven *et al.* [93] reported the *in vitro* anticancer activities of Cu(II) complexes of a series of quinolin-2(1*H*)-one-derived Schiff base against Human hepatic carcinoma cell line, Hep-G2 while cisplatin was used as a standard drug. Compound (7*E*)-7-(3-ethoxy-2-hydroxybenzylideneamino)-4-methylquinolin-2(1*H*)-one was reported as a potential therapeutic agent [92].

2.4.4 Anticancer properties of ruthenium Schiff base complexes

Raja *et al.* [72] reported the *in vitro* cytotoxicity study of hexa-coordinated ruthenium(II) Schiff base complexes of the sort [RuCl(CO)(B)L] (showing that B = PPh_3/ $AsPh_3$/ py and L = monobasic tridentate Schiff base ligand obtained by the build-up of salicylaldehyde with 4-aminoantipyrine. The IC_{50} value for the ligand and $[RuCl(CO)(PPh_3)L]$ complex were 52.3 and 31.6 μM respectively [72]. Ruthenium(III) complexes of three tetradentate Schiff bases bearing N_2O_2 donors resulting from ethane-1,2-diamine, 4-acetylresorcinol, acetylacetone and 1-phenylbutane-1,3-dione articulated as

[$RuCl(LL^1)(H_2O)$], [$RuCl(LL^2)(H_2O)$] and [$RuCl(LL^3)(H_2O)$] have been reported to exhibit moderate to strong scavenging activity on DPPH and ABTS radicals [196].

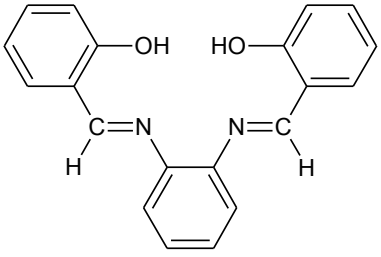
The *in vitro* anticancer examinations of the Ru(III) complexes and parthenolide (standard) with concentrations extending from 0.01 to 100 μM were investigated using three malignancy cell lines: human renal cancer cell (TK-10), human melanoma cancer cell (UACC-62) and human breast cancer cell (MCF-7) utilizing the SRB test. It was established that complexes of Ru(III) demonstrated low to moderate *in vitro* antiproliferative impact when contrasted to parthenolide (standard drug) against the selected cancerous cell lines [196].

Ruthenium complexes are efficient in DNA binding [197]. MTT assay has been used for *in vitro* anticancer activity evaluation of novel ruthenium (III) Schiff base complexes of the sort [$RuX_2(PPh_3)_2(L)$] (where X = Cl or Br; L = monobasic bidentate Ligand) [198]. An octahedral geometry was proposed for all the Ru(III) complexes. The compounds were evaluated for antimicrobial activity, likewise the DNA–binding studies of the complexes by electronic spectra [198].

Three new mononuclear ruthenium(III) compounds comprising Schiff base (HL) have been prepared, characterized by spectroscopic studies [199]. Anticancer study of the Schiff base (HL) and the complex [$RuCl_2(AsPh_3)L$] against human cervical cancer cell line (HeLa) using the MTT test was also reported. The IC_{50} values for the free ligand (HL) and complex are 52.3 and 37.8 μM, respectively, however, these values were found to be low when compared to the standard anticancer drug: cisplatin, with IC_{50} value of 16.7 μM [199].

2.5 Miscellaneous application of symmetrical and asymmetrical Schiff bases and their complexes

Abd-Elzar [200] reported the spectroscopic characterization of tetradentate Schiff bases with a N_2O_2 donor atom set formed by the 1:2 molar condensation of *o*-phenylenediamine with corresponding aldehyde, to afford: *N,N′*-bis(salicyldhyde)-*o*-phenylenediamine **(37)**, *N,N′*-bis(*o*-hydroxyacetophenone)-*o*-phenylenediamine **(38)**, *N,N′*-bis(2-hydroxy-1-naphthaldhyde)-*o*-phenylenediamine **(39)**, and their nickel, copper and zinc complexes. The spectroscopic revealed that the metal ions are bounded to the Schiff bases via the phenolic oxygen and the imino nitrogen and confirms a square-planar geometry for these complexes [200].



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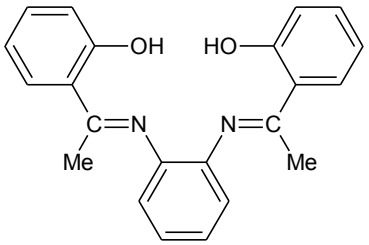
N,N′-bis(salicyldhyde)-*o*-phenylenediamine



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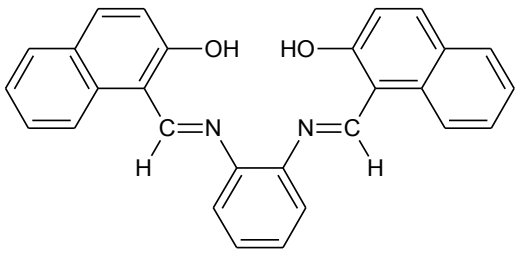
N,N′-bis(*o*-hydroxyacetophenone)-*o*-phenylenediamine'

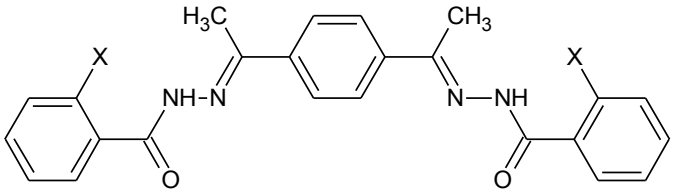


(39)

N,N′-bis(2-hydroxy-1-naphthaldhyde)-*o*-phenylenediamine

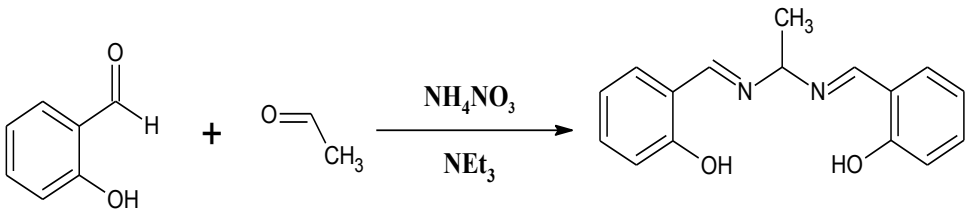
Complexation of Ni(II) and VO(II) complexes with Schiff base of sulfamethoxazole [4-amino-N-(5-methyl-3-isoxazolyl) benzenesulfonamide] and salicylaldehyde, acting as a

bidentate with N, O donor atoms has been reported [201]. Gup and Kirkan [202] reported synthesis, spectroscopic investigations of mixed-ligand and polymeric dinuclear Cu(II), Ni(II) complexes by means of tetradentate N_2O_2 donor ligands: 1,4-bis(1-anthranoylhydrazonoethyl) benzene (L^1), 1,4-bis(1-salicyloylhydrazonoethyl)benzene (L^2) and *N,N′*-bidentate heterocyclic base [1,10-phenonthroline (phen)] of the type: $[M_2L(phen)_2]Cl_2$ (L = L^1 or L^2) **(40)**, The impact of fluctuating pH and solvent on the absorption behaviour of the Schiff base ligands and complexes was examined [202].



Where L^1: X = $-NH_2$, L^2: X = -OH **(40)**

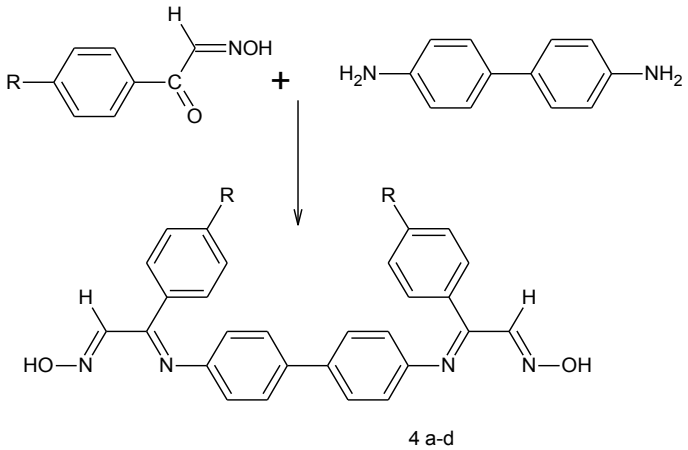
In 2013, Ammar and Alaghaz [203] reported the spectroscopic, characterization and potentiometric investigations of tetradentate $[N_2O_2]$ Schiff base complexes of the kind: $[M(BHBDAE)(H_2O)_2]$ where M = Co(II), Zn(II), Ni(II) and Cu(II); BHBDAE; *N,N′*-bis(2–hydroxybenzylidene)–1,1–diaminoethane (Scheme 2.5) **(41)**. The structural properties of the compounds were elucidated by IR, magnetic moment, ^1H NMR, EPR as well as electronic spectroscopy information. Protonation constants of Schiff base ($H_2BHBDAE$) and stability constants of the binary Co(II), Zn(II), Ni(II) and Cu(II) complexes were resolved potentiometrically in 50 % DMSO–water media at 37 °C and ionic strength 0.10 mol dm^{-3} sodium nitrate. The results showed that the metal chelates stability follows the order: Co < Ni < Cu < Zn. The complex exhibited an anisotropic EPR spectra typical for hexagonal geometry of copper(II) complex [203].



(41)

Scheme 2.5: Synthesis of *N,N′*-bis(2–hydroxybenzylidene)diamino-1,1-ethane

Thakkar and Bootwala [204] have recounted the synthesis and characterization of binuclear Fe(II), Co(II) and Ni(II) complexes of tetradentate of Schiff base (Scheme 2.6): bis(2-hydroxyimino-1-(4-R-phenyl)-1-ethylidene)benzidine, R = H, Cl, Br, CH_3 **(42)**. Substitution in the phenyl ring produces shifts in the azomethine stretching vibration $\nu_{C=N}$ and varies as Fe > Co > Ni indicating extensive delocalization of electrons from the highly populated d-orbitals into π* orbitals of the ligand. The magnetic moments and electronic spectra indicate octahedral geometry for the complexes [204].



4 a-d

R	
4a; R = H	$(HINAP)_2$ bz
4b; R = Cl	(*p* - Cl - $HINAP)_2$ bz
4c; R = Br	(*p* - Br - $HINAP)_2$ bz
4d; R = CH_3	(*p* - CH_3 - $HINAP)_2$ bz

(42)

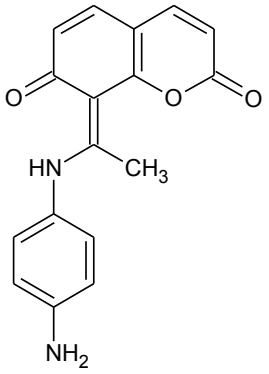
Scheme 2.6: Preparation Scheme for: bis(2-hydroxyimino-1-(4-R-phenyl)-1-ethylidene)benzidine

Fakhari *et al.* [205] reported the synthesis and usage of *N,N′*-bis(3-methylsalicylidene)-ortho-phenylenediamine ligand (MSOPD), in the nickel spectrophotometric determination. MSOPD was found to interact at room temperature with nickel ion forming 1:1 mole proportion complex at a pH of 8. The apparent molar absorptivity was observed to be 9.5×10^4 l mol^{-1} cm^{-1} at a wavelength of 430 nm. Nickel in some usual food samples were examined, utilizing a detection limit of 1.36×10^{-8} M [205].

Pre-concentration of Cu(II), Ni(II), Cd(II), and Zn(II) via a minicolumn loaded with modified silica gel and (*N,N′*-bis(salicylene)-phenylene-1,3-diamine Schiff' base have been proposed [206]. The proposed technique has been utilized for the examination of the metal ions in normal water samples and standard reference aluminium blend material.

In 2012, Aazam *et al.* [207] reported the preparation of mononuclear Zn(II), Pd(II), Cu(II), Ni(II) and Cd(II) metal complexes of Schiff-base ligand **(43)** got from 8-acetyl-7-hydroxycoumarin and *P*-phenylenediamine. The spectra data revealed that Zn(II) and Ni(II) complexes are octahedral, tetrahedral geometry for Cd(II) complex, Cu(II) and Pd(II) complexes exhibit square planar geometry.

The Schiff base displayed photoluminescence emanating from intraligand (π–π*) transitions. Metal-mediated enrichment was reported to be observed upon the complexation of the ligand with Zn(II) and Cd(II), whereas metal-mediated fluorescence quenching occurs in Cu(II), Pd(II) and Ni(II) complexes. These properties could be exploited for the detection and spectrofluorimetric determination of Zn(II) and Cd(II) in environmental, biological and pharmaceutical formulations [207].



(43)

Unsymmetrical salen-type ligands integrating two distinctive benzylidene moieties and a diamine support have been synthesized in high return (80–90%) under gentle environments via a stepwise manner [208]. Anhydrous hydrochloric acid was utilized to specifically shield one amino assemblage of the adjacent diamine backbone followed by the reaction of an equivalent of a different benzaldehyde derivatives such as (2-methoxy-3,5-bis(tert-butyl); 2-hydroxy-3,5-dibromo; 2-hydroxy-3,5-dinitro; 2-hydroxy-3-tert-butyl; 2-hydroxy-3-tert-butyl-5-dimethylamino; 2-hydroxy-3-tert-butyl-5-iso-propoxy) in the presence of triethylamine to afford an unsymmetrical salen-type ligand. The method of reaction proposed by the authors allows facile access to a plethora of salen-type Schiff base ligands with easily tuneable steric and electronic properties [208].

In 2015, Yamada and co-workers [36] reported the N_2O_2 ligands: H_2L^1 and H_2L^2, to act as bis-bidentate ligands and react with zinc(II) ions, which prefer four-coordinate tetrahedral environment, to form dinuclear complexes formulated as $[Zn_2(L^1)_2]$ and $[Zn_2(L^2)_2]$, respectively. Both of the zinc(II) centres in $[Zn_2(L^1)_2]$ are considerably distorted from the ideal tetrahedron, while the distortions in $[Zn_2(L^2)_2]$ are less due the flexibilities around the azomethine groups in H_2L_2. The two complexes exhibited yellowish-green light emitters on exposure to ultraviolet light and therefore, potentially applicable for modelling

active sites of the hydrolytic enzymes, where the zinc(II) centres are ligated by hard donors such as nitrogen and/or oxygen [36].

2.6 Summary of the Chapter

The huge research on Schiff base metal complexes has given rise to several new molecules in the past decades that have been of biological importance. Schiff base ligands and their biologically active complexes were described to provide potential sites as biochemically active compounds due to their biological properties. Schiff base–transition and inner-transition metal complexes are one of such adaptable and thoroughly studied compounds used as model molecules for biological probes, analytical tools, in pharmaceutical as well as chemical sciences. Also, metal based (synthetically prepared compound) antioxidants that can be measured using the scavenging ability of the compound with respect to Schiff base metal complexes to trap free radicals, protect living organisms and cells from damage caused by oxidative stress or scavenge free radicals. It is expected that progress will be experienced in the field of disease control upon the utilization of the Schiff' base-transition metal complexes as future chemotherapeutic agents.

Chapter Three

Experimental

CHAPTER THREE

EXPERIMENTAL

3.0 Background to the Chapter

This chapter describes the synthesis of symmetrical and unsymmetrical tetradentate Schiff bases; tridentate unsymmetrical Schiff bases that were used for the preparation of ruthenium(III), zinc(II), cobalt(II), nickel(II) and copper(II)-Schiff base complexes in Chapters Four and Five. Section 3.1 gives the list of reagents and chemicals used in this research. Sections 3.2.1 and 3.2.2 explain the method utilized for the synthesis of symmetrical and unsymmetrical Schiff bases while 3.2.3 and 3.2.4 explain the preparation of ruthenium(III) complexes. This involved both the synthesis of both the tetradentate ruthenium(III)-Schiff base complexes and tridentate ruthenium(III)-Schiff base complexes. In sections 3.2.4, synthesis of the symmetrical and unsymmetrical tetradentate zinc(II), cobalt(II), nickel(II) and copper(II) complexes were reported.

Characterization techniques utilized in the study are described in section 3.3 and 3.4. The biological methodology of the Schiff bases and their metal complexes are presented in sections 3.4 for the antimicrobial studies; 3.5 for Antioxidant assay; while section 3.6 contain the *in vitro* antiproliferative activity for the ruthenium(III)-Schiff base complexes.

3.1 Materials

All reagents and chemicals were of analytical grade and used without further purification. Chemicals utilized for the preparation of Schiff base ligands are: 2,4-

pentanedione (Fluka), Ethylenediamine (Merck), 2',4'-dihydroxyacetophenone (Aldrich), 1-phenylbutane-1,3-dione (Aldrich), Benzaldehyde (Fluka), Benzoyl chloride (Merck), acetanilide (Merck), Acetophenone (BDH), Vanillin (4-hydroxy-3-methoxybenzaldehyde) (Sigma-Aldrich), 4-methoxybenzaldehyde, Veratraldehyde (3,4-dimethoxybenzaldehyde) (Sigma-Aldrich), Methyladehyde (Sigma).

Metal Salts: Cobalt(II) acetate tetrahydrate, copper(II) acetate dihydrate, nickel(I1) acetate tetrahydrate, ruthenium(III) chloride, zinc(lI) acetate dihydrate (Merck).

Antioxidants: Ascorbic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Rutin hydrate, butylated hydroxytoluene (BHT), were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Solvents: Absolute Ethanol, methanol, distilled water, chloroform, acetonitrile, Dimethylsulfoxide, dichloromethane, *N,N′*-dimethylformamide, acetone, diethylether.

3.2 Synthesis

3.2.1 Synthesis of tetradentate Schiff bases

3.2.1.1 Preparation of symmetrical tetradentate Schiff base *N,N′*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine ligand

The ligand was prepared following a literature method [200]. An ethanol solution (20 mL) containing ethane-1,2-diamine (10 mmol, 0.601 g) was added slowly to a stirring ethanolic solution (50 mL) containing 2',4'-dihydroxyacetophenone (20 mmol, 3.043 g). The resulting light brown mixture was reflux for 3 h, and left to stand at room temperature overnight. The obtained precipitate was filtered and washed with ethanol, followed by

recrystallization in warm ethanol and air-dried to give a brownish-yellow solid and further dried in a desiccator over anhydrous $CaCl_2$.

[HLL^1, ehopd] = *N,N′*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine ligand.

3.2.1.2 Preparation of unsymmetrical tetradentate Schiff bases *N*-(2',4'-dihydroxy-phenylethylidene)-*N′*-(1,3-butanedioneethylidene)ethylenediamine ligand

The ligand was prepared following a literature method [117]. Ethylenediamine (15 mmol, 0.902 g) in 30 mL ethanol was slowly added to ethanol solution (40 mL) containing 2',4'-dihydroxyacetophenone (15 mmol, 2.282 g), followed by the slow addition of acetylacetone (15 mmol, 1.502 g) dissolved in 30 mL ethanol over some period of time. The resulting coloured mixture was refluxed with stirring for 4 h. The mixture was left standing with continuous stirring for approximately 24 hours at room temperature and the subsequent precipitate was separated by filtration, washed with ethanol, followed by recrystallization in ethanol, air-dried to give a solid and dried over anhydrous $CaCl_2$.

[HLL^2, ehata] = *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedioneethylidene)ethylene diamine ligand.

3.2.1.3 Preparation of unsymmetrical tetradentate Schiff bases *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedionephenylidene)ethylenediamine ligand

The ligand was prepared following a literature method [117]. Ethylenediamine (15 mmol, 0.902 g) in 30 mL ethanol was slowly added to ethanol solution (40 mL) containing 2',4'-dihydroxyacetophenone (15 mmol, 2.282 g), followed by the slow addition of 1-phenylbutane-1,3-dione (15 mmol, 2.433 g) dissolved in 40 mL ethanol over some period of

time. The resulting coloured mixture was refluxed with stirring for 4-5 h. The mixture was left standing with continuous stirring for approximately 24 hours at room temperature and the subsequent precipitate was separated by filtration, washed with ethanol, followed by recrystallization in ethanol, air-dried to give a solid and dried over anhydrous $CaCl_2$.

[HLL^3, ehbta] = *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedionephenylidene) ethylenediamine ligand.

3.2.2 Synthesis of tridentate unsymmetrical Schiff bases

3.2.2.1 Synthetic procedure for compounds [HLL^4, ehmta] - [HLL^7, ehacp] ligands

The ligand was prepared following a literature method [117]. Ethylenediamine (15 mmol, 0.902 g) in 20 mL ethanol was gently added to ethanol solution (30 mL) containing 2',4'-Dihydroxyacetophenone (15 mmol, 2.282 g), permitted to stir for 60 minutes at room temperature, then followed by drop-wise addition for 10-15 minutes of appropriate aldehyde: methylaldehyde (15 mmol, 0.451 g); benzaldehyde (15 mmol, 1.592 g); 4-methoxy benzaldehyde (15 mmol, 2.043 g); acetophenone (15 mmol, 1.802 g) dissolved in 20-30 mL ethanol at room temperature. The resulting mixture was refluxed with stirring for 3-4 h, and left to stir further for 22-24 hours at room temperature to give a crystalline solid after suction filtration, washed with ethanol. The crude product was recrystallized from warm ethanol. The products were dried in the vacuum at 50 ^oC overnight to give the required product and further dried in a desiccator over anhydrous $CaCl_2$.

[HLL^4, ehmta] = *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(methylidene)ethylenediamine ligand.

[HLL^5, ehben] = *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(benzylidene)ethylenediamine ligand.

[HLL^6, ehmez] = *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(4-methoxybenzylidene)ethylene-diamine ligand.

[HLL^7, ehacp] = *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1-phenylethylidene)ethylene-diamine ligand.

3.2.2.2. Synthetic procedure for compounds [HLL^8, ehacn] - [HLL^{11}, ehbzc] ligands

Ethylenediamine (15 mmol, 0.902 g) in 20 mL ethanol was gently added to ethanol solution (30 mL) containing 2',4'-Dihydroxyacetophenone (15 mmol, 2.282 g), allowed to stir for 60 minutes at room temperature, then followed by drop-wise addition for 10-15 minutes of appropriate aldehyde: acetanilide (15 mmol, 2.027 g); 4-hydroxy-3-methoxy benzaldehyde (15 mmol, 2.282 g); 3,4-dimethoxy benzaldehyde (15 mmol, 2.493 g); benzoylchloride (15 mmol, 2.108 g) dissolved in 30 mL ethanol at room temperature and stirred for 120 minutes. The mixture was left standing with continuous stirring for approximately 36 hours at room temperature to give a crystalline solid after suction filtration, washed with ethanol. The crude product was recrystallized from warm ethanol. The products were dried in the vacuum at 50 ^oC overnight to give the required product.

[HLL^8, ehacn] = N-(2',4'-dihydroxyphenylethylidene)-N'-(phenylethanimidamide)ethylene diamine ligand.

[HLL^9, ehvan] = *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(4-hydroxy-3-methoxybenzylidene) ethylenediamine ligand.

[HLL^{10}, ehvet] = *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(3,4-dimethoxybenzylidene) ethylenediamine ligand.

[HLL^{11}, ehbzc] = *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(benzenecarboximidoylchloride) ethylenediamine ligand.

3.2.3 Preparation of ruthenium(III) complexes

3.2.3.1 Preparation of *N,N′*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine complex of ruthenium(III)

A general approach was followed for the synthesis of all the ruthenium(III) complexes [209]. All operations were carried out under strictly anhydrous conditions. The various complexes were prepared by the addition of (0.5 mmol, 0.242 g) of $RuCl_3 \cdot 3H_2O$ dissolved in about 15 mL of Absolute ethanol, into a hot ethanolic solution (20 mL) of (0.5 mmol, 0.164 g) of *N,N′*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine ligand [HLL^1, ehopd] in molar ratio (1:1). The colour of the complexes changed immediately. The resulting mixture was then refluxed for 6 hours. The precipitated solids were allowed to cool and filtered off from the reaction mixture, thoroughly washed with absolute ethanol and then with (3 x 5 mL) diethyl ether, dried over anhydrous calcium chloride.

3.2.3.2 Preparation of *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedione ethylidene)ethylenediamine complex of ruthenium(III)

A general approach was followed for the synthesis of all the ruthenium(III) complexes [209]. All operations were carried out under strictly anhydrous conditions. The various complexes were prepared by the addition of (0.5 mmol, 0.242 g) of $RuCl_3 \cdot 3H_2O$ dissolved in about 15 mL of Absolute ethanol, into a hot ethanolic solution (20 mL) of *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedioneethylidene)ethylenediamine ligand [HLL^2, ehata] (0.5 mmol, 0.138 g) in molar ratio (1:1). The colour of the complexes changed immediately. The resulting mixture was then refluxed for 6 hours. The precipitated solids were allowed to cool and filtered off from the reaction mixture, thoroughly washed with

absolute ethanol and then with (3 x 5 mL) diethyl ether, dried over anhydrous calcium chloride.

3.2.3.3 Preparation of *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedione phenylidene)ethylenediamine complex of ruthenium(III)

A general approach was followed for the synthesis of all the ruthenium(III) complexes [209]. All operations were carried out under strictly anhydrous conditions. The various complexes were prepared by the addition of (0.5 mmol, 0.242 g) of $RuCl_3 \cdot 3H_2O$ dissolved in about 15 mL of Absolute ethanol, into a hot ethanolic solution (20 mL) of *N*-(2',4'-di hydroxyphenylethylidene)-*N′*-(1,3-butanedionephenylidene)ethylenediamine ligands [HLL^3, ehbta] (0.5 mmol, 0.169 g) in molar ratio (1:1). The colour of the complexes changed immediately. The resulting mixture was then refluxed for 6 hours. The precipitated solids were allowed to cool and filtered off from the reaction mixture, thoroughly washed with absolute ethanol and then with (3 x 5 mL) diethyl ether, dried over anhydrous calcium chloride.

3.2.3.4 Preparation of unsymmetrical tridentate ruthenium(III) complexes with ligands: [HLL^4, ehmta] - [HLL^{11}, ehbzc]

A general approach was followed for the synthesis of all the ruthenium(III) complexes [73, 209]. All operations were carried out under strictly anhydrous conditions. The various complexes were prepared by the addition of (0.5 mmol, 0.242 g) of $RuCl_3 \cdot 3H_2O$ dissolved in about 15 mL of Absolute ethanol, into a hot ethanolic solution (20 mL) of (0.5 mmole) of unsymmetrical ligands: (0.5 mmol, . 0.103 g) *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-

(methylidene)ethylenediamine [HLL^4, ehmta]; (0.5 mmol, 0.141 g) *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(benzylidene)ethylenediamine [HLL^5, ehben]; (0.5 mmol, 0.156 g) *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(4-methoxybenzylidene)ethylenediamine [HLL^6, ehmez]; (0.5 mmol, 0.148 g) *N*-(2',4'-dihydroxyphenylethylidene)-N'-(1-phenylethylidene)ethylenediamine [HLL^7, ehacp]; (0.5 mmol, 0.156 g) *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(phenylethanimidamide)ethylenediamine [HLL^8, ehacn]; (0.5 mmol, 0.164 g) *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(4-hydroxy-3-methoxybenzylidene) ethylenediamine [HLL^9, ehvan]; (0.5 mmol, 0.171 g) *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(3,4-dimethoxybenzylidene)ethylenediamine [HLL^{10}, ehvet]; (0.5 mmol, 0.158 g) *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(benzenecarboximidoylchloride)ethylenediamine [HLL^{11}, ehbzc] in molar ratio (1:1). The colour of the complexes changed immediately. The resulting mixture was then refluxed for 6 hours. The precipitated solids were allowed to cool and filtered off from the reaction mixture, thoroughly washed with absolute ethanol and then with (3 x 5 mL) diethyl ether, dried over anhydrous calcium chloride.

3.2.4 Preparation of the metal(II) complexes

3.2.4.1 Preparation of *N,N′*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine complexes of zinc(II), cobalt(II), nickel(II) and copper(II)

A general approach was followed for the synthesis of all the metal(II) complexes [191, 200]. The complexes were prepared by the addition of (1.5 mmol, 0.329 g) $Zn(CH_3COO)_2{\cdot}2H_2O$; (1.5 mmol, 0.300 g) $Cu(CH_3COO)_2{\cdot}2H_2O$; (1.5 mmol, 0.373 g) $Ni(CH_3COO)_2{\cdot}4H_2O$; or (1.5 mmol, 0.374 g) $Co(CH_3COO)_2{\cdot}4H_2O$ dissolved in about 30 mL of 40% ethanol (v/v) solution, into a hot ethanolic solution (30 mL) of (1.5 mmol, 0.493 g) of

N,N′-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine ligand [HLL^1, ehopd] in molar ratio (1:1). The colour of the complexes changed in a few minutes. The resulting mixture was then refluxed for 3 hours and allowed to cool with continuous stirring. The precipitated solids were filtered off from the reaction mixture, thoroughly washed with ethanol and then with diethyl ether, dried over anhydrous calcium chloride.

3.2.4.2 Preparation of *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedione ethylidene)ethylenediamine complexes of zinc(II), cobalt(II), nickel(II) and copper(II)

The complexes were prepared by the addition of 1.5 mmole of (1.5 mmol, 0.329 g) $Zn(CH_3COO)_2 \cdot 2H_2O$; (1.5 mmol, 0.300 g) $Cu(CH_3COO)_2 \cdot 2H_2O$; (1.5 mmol, 0.373 g) $Ni(CH_3COO)_2 \cdot 4H_2O$; or (1.5 mmol, 0.374 g) $Co(CH_3COO)_2 \cdot 4H_2O$ dissolved in about 30 mL of 40% ethanol (v/v) solution, into a hot ethanolic solution (30 mL) of (1.5 mmol, 0.415 g) of *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedioneethylidene)ethylenediamine ligand [HLL^2, ehata] in molar ratio (1:1). The colour of the complexes changed in a few minutes. The resulting mixture was then refluxed for 3 hours and allowed to cool with continuous stirring. The precipitated solids were filtered off from the reaction mixture, thoroughly washed with ethanol and then with diethyl ether, dried over anhydrous calcium chloride.

3.2.4.3 Preparation of *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedione phenylidene)ethylenediamine complexes of zinc(II), cobalt(II), nickel(II) and copper(II)

The complexes were prepared by the addition of 1.5 mmole of (1.5 mmol, 0.329 g) $Zn(CH_3COO)_2 \cdot 2H_2O$; (1.5 mmol, 0.300 g) $Cu(CH_3COO)_2 \cdot 2H_2O$; (1.5 mmol, 0.373 g) $Ni(CH_3COO)_2 \cdot 4H_2O$; or (1.5 mmol, 0.374 g) $Co(CH_3COO)_2 \cdot 4H_2O$ dissolved in about 30 mL

of 40% ethanol (v/v) solution, into a hot ethanolic solution (30 mL) of (1.5 mmol, 0.507 g) of *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedionephenylidene)ethylenediamine ligand [HLL^3, ehbta] in molar ratio (1:1). The colour of the complexes changed in a few minutes. The resulting mixture was then refluxed for 3 hours and allowed to cool with continuous stirring. The precipitated solids were filtered off from the reaction mixture, thoroughly washed with ethanol and then with diethyl ether, dried over anhydrous calcium chloride.

3.3 Characterization techniques

3.3.1 Microanalysis (CHN analysis)

Carbon, hydrogen and nitrogen analysis was obtained using Perkin-Elmer model 2400 Series CHNS analyzer.

3.3.2 Melting Point Test

The melting point and decomposition temperature of both the ligands and complexes were recorded with Stuart melting Point (SMP 11). The melting point is a vital physical property of a compound. It can be used to identify a substance and as an indication of its purity.

3.3.3 Fourier Transform Infrared spectroscopy (FT-IR)

FT-IR spectra were collected on Perkin Elmer Paragon 2000 spectrophotometer in the region 4000–400 cm^{-1} using KBr disc method.

3.3.4 Conductivity Measurement

The molar conductance of the complexes was determined in either DMF at room temperature utilizing Crison EC- Meter Basic 30+ conductivity cell.

3.3.5 Electronic absorption spectra

The electronic absorption spectra of the ligands and the complexes within UV-Visible region were recorded on a Perkin Elmer Lambda-25 UV-Vis spectrometer in the region 200–1000 nm.

3.4. *In vitro* antimicrobial studies

3.4.1 Bacterial strains utilized in this study

The reference strains used in this study were selected based on their pathological effects on human and food product deterioration. Six bacteria strains including three Gram-positive bacteria, *viz: Staphylococcus aureus* (ATCC 25923), *Streptococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702) and three Gram-negative bacteria *viz: Pseudomonas aeruginosa* (ATCC 19582), *Escherichia coli* (ATCC 25922), and *Shigella flexineri* (KZN). They were obtained from the Microbiological unit of the Botany Department, University of Fort Hare.

3.4.2 Antimicrobial action of the synthesised compounds and Minimum Inhibitory Concentration (MIC)

Antibacterial action of the ligands and complexes were investigated by the Agar well diffusion method with little modification following the procedure previously reported [210]. The bacteria isolates were sub-cultured on nutrients agar plates and incubated at 37 ^{o}C for 24 hours. A loop full of bacteria cells from the nutrient agar plates was incubated into 50 mL of a nutrient broth in a 250 mL sidearm Erlenmeyer flask and incubated at 37 ^{o}C for 18 hours with vigorous shaking. Using a sterile glass spreader, 100 µL of 18 h bacterial cultures were used to spread a bacterial lawn on nutrient agar. The Minimum Inhibitory Concentration (MIC) of the synthesised compounds and standard drugs were determined using agar dilution method as described by the National Committee for Clinical Laboratory Standards [211].

The bacterial strains were grown at 37 ^{o}C overnight and maintained on nutrient agar. Inoculums of the test organisms were prepared in normal saline (9 g L^{-1}) compared with 0.5 McFarland standard to attain 5 x 10^{5} (CFU mL^{-1}). The suspension was then used to inoculate sterile petri plates of 9.0 cm diameter in which the test organisms were grown. Stock solutions of the compounds were prepared in dimethyl sulfoxide (DMSO) and further diluted in MHB agar at 50 ^{o}C to give final concentrations ranging from 0.312-10 mg/ mL; after pouring into plates and allow the agar to set, plates were inoculated with standardized inocula of the test bacteria, and further incubated at 37 ^{o}C for 24 h under aseptic conditions. The procedure was repeated for ciprofloxacin and amoxicillin drugs (standard antibacterial agents). The MIC was recorded as the lowest concentration at which no visible growth was observed.

3.5. Antioxidant assay

3.5.1. Scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

The antioxidant activity of Schiff base ligands and the synthesized M(III)/ M(II) complexes were studied spectrophotometrically by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method. DPPH is known as a stable commercially available free radical, soluble in methanol to give a violet solution, which upon reduction by an antioxidant changes to a corresponding light yellow to yellow colour. The free radical scavenging effects of all the metal compounds and Schiff bases with the DPPH radical were evaluated as previously described with slight modification [147, 212].

A solution of 0.4 mM DPPH in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL DMF solutions of Schiff bases and M(II)/ M(III) complexes with various concentrations (100, 200, 300, 400, and 500 μg/mL). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Rutin and ascorbic acid (Vitamin C) are used as standard drugs. The actual decrease in absorption was measured against that of the control. All test and analysis were run in triplicates and the results obtained were averaged. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \; X \; 100$$

Where

$Abs_{control}$ is the absorbance of DPPH radical + DMF;
Abs_{sample} is the absorbance of DPPH radical + sample [test samples/ standard].

3.5.2. ABTS: 2,2'-azino-bis-(-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

ABTS scavenging ability of the metal compounds and Schiff bases adopted a previously described method by Adedapo and co-worker [213]. The working solution was prepared by mixing two stock solutions of 7 mM ABTS solution and 2.4 mM potassium persulfate solution in equal amounts (1:1) and allowed solution to react in the dark for 12 h at room temperature. The resulting solution was further diluted by mixing 1 mL $ABTS^+$ solution to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer.

Test samples (1 mL) were allowed to react with 1 mL of the $ABTS^+$ solution, followed by the absorbance reading at 734 nm after 7 min using the spectrophotometer. The ABTS scavenging capacity of the test compounds was compared with that of Rutin and Butylated hydroxyl toluene (BHT) (Standard drugs). All test and analysis were run in triplicates and the results obtained were averaged. The percentage inhibition was calculated as ABTS radical scavenging activity using the following equation:

$$(\%)\ \text{Inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\ X\ 100$$

Where

$Abs_{control}$ is the absorbance of ABTS radical + DMF;
Abs_{sample} is the absorbance of ABTS radical + sample [test samples/ standard].

3.6. *In vitro* antiproliferative activity

3.6.1 Cell lines and culture conditions

Human renal cancer cell lines (TK-10), human melanoma cancer cell line (UACC-62) and human breast cancer cell line (MCF-7) were obtained from NCI in a framework of collaborative research program between CSIR and NCI. Cell lines were routinely maintained as a monolayer cell culture at 37.0°C with 5% CO_2, 95% air and 100% relative humidity in RPMI medium which is supplemented with 5% fetal bovine serum (FBS), 2 mM L−glutamine and 50 μg mL^{-1} gentamicin. The SRB test has been adopted for this research *in vitro* cell line screening and was executed at the CSIR in agreement with the protocol of the Drug Evaluation Branch, NCI.

3.6.2 Cell viability assay

Cell viability was examined by (Sulforhodamine B) SRB assay as previously described [214-215]. The cells (TK-10, UACC-62 and MCF-7) (3-19 passages) were inoculated into 96-well microtitre plates at plating densities of 7-10 000 cells/well and were incubated for 24 h. After 24 h, the cells were treated with the experimental compounds which were previously dissolved in DMSO and diluted in medium to produce 5 concentrations of 0.01, 0.1, 0, 10 and 100 μM. Cells without drug addition served as control. The blank contains complete medium without cells.

Parthenolide was used as a standard. The plates were incubated for 48 h, after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Thereafter, the unbound dye was

removed and protein-bound dye was extracted with 10 mM Tris base for optical density determination at the wavelength 540 nm using a multiwell spectrophotometer. The Z'-factor coefficient was adapted to monitor the quality of immunocytochemical assays such as the Sulforhodamine B (SRB).

3.6.3 Statistical analysis

Statistical investigation of information was performed using Microsoft statistical took package. IC_{50} (Inhibitory Concentration 50%) is the concentration of a given compound that is require for 50% inhibition *in vitro*. 50% of cell growth inhibition (IC_{50}) and radicals' inhibition (IC_{50}) was determined by non-linear regression. The obtained values were presented as means ± SD.

3.8 Summary of the Chapter

All reagents and chemicals used for the synthesis of the Schiff bases and their metal complexes are presented in this chapter. The syntheses of the compounds were successfully carried out as detailed. Three tetradentate ligands denoted as: [HLL^1, ehopd], [HLL^2, ehata], [HLL^3, ehbta] and eight tridentate ligands as: [HLL^4, ehmta], [HLL^5, ehben], [HLL^6, ehmez], [HLL^7, ehacp], [HLL^8, ehacn], [HLL^9, ehvan], [HLL^{10}, ehvet], [HLL^{11}, ehbzc] were synthesized. Three Ru(III) complexes with tetradentate ligands were synthesized and eight tridentate Ru(III) complexes were also prepared. Twelve metal(II) complexes of tetradentate ligands were prepared using Zn(II), Co(II), Ni(II) and Cu(II) salts. The Schiff base ligands and the corresponding metal complex were characterized and evaluated for its antibacterial, antioxidant or anticancer potential.

Further explanation on the synthesis and characterizations of ruthenium(III) and metal(II)-Schiff base complexes are explained in chapters four and five while biological activities of the synthesised compounds are presented in chapter six.

Chapter Four

Synthesis and characterization of metal complexes of symmetrical Schiff base

Part of Chapter Four has been published as:

Ejidike, I.P.; Ajibade, P.A. Synthesis, characterization, *in vitro* antioxidant and anticancer studies of ruthenium(III) complexes of symmetric and asymmetric tetradentate Schiff bases, *Journal of Coordination Chemistry*, **2015**, *68*, 2552-2564.

CHAPTER FOUR

SYNTHESIS AND CHARACTERIZATION OF METAL COMPLEXES OF SYMMETRICAL SCHIFF BASE

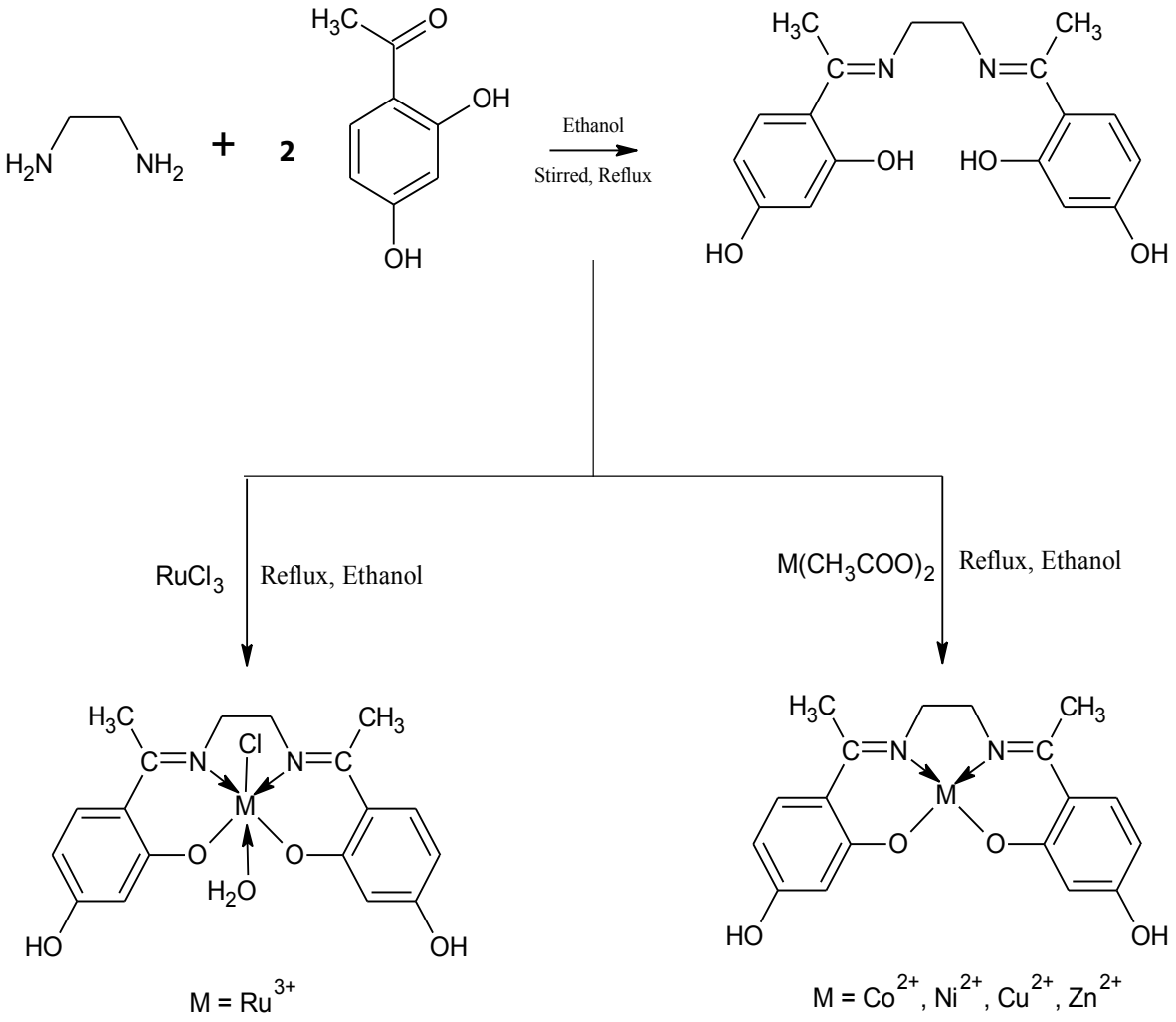
4.0 Background to the Chapter

Developments of coordination compounds containing nitrogen and oxygen donor ligands have been of research interest owing to their steric and electronic features in the last few years [74]. The fascinating electron-transfer properties exhibited by ruthenium, has increased interest of the chemists in the study of this metal complexes. An interesting aspect is the variety in the coordination environment around ruthenium ion that plays a vital role in modulating the redox properties of the ruthenium complexes. Also, the spectroscopic and biological studies of metal(II) complexes containing nitrogen and oxygen donor's ligands have been reported by different researchers [23, 114-122].

Schiff base ligands and their metal complexes have been explored in the last few decades due to their antimicrobial and anticancer properties [100-102]. In light of the literature survey, there have been a vast application of ruthenium, zinc, copper, cobalt, nickel Schiff base complexes in biological field. Hence, the present investigation seek to synthesize and characterize symmetrical Schiff base complexes of ruthenium(III), zinc(II), cobalt(II), nickel(II), and copper(II) and study their biological activities. Sections 4.1 and 4.2 describe the synthesis of the symmetrical tetradentate Schiff base and its metal complexes. Spectroscopic analyses are detailed in sections 4.3-4.5.

4.1 Preparation and characterization of the symmetrical Schiff base ligand

The Schiff base ligand was obtained from the condensation reaction of ethylenediamine with 2',4'-dihydroxyacetophenone in a 1:1 mole ratio under anhydrous refluxing environment for 3 h, and left to stand at room temperature overnight [HLL^1, ehopd], resulting in the elimination of the hydroxyl group and formation of a >C=N bond, while the obtained product is called imine [216]. The ligand involves an aliphatic bridge and isolated as brownish-yellow solid in good yield (Scheme 4.1).



Scheme 4.1: Preparation of the symmetrical Schiff base [HLL^1, ehopd] and its metal complexes

Table 4.1: Analytical data and physical properties of symmetrical Schiff base and its metal complexes

Compounds	Empirical Formula	F. Wt (g)	Colour	Yield (%)	D.T. (°C)	Condut. (μScm^{-1})	Microanalysis (Calc.) %C	%H	%N
H_2Ll^1, ehopd	$C_{18}H_{20}N_2O_4$	328.36	Brownish-yellow	76.52	244-245	−	65.73 (65.84)	6.28 (6.14)	8.71 (8.53)
$RuLL^1$ Ru(opd)	$C_{18}H_{24}N_2O_7RuCl$	516.92	Dark-green	60.28	239-240	47.4	42.08 (41.82)	4.43 (4.68)	5.31 (5.42)
$CoLL^1$ Co(opd)	$C_{18}H_{22}N_2O_6Co$	421.32	Grey	63.86	223-224	2.61	51.48 (51.31)	5.52 (5.26)	6.46 (6.65)
$NiLL^1$ Ni(opd)	$C_{18}H_{22}N_2O_6Ni$	421.07	Reddish-brown	65.94	242-243	2.28	51.16 (51.34)	5.45 (5.27)	6.39 (6.65)
$CuLL^1$ Cu(opd)	$C_{18}H_{22}N_2O_6Cu$	425.93	Dark-purple	74.11	240-241	1.58	50.93 (50.76)	5.02 (5.21)	6.74 (6.58)
$ZnLL^1$ Zn(opd)	$C_{18}H_{20}N_2O_5Zn$	409.75	Lemon yellow	72.11	242-243	1.70	52.59 (52.76)	5.08 (4.92)	7.11 (6.84)

4.2 Synthesis and characterization of symmetrical tetradentate metal complexes

The symmetrical tetradentate Schiff base metal complexes were obtained by refluxing together the appropriate symmetrical Schiff base [HLL^1, ehopd] with either ruthenium(III) chloride or metal(II) acetate for different metals in ethanol as shown in Scheme 4.1. The compounds were obtained pure and in good yield. The metal complexes were not melting but rather decomposed at temperature greater than 220 °C. The analytical data, percentage yields, colour, and melting points of the compounds are presented in Table 4.1.

4.3 Molar conductivity measurement

4.3.1 Molar conductivity measurement of *N,N′*-bis(2',4'-dihydroxyphenylethylidene) ethylenediamine complex of ruthenium(III)

The molar conductivity ($\Lambda\mu$) value of the [Ru(opd)] complex (Table 4.1) in 10^{-3} molL^{-1} DMF solution at room temperature, being 47.4 μScm^{-1} indicates that the compound is non-electrolyte in solution [217-218].

4.3.2 Molar conductivity measurements of *N,N′*-bis(2',4'-dihydroxyphenylethylidene) ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The molar conductance measurement ($\Lambda\mu$) of the Zn(II), Co(II), Ni(II) and Cu(II) complexes in DMF solution as listed in Table 4.1 are in the range 1.58 - 2.28 μScm^{-1} signifying non-electrolytic character of the synthesised complexes [217, 219]. Furthermore, low molar conductivity of the complexes could be due to low ionic mobility of the anionic coordination sphere possessing the bulky size [230].

4.4 Infrared spectra studies

Fourier Transform Infrared Spectroscopy (FT-IR), an instrument based on the fundamental principles of molecular spectroscopy was utilized in order to identify the presence of certain functional groups in the synthesized compounds, and as an approach to confirm the identity of the compounds through the collection of absorption frequencies. The selected vibrational stretching frequencies are presented in Table 4.2 and Figures 4.1 - 4.3.

4.4.1 Infrared spectra of *N,N′*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine complex of ruthenium(III)

The relevant FT-IR data of the ligand and the metal complex are presented in Table 4.2 and the spectra presented in Figure 4.1. The identified bands have been categorized by those emanating from the ligand *N,N′*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine [HLL^1, ehopd] and those emerging from the bounds established between metal ion and the ligand coordinating sites by means of comparison with those already reported in the literature on similar compounds [191, 203, 220]. The FT-IR spectra of the free ligand showed bands in the region 3475, 1616 and 1267, 1173 cm^{-1} assignable to ν(OH), ν(C=N), ν(C-O) respectively [191, 209, 220].

Table 4.2: Selected infrared spectra bands of symmetrical Schiff base and its metal complexes

Compound	ν(OH)/ν(H_2O)	ν(C=N)	ν(C-O)	ν(Ru-N)	ν(Ru-O)
H_2Ll^1, ehopd	3475	1616	1267, 1173	-	-
$RuLL^1$ Ru(opd)	3435	1622	1244, 1170	535	437
$CoLL^1$ Co(opd)	3416	1618	1249, 1158	474	407
$NiLL^1$ Ni(opd)	3416	1618	1251, 1158	482	441
$CuLL^1$ Cu(opd)	3416	1620	1247, 1153	474	424
$ZnLL^1$ Zn(opd)	3419	1617	1257, 1169	536	437

The free Schiff base ligand [HLL^1, ehopd] showed a very strong absorption in the 1616 cm^{-1} range in the IR spectra which is a typical of the azomethine ν(C=N) group (Table

4.2). In the Schiff base complex, this absorption were shifted to the 1622 cm^{-1} region signifying the coordination of the Schiff bases via the nitrogen atom in accordance with the coordination of the azomethine function to the metal ion for all the complexes [130, 221] and this shifting of the wavenumber is expected because of the coordination of the nitrogen atom of the azomethine group to the ruthenium metal ion, thereby reducing electron density in the azomethine link [133, 209]. A medium band corresponding to phenolic oxygen ν(C–O) is observed at 1267, 1173 cm^{-1} for the free ligand. Upon chelation, this band is shifted to lower frequency in the regions 1244, 1170 cm^{-1} for the ruthenium(III) Schiff base complex [130, 222].

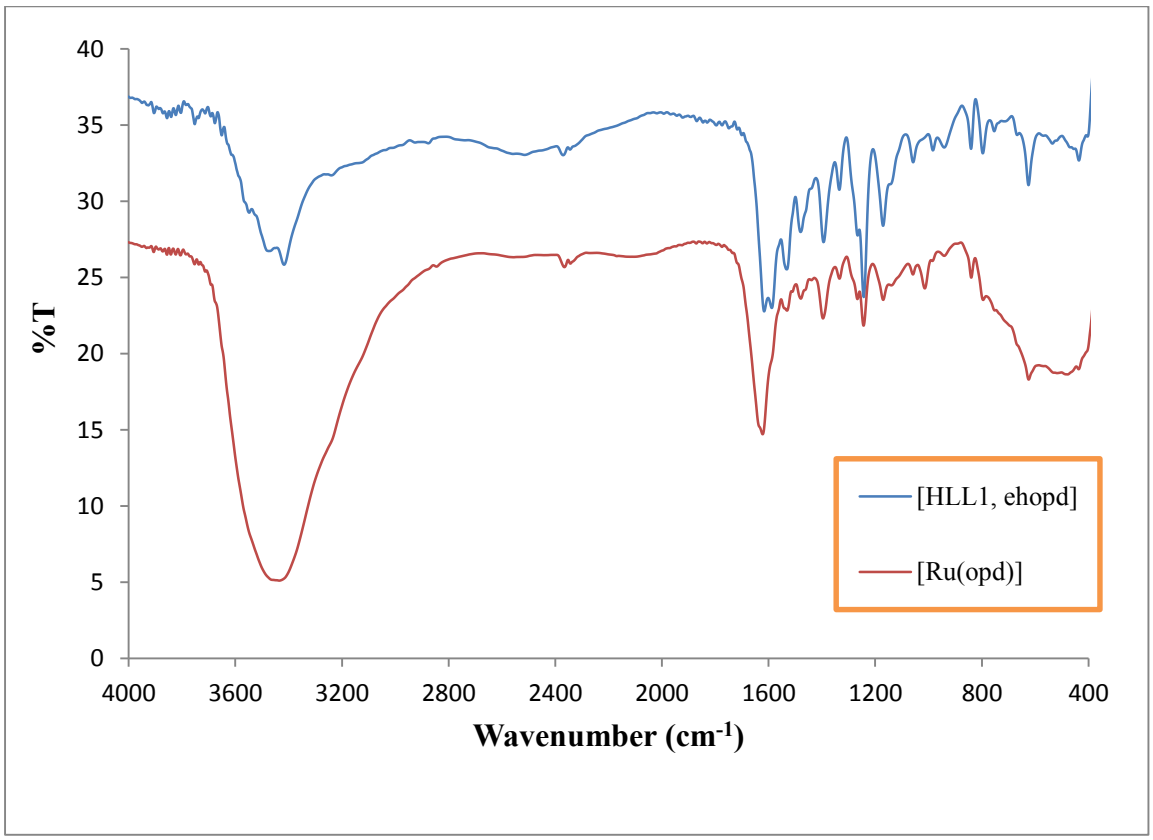


Figure 4.1: FTIR spectra of symmetrical ligand [HLL^1, ehopd] and its complex [Ru(opd)]

This indicates the enolisation of >C=O followed by deprotonation and complexation with metal and the destruction of keto group presumably viz., enolisation and ketolisation bonding of the ligand through the resulting enolate and ketolate oxygen. This is further supported by the disappearance of the ν(OH) in the range 3475 cm^{-1} in the complex. The presence of coordinated water in the complex was seen in the regions 3435 and 846 cm^{-1}. These absorptions are due to ν(O–H) stretching and ν(O–H) rocking vibrations and further confirmed the presence of non-ligand assignable to the rocking mode of H_2O [116, 220, 223]. In the low frequency region, the bands in the region 535 cm^{-1} and 437 cm^{-1}, probably due to the formation of ν(Ru–N) and ν(Ru–O) vibrations respectively [200, 224] were observed.

4.4.2 Infrared spectra of *N,N′*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The FT-IR data of the ligand and complexes were compared and assigned. The spectra are presented in Figures 4.2 and 4.3. The ligand *N,N′*-bis(2',4'-di hydroxyphenylethylidene)ethylenediamine [HLL^1, ehopd] spectrum displayed a broad and weak band at 2678-2800 cm^{-1}, and was assigned to the ν(OH) in hydrogen-bonded O-H in plane bending vibration as hydrogen-bonding fragment in free ligand [200, 203, 225]. The selected compounds characteristics IR frequencies are presented in Table 4.2. A strong band at 3475 cm^{-1} was due to ν (OH) ligand stretching vibrations.

In the complexes spectra, a broad band at ~3420 cm^{-1} signifies the ν(OH) stretching of the coordinated H_2O molecules associated with the complexes [124,186,191]. An intense band was observed at 1616 cm^{-1} in the free ligand due to stretching vibration of the -HC=N- ν(C=N) [116, 179, 187]. There was a positive shift towards higher frequencies in the metal

complexes spectra, this shifting towards higher frequency can be due to ν(C=N) within 1617-1620 cm^{-1} regions signifying the interaction of (>C=N) nitrogen to the metal ion as a result of rear donation of π-electrons by the metals [126, 179].

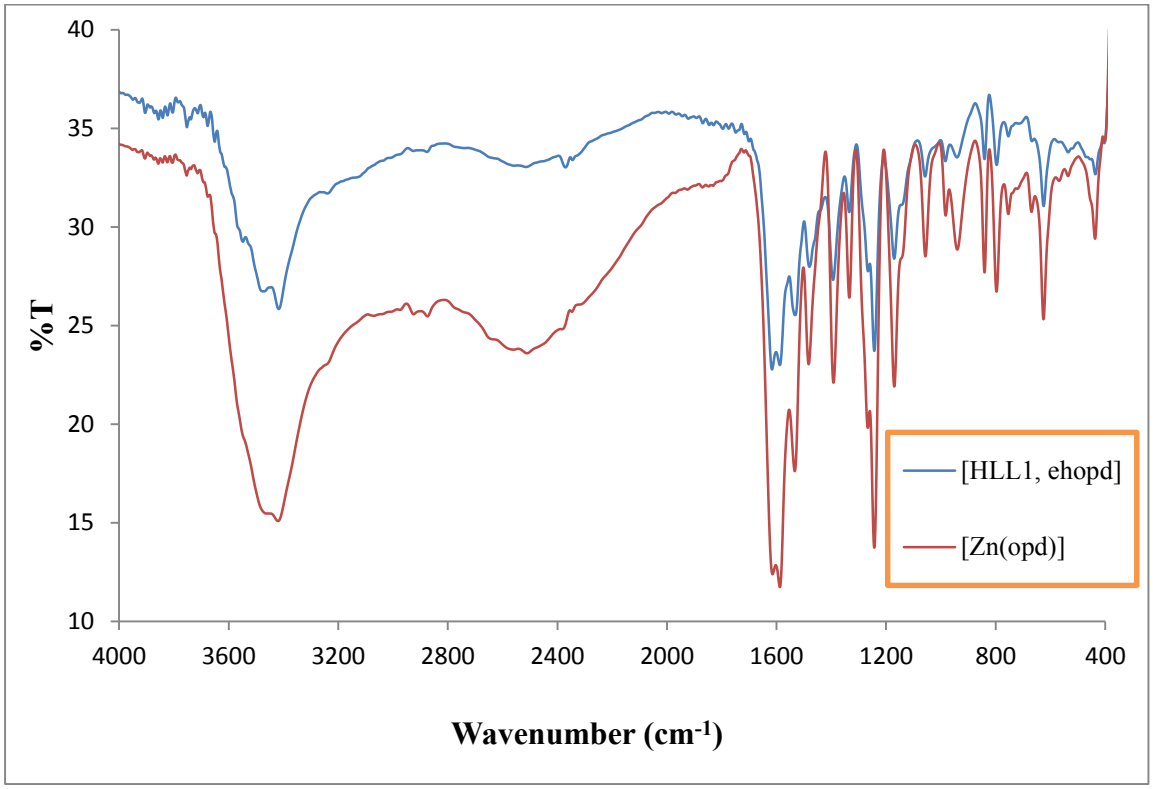


Figure 4.2: FTIR spectra of symmetrical ligand [HLL^1, ehopd] and its complex [Zn(opd)]

The disappearance of the stretching frequency observed in the ligand at 2678 - 2800 cm^{-1} in the complexes, indicates the contribution of phenolic OH in bounding sphere. The stretching vibration of the phenolic ν(C-O) showed at 1267 cm^{-1} in the free Schiff base [187, 191] shifted to higher frequencies in the region 1334 - 1338 cm^{-1} in the complexes after complexation. This shifts further support the coordination of the phenolic oxygen leading to the formation of C-O-M bond [200, 226] (M = Ni, Co, Cu and Zn). There was consistency in the aromatic ring vibrations ν(C=C) in all derivatives, hence, no shift was observed after complex formation [227].

Weak bands observed at the lower frequency region 474 - 536 cm^{-1} in the complexes were ascribed to ν(M-N) vibrations: 482 cm^{-1} ν(Ni-N); 474 cm^{-1} ν(Co-N); 474 cm^{-1} ν(Cu-N); 536 cm^{-1} ν(Zn-N) bonds while region 407 - 437 cm^{-1} is due to ν(M-O) vibrations: 441 cm^{-1} ν(Ni-O); 407 cm^{-1} ν(Co-O); 424 cm^{-1} ν(Cu-O); 437 cm^{-1} ν(Zn-O) bonds [186-187, 191]. The presence of coordinated water within 842 - 850 cm^{-1} further confirmed the presence of non-ligand due to the rocking vibration of H_2O within the compound lattice [116, 223, 228]. The data from infrared spectra suggest the metal ions to ligand chelation occurred via the phenolic oxygen atoms of the 4-acetylresorcinol moiety and that of imino N atom of the ligand (C=N) [200, 203].

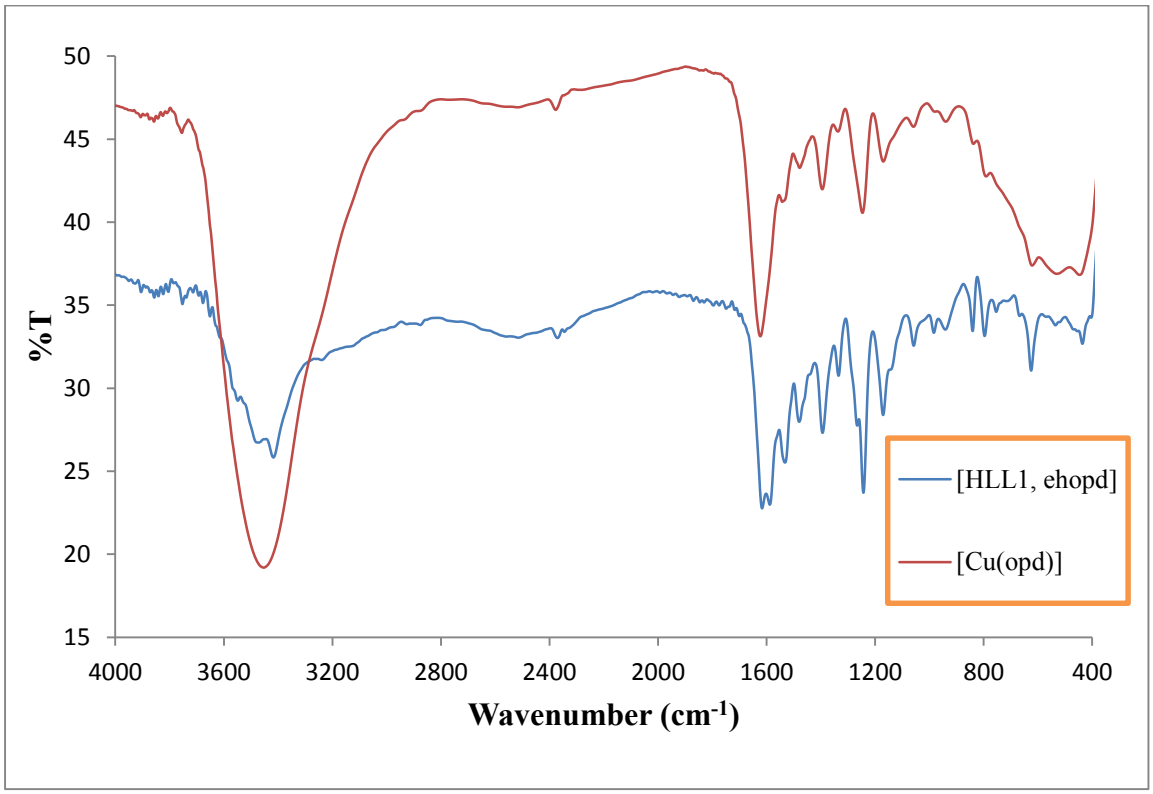


Figure 4.3: FTIR spectra of symmetrical ligand [HLL^1, ehopd] and its complex [Cu(opd)]

4.5 Electronic spectra of the compounds

The electronic spectra of the metal complexes in DMF were measured in the regions 200-1000 nm (50 000 - 10 000 cm^{-1}) at room temperature. The results are presented in Table 4.3 and the spectra in Figures 4.4 and 4.5.

Table 4.3: Electronic spectra data of symmetrical tetradentate ruthenium(III) and metal(II) complexes

Complexes	Solvent	*d-d* transitions/ nm (cm^{-1})	C.T. nm (cm^{-1})	Ligand nm (cm^{-1})
RuLL[1] Ru(opd)	DMF	612 (16 340)	452 (22 124)	282 (35 461) 314 (31 847) 390 (25 641)
CoLL[1] Co(opd)	DMF	631 (15 848)	392 (25 510)	314 (31 847) 374 (26 738)
NiLL[1] Ni(opd)	DMF	434 (23 042) 560 (17 857)	403 (24 814)	308 (32 468) 338 (29 586) 382 (26 178)
CuLL[1] Cu(opd)	DMF	447 (22 372) 559 (17 889)	389 (25 707)	315 (31 746) 362 (27 625)
ZnLL[1] Zn(opd)	DMF		444 (31 847)	285 (35 088) 314 (31 847) 381 (22 523)

C.T. = Charge transfer

4.5.1 Electronic spectra of *N,N'*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine complex of ruthenium(III)

The complex showed three to four bands in the region 280 - 618 nm (35 715 - 16 181 cm^{-1}) as presented in Table 4.3 and spectrum displayed in Figure 4.4. Ruthenium(III) ground state is

$^2T_{2g}$ and the initially excited doublet levels in the trend of increasing energy are $^2A_{2g}$ and $^2T_{1g}$, which arise from $t_{2g}^4 e_g^1$ configuration. The spectra profiles lower than 400 nm (25 000 cm^{-1}) are very similar, referred to as the ligand-centred transitions [HLL^1, ehopd]. These bands have been designated as $\pi^* \leftarrow \pi$ and $\pi^* \leftarrow$ n transitions of the benzene ring and the double bond of the azomethine group [133, 229].

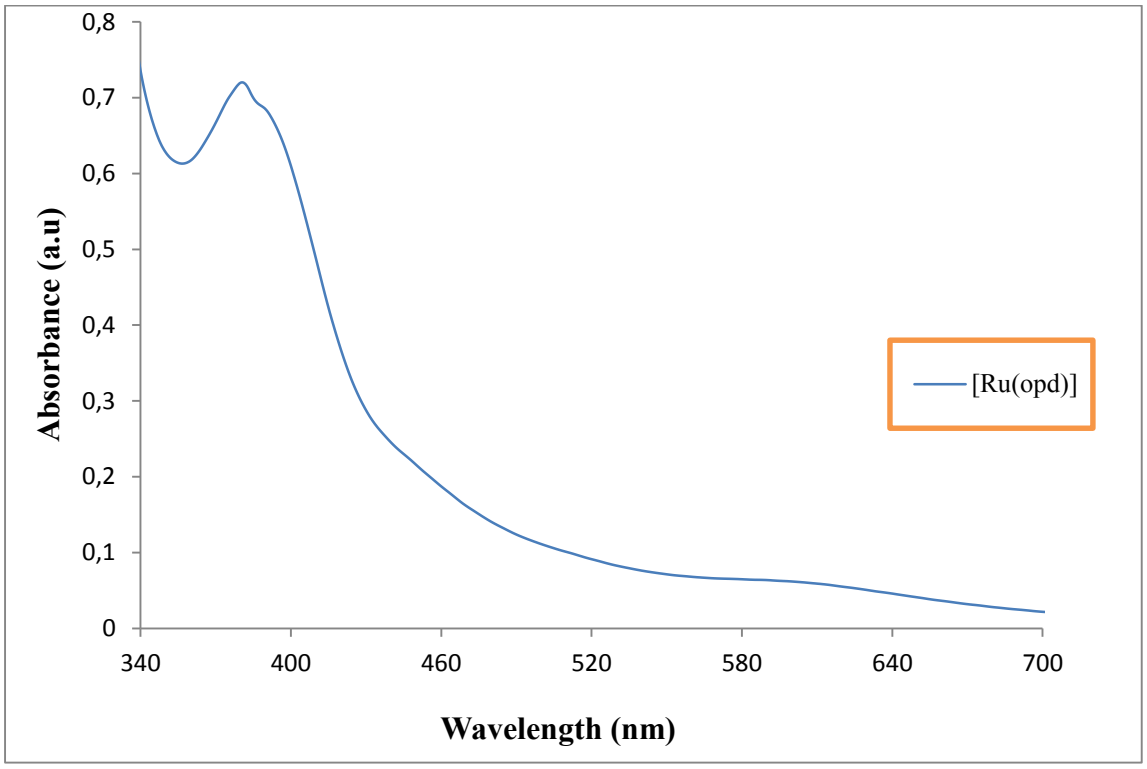


Figure 4.4: Electronic spectrum of symmetrical tetradentate [Ru(opd)] complex

In a d^5 system, such as the ruthenium(III) metal ion which has moderately high oxidizing properties, the charge transfer bands of the type $L_{\pi y} \rightarrow T_{2g}$ are observable in the low energy region, and obscures the weaker bands due to d-d transitions [230]. It therefore becomes difficult to assign clearly the bands of ruthenium(III) complexes that appeared in the visible region [73, 230]. However, extinction coefficient for the band in the 612 nm (16 340 cm^{-1}) region was observed to be very low as compared to that of charge transfer band.

Hence, the band around 612 nm (16 340 cm^{-1}) have been assigned to $^2T_{2g} \rightarrow {}^2A_{2g}$ transition which is in congruity with assignment made for similar octahedral ruthenium(III) complexes [209, 229-231]. In general, the electronic spectra of the complex is representative of an octahedral environment around ruthenium(III) ions for [Ru(opd)]. The band in the region 452 nm (22 124 cm^{-1}) has been assigned to charge transfer transition [209].

4.5.2 Electronic spectra of *N,N′*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The types of electronic transitions existing within the solution of the compounds are presented in Table 4.3 and the spectra displayed in Figure 4.5. The free ligand [HLL^1, ehopd] spectrum exhibits three absorption bands at 284 nm (35 212 cm^{-1}), 309 nm (32 363 cm^{-1}) and 383 nm (26 110 cm^{-1}). The first two bands at 284 nm and 309 nm are due to aromatic $\pi^* \leftarrow \pi$ and imino $\pi^* \leftarrow \pi$ transitions respectively [232-233], and were not affected upon metal coordination. $\pi^* \leftarrow$ n transition is attributed to the third absorption band in the ligand spectra (383 nm), while a shift to longer wavelength with increased intensity observed in the metal complexes spectra can be attributed to the lone pair electron donated by the (>C=N) nitrogen atoms of the ligand to the vacant orbitals of metal ions (M ← N) [200, 228].

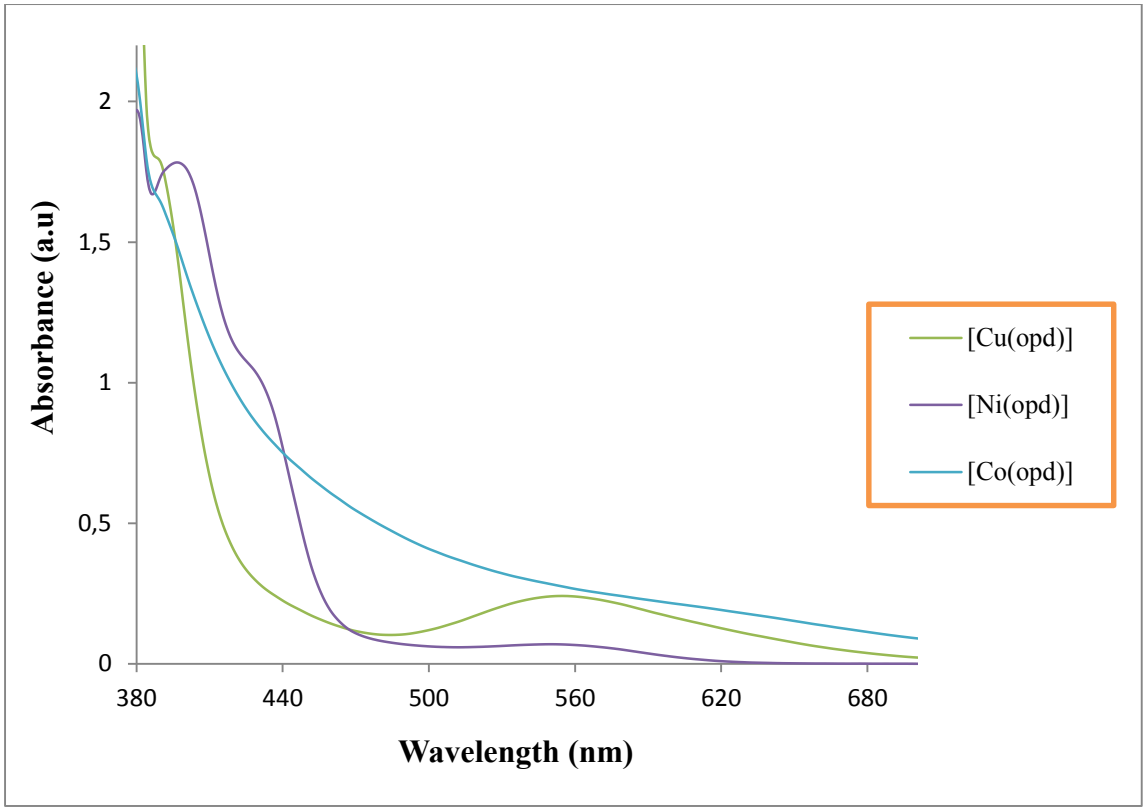


Figure 4.5: Electronic spectra of symmetrical tetradentate Cu(opd), Ni(opd) and Co(opd) complexes

The absorption spectra of tetrahedral Co(II) complexes in the visible region is reported to exhibit one absorption band due to $^4A_2(F) \rightarrow {}^4T_1(P)$ transition [231]. Spectrum of the present [Co(opd)] complex showed a peak with λ_{max} value of 15 848 cm^{-1}, suggesting a tetrahedral geometry of the ligand around the Co(II) ion. Hence, [Co(opd)] complex is assigned tetrahedral geometry.

Complexes of Ni(II) have been reported to possess a coordination geometry ranging from octahedral to tetrahedral/ square planar geometries [191], with absorption spectra often times described as characteristic of complicated equilibria containing the diverse structural types [235]. The absorption spectrum of the present [Ni(opd)] complex exhibited a band at

403 nm (24 814 cm^{-1}) attributed to charge-transfer transitions L → M (LMCT) and two absorption bands at 403 nm (23 042 cm^{-1}) and 560 nm (17 857 cm^{-1}) due to $^1A_{1g} \rightarrow {}^1A_{2g}$ and $^1A_{1g} \rightarrow {}^1B_{1g}$ transitions respectively. With regards to this transition, [Ni(opd)] complex is assigned a square planar geometry [228]. The observed electronic transitions and reddish-brown colour of the complex further confirms square-planar geometry for the nickel complex [236].

Copper(II) complex [Cu(opd)] in the visible region displayed two spectroscopic bands at 559 nm (17 889 cm^{-1}) and 447 nm (22 372 cm^{-1}) due to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions. A broad band centred was observed at 559 nm, this absorption prefer the square-planar geometry [231].

Generally, zinc complexes possesses completely filled d^{10} electronic configuration. Therefore, does not exhibit *d-d* electronic transition, however exhibits charge transfer. [Zn(opd)] complex displayed a band at 444 nm (31 847 cm^{-1}) ascribed to the LMCT transition, hence zinc complex possess a tetrahedral geometry [237-238].

4.6 Summary of the Chapter

Empirical formula of the Ru(III), Co(II), Ni(II), Cu(II) and Zn(II) with [HLL^1, ehopd] ligand were derived on the basis of elemental analysis, which further confirms the proposed structures of the complexes as given in Scheme 4.1. The synthesized Schiff base obtained from ethane-1,2-diamine and 2',4'-dihydroxyacetophenone acts as dibasic, tetradentate ligand. The ligand acting as ONNO tetradentate coordinate Ru(III), Co(II), Ni(II), Cu(II) and Zn(II) ions through nitrogen of >C=N group and phenolic oxygen donor (Figures 4.1-4.3). Further

evidence was obtained from IR and UV spectra data. Molar conductivity values of the complexes gave insight into the non-electrolytic behaviour of the complexes in DMF solution. In [Ru(opd)], octahedral geometry around the Ru(III) ions is completed by H_2O and a Cl^- in addition to one oxygen and two nitrogen of ehopd ligand. Thus, possess an octahedral geometry. Metal(II) ions to ligand bonding has been confirmed by the electronic spectra evaluation revealing [Co(opd)] complex with tetrahedral geometry. Square planar geometry have been assigned for [Ni(opd)] and [Cu(opd)] complexes (Figures 4.4 - 4.5).

Chapter Five

Synthesis and characterization of metal complexes of unsymmetrical Schiff base

Part of Chapter Five has been published as:

Ejidike, I.P.; Ajibade, P.A. Synthesis, characterization, antioxidant, and antibacterial studies of some metal(II) complexes of tetradentate Schiff base ligand: (4*E*)-4-[(2-{(*E*)-[1-(2,4-dihydroxyphenyl)ethylidene]amino}ethyl)imino]pentan-2-one. *Bioinorganic Chemistry and Applications*, **2015**, *2015*, 890734, 1-9.

Ejidike, I.P.; Ajibade, P.A. Synthesis, characterization and biological studies of metal(II) complexes of (3*E*)-3-[(2-{(*E*)-[1-(2,4-dihydroxyphenyl)ethylidene]amino}ethyl)imino]-1-phenylbutan-1-one Schiff base. *Molecules*, **2015**, *20*, 9788-9802.

Ejidike, I.P.; Ajibade, P.A. Ruthenium(III) complexes of heterocyclic tridentate (ONN) Schiff base: Synthesis, characterization and its biological properties as an antiradical and antiproliferative agent. *International Journal of Molecular Science* 2016, *17*, 60; doi:10.3390/ijms17010060

CHAPTER FIVE

SYNTHESIS AND CHARACTERIZATION OF METAL COMPLEXES OF UNSYMMETRICAL SCHIFF BASE

5.0 Background to the Chapter

There has been an increase interest in the preparation and characterization of unsymmetrical Schiff base ligands and their metal complexes in recent times. This is partly related to the fact that the systematic evaluation of these complexes may give some insight into the potentials for biological applications [80-85]. Several symmetrical tetradentate bis-type Schiff bases of the sort: 1,2-diamines with (*o*/*p*)-hydroxyl aldehyde/ketone have been synthesized and studied. However, there are few reports on the synthesis of unsymmetrical tetradentate Schiff bases derived from 1,2-diamines and different aldehydes/ketones [116, 117].

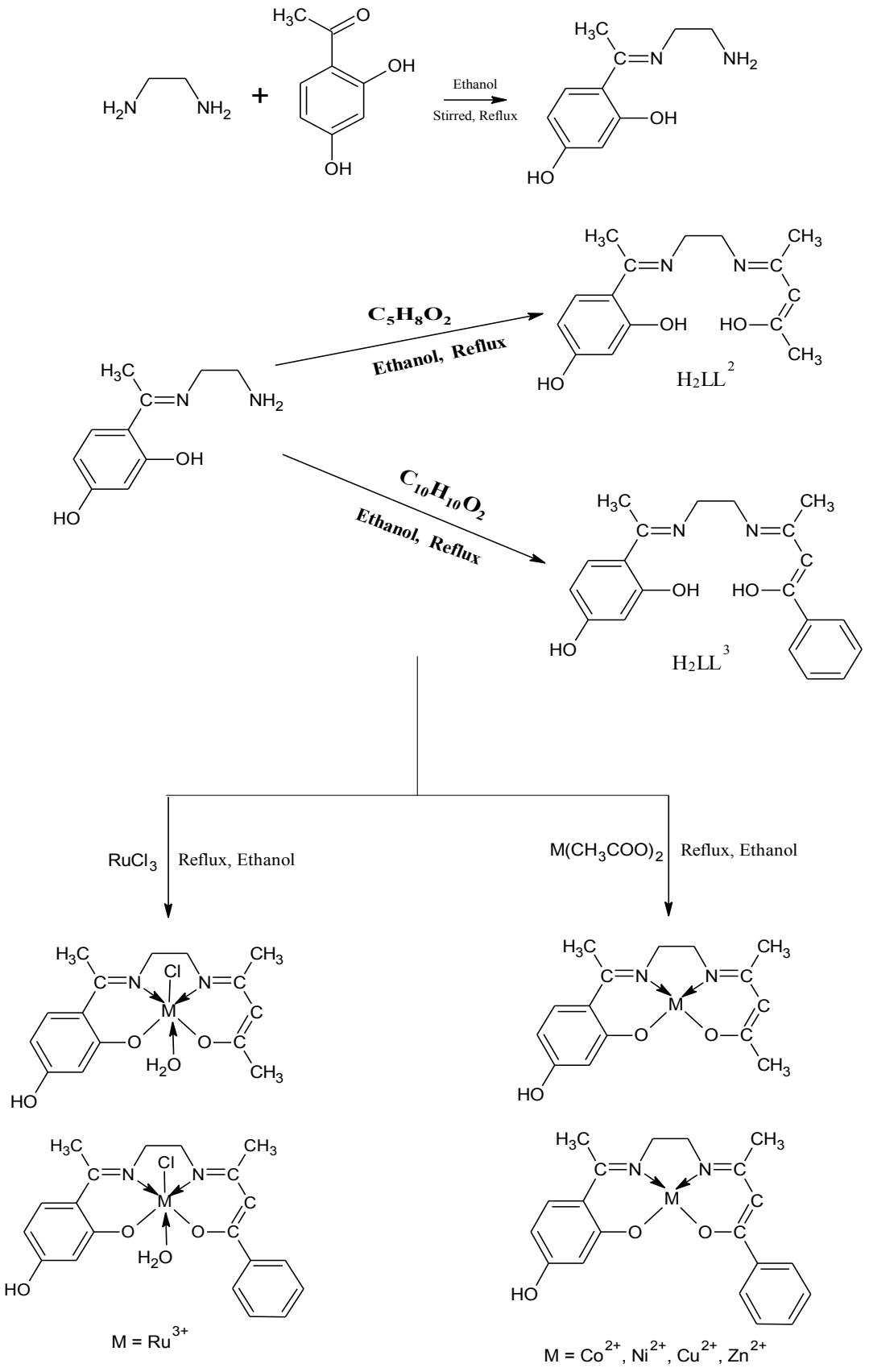
Unsymmetrical tetradentate Schiff bases have exhibited some noteworthy biological activity and desirable physicochemical, stereochemical, electrochemical, structural and catalytic properties [218]. Unsymmetrical ligands possess clearly many advantages over their corresponding symmetrical counterparts in composition elucidation, binding sites of metal ion geometry in metalloproteins and selectivity of natural system with synthetic materials [127]. In unsymmetrical Schiff base metal complexes, modification of the coordinating centre environment can be achieved by introducing different substituents to the ligand, thus providing a useful range of steric and electronic properties necessary for fine-tuning of their structures and reactivity. With this observation, the chapter describes the syntheses and characterization of unsymmetrical Schiff bases (Scheme 5.1) and their ruthenium(III), zinc(II), cobalt(II), nickel(II), and copper(II) complexes. Sections 5.1 and 5.2 describe the

synthesis of the unsymmetrical tetradentate Schiff base and its metal complexes, while the molar conductivity, FT-IR and UV-vis analysis are detailed in sections 5.3 - 5.5.

The preparation of unsymmetrical tridentate Schiff base and its ruthenium(III) complexes is presented in section 5.6. In this section, eight derivatives of 4-[(1*E*)-*N*-(2-aminoethyl)ethanimidoyl]benzene-1,3-diol (Scheme 5.2) were studied in order to understand the effect of methyl, methoxy, chloride, phenyl, hydroxyl on the stability, conductivity and biological activities of nitrogen atoms of the azomethine on metal coordination. Spectroscopic analyses for unsymmetrical tridentate ruthenium(III) Schiff base complexes were explained in details in sections 4.3 - 4.5. The schematic representations of the eight ligands coordinated to Ru^{3+} ion are shown in Scheme 5.3.

5.1 Preparation of the unsymmetrical Schiff bases

The unsymmetrical tetradentate Schiff base ligands were prepared in a 1:1:1 molar ratio of 2',4'-dihydroxyacetophenone, ethane-1,2-diamine, acetylacetone or 1-phenylbutane-1,3-dione, stirred for few hours and allowed to stand with continuous stirring for approximately 24 hours at room temperature (Scheme 5.1, HLL^2 and HLL^3). A brownish-yellow to orange-brown solid in high yield (Table 5.1) were obtained and decomposed at 211 - 245 ^oC. The scheme for the preparation of the unsymmetrical tridentate Schiff bases were similar to that of unsymmetrical tetradentate Schiff base ligands but different substituted aldehyde were used (Scheme 5.2, HLL^4-HLL^{11}) with decomposition temperature of 219 - 249 ^oC (Table 5.1). In the course of the ligands preparation, some factors were observed to affect the methodology which includes (a) the sequence of reagents addition, (b) the temperature of the starting material solution, (c) the reaction time, (d) the nature of the carbonyl group.



Scheme 5.1: Preparation of the unsymmetrical Schiff bases and their metal complexes

Table 5.1: Analytical data and physical properties of unsymmetrical Schiff bases and ruthenium(III) complexes

Comp-ounds	Empirical Formula	F. Wt (g)	Colour	Yield (%)	D.T. (°C)	Condut. (μScm^{-1})	Microanalysis (Calc.) %C	%H	%N
H_2LL^2, ehata	$C_{15}H_{19}N_2O_3$	275.32	Golden yellow	61.23	235-236	–	65.26 (65.44)	7.13 (6.96)	9.98 (10.17)
$RuLL^2$ Ru(ata)	$C_{15}H_{23}N_2O_6RuCl$	463.88	Darkish-green	76.20	228-229	30.6	38.75 (38.84)	4.81 (5.00)	5.83 (6.04)
H_2LL^3, ehbta	$C_{20}H_{21}N_2O_3$	337.40	Orange-brown	74.17	211-212	–	70.96 (71.20)	5.13 (5.27)	8.09 (8.30)
$RuLL^3$ Ru(bta)	$C_{20}H_{25}N_2O_6RuCl$	525.95	Darkish-green	53.67	231-232	23.8	45.88 (45.67)	4.56 (4.79)	5.18 (5.33)
HLL^4, ehmta	$C_{11}H_{14}N_2O_2$	206.24	Darkish-yellow	88.82	218-219	–	63.98 (64.06)	7.02 (6.84)	13.49 (13.58)
$RuLL^4$ Ru(mta)	$C_{11}H_{19}N_2O_5RuCl_2$	431.26	Brownish-green	59.04	211-212	41.2	30.41 (30.64)	4.28 (4.44)	6.23 (6.50)
HLL^5, ehben	$C_{17}H_{18}N_2O_2$	282.34	Dark-yellow	71.5	233-234	–	72.17 (72.32)	6.31 (6.43)	9.86 (9.92)
$RuLL^5$ Ru(ben)	$C_{17}H_{21}N_2O_4RuCl_2$	489.34	Darkish-green	66.02	228-229	32.0	42.01 (41.73)	4.18 (4.33)	5.51 (5.72)
HLL^6, ehmez	$C_{18}H_{20}N_2O_3$	312.36	Yellowish-brown	65.7	235-236	–	69.16 (69.21)	6.34 (6.45)	8.91 (8.97)
$RuLL^6$ Ru(mez)	$C_{18}H_{23}N_2O_5RuCl_2$	519.37	Darkish-green	68.28	232-233	45.2	41.51 (41.63)	4.70 (4.46)	5.25 (5.39)
HLL^7, ehacp	$C_{18}H_{20}N_2O_2$	296.36	Pale yellow	67.6	247-248	–	72.95 (72.98)	6.80 (6.71)	9.45 (9.40)
$RuLL^7$ Ru(acp)	$C_{18}H_{23}N_2O_4RuCl_2$	503.37	Darkish-green	63.35	231-232	34.7	43.14 (42.95)	4.82 (4.61)	5.35 (5.57)
HLL^8, ehacn	$C_{18}H_{21}N_3O_2$	311.38	Golden yellow	69.0	251-252	–	69.39 (69.43)	6.76 (6.80)	13.41 (13.49)
$RuLL^8$ Ru(acn)	$C_{18}H_{24}N_3O_4RuCl_2$	518.38	Dark-green	60.36	238-239	31.8	41.43 (41.71)	4.54 (4.67)	8.29 (8.11)
HLL^9, ehvan	$C_{18}H_{20}N_2O_4$	328.36	Brownish-red	73.8	209-210	–	65.79 (65.84)	6.11 (6.14)	8.45 (8.53)
$RuLL^9$ Ru(van)	$C_{18}H_{23}N_2O_6RuCl_2$	535.37	Darkish-green	61.90	218-219	30.5	40.58 (40.38)	4.21 (4.33)	5.44 (5.23)
HLL^{10}, ehvet	$C_{19}H_{22}N_2O_4$	342.39	Brownish-yellow	66.0	222-223	–	66.61 (66.65)	6.42 (6.48)	8.09 (8.18)
$RuLL^{10}$ Ru(vet)	$C_{19}H_{25}N_2O_6RuCl_2$	549.39	Darkish-green	58.43	226-227	30.1	41.29 (41.54)	4.32 (4.59)	4.98 (5.10)
HLL^{11}, ehbzc	$C_{17}H_{17}ClN_2O_2$	316.78	Brownish-yellow	64.2	234-235	–	64.44 (64.46)	5.36 (5.41)	8.76 (8.84)
$RuLL^{11}$ Ru(bzc)	$C_{17}H_{20}N_2O_4RuCl_3$	523.79	Dark-green	55.65	228-229	38.8	39.11 (38.98)	3.67 (3.85)	5.11 (5.35)

Table 5.2: Analytical data and physical properties of unsymmetrical tetradentate cobalt(II), nickel(II), copper(II) and zinc(II) complexes

Comple-xes	Empirical Formula	F. Wt (g)	Colour	Yield (%)	D.T. (°C)	Condut. (μScm^{-1})	Microanalysis (Calc.) %C	%H	%N
$CoLL^2$ Co(ata)	$C_{15}H_{23}N_2O_6Co$	368.29	Darkish-brown	69.30	232-233	3.57	46.81 (46.64)	6.19 (6.00)	7.50 (7.25)
$CoLL^3$ Co(bta)	$C_{20}H_{27}N_2O_7Co$	466.38	Darkish-green	52.90	199-200	6.70	51.27 (51.51)	6.09 (5.84)	6.20 (6.01)
$NiLL^2$ Ni(ata)	$C_{15}H_{21}N_2O_5Ni$	368.03	Reddish-brown	76.20	191-192	4.47	49.11 (48.95)	5.52 (5.75)	7.44 (7.61)
$NiLL^3$ Ni(bta)	$C_{20}H_{25}N_2O_6Ni$	448.12	Darkish-yellow	73.20	210-211	4.31	53.81 (53.61)	5.48 (5.62)	6.33 (6.25)
$CuLL^2$ Cu(ata)	$C_{15}H_{21}N_2O_5Cu$	372.89	Dark-purple	77.27	229-230	3.91	48.55 (48.32)	5.43 (5.68)	7.23 (7.51)
$CuLL^3$ Cu(bta)	$C_{20}H_{23}N_2O_5Cu$	434.96	Brownish-purple	83.85	208-209	2.85	55.09 (55.23)	5.52 (5.33)	6.20 (6.44)
$ZnLL^2$ Zn(ata)	$C_{15}H_{19}N_2O_4Zn$	356.71	Lemon yellow	65.28	226-227	3.22	50.23 (50.51)	5.08 (5.37)	8.03 (7.85)
$ZnLL^3$ Zn(bta)	$C_{20}H_{23}N_2O_5Zn$	436.79	Lemon brown	64.80	189-190	3.26	55.18 (55.00)	4.98 (5.31)	6.63 (6.41)

5.2 Unsymmetrical tetradentate Schiff bases and its metal complexes

The unsymmetrical tetradentate Schiff base [H_2LL^2, ehata], [HLL^3, ehbta] involves an aliphatic bridge and isolated as golden yellow to orange brown solid in good yield. Its metal complexes were obtained by refluxing together the appropriate unsymmetrical Schiff base with the equivalent ruthenium(III) or metal(II) salts for different metals in ethanol as shown in Scheme 5.1. The compounds were isolated in good yield and were not melting but rather decomposing at temperature greater than 190 °C.

The analytical data, percentage yields, colour and melting points of the complexes are presented in Tables 5.1 and 5.2.

N-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedioneethylidene)ethylenediamine [H_2LL^2, ehata], *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedionephenylidene)ethylene diamine [HLL^3, ehbta]

5.3 Molar conductivity measurement

5.3.1 Molar conductivity measurements of unsymmetrical tetradentate ruthenium(III) complexes with [HLL^2, ehata] and [HLL^3, ehbta] ligands

The molar conductivity ($\Lambda\mu$) value of the [Ru(ata)] and [Ru(bta)] complexes (Table 5.1) in 10^{-3} $molL^{-1}$ DMF solution at room temperature are 23.8 and 30.6 μScm^{-1} respectively (Scheme 5.1) indicates they are non-electrolyte in solution [217, 218].

5.3.2 Molar conductivity measurements of *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedioneethylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The conductivity values ($\Lambda\mu$) of the [Zn(ata)], [Co(ata)], [Ni(ata)] and [Cu(ata)] complexes in DMF solution as listed in Table 5.1, were in the range 3.22 - 4.47 μScm^{-1} signifying non-electrolytic character of the synthesised complexes (Scheme 5.1) [217, 219].

5.3.3 Molar conductivity measurements of *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedionephenylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The conductivity values ($\Lambda\mu$) of the metal chelate: [Zn(bta)], [Co(bta)], [Ni(bta)] and [Cu(bta)] solution in DMF for ~10^{-3} M as shown in Table 5.1 gave low conductance ranging

from 2.85 - 6.70 μScm^{-1} and this supports non-electrolyte nature of the complexes in solution (Scheme 5.1) at room temperature [217].

5.4 Infrared spectra studies

The selected vibrational stretching frequencies of the unsymmetrical Schiff base ligands and their corresponding complexes are presented in Tables 5.3 - 5.4 and the spectra in Figures 5.1 - 5.6.

Table 5.3: Selected infrared spectra bands of unsymmetrical Schiff bases and ruthenium(III) complexes

Compounds	ν(OH)/ν(H_2O)	ν(C=N)	ν(C-O)	ν(Ru-N)	ν(Ru-O)
H_2LL^2, ehata	3475	1612	1243, 1171	-	-
$RuLL^2$ Ru(ata)	3419	1621	1243, 1168	522	428
H_2LL^3, ehbta	3479	1605	1288, 1241	-	-
$RuLL^3$ Ru(bta)	3430	1623	1258, 1138	519	415
HLL^4, ehmta	3470	1608	1254, 1170	-	-
$RuLL^4$ Ru(mta)	3422	1622	1284, 1173	477	418
HLL^5, ehben	3473	1613	1241, 1170	-	-
$RuLL^5$ Ru(ben)	3442	1629	1249, 1172	520	435
HLL^6, ehmez	3471	1615	1244, 1171	-	-
$RuLL^6$ Ru(mez)	3449	1621	1250, 1181	519	476
HLL^7, ehacp	3472	1614	1242, 1168	-	-
$RuLL^7$ Ru(acp)	3418	1620	1251, 1173	481	427

HLL[8], ehacn	3477	1616	1240, 1167	-	-
RuLL[8] Ru(acn)	3436	1621	1242, 1170	520	438
HLL[9], ehvan	3476	1619	1243, 1163	-	-
RuLL[9] Ru(van)	3422	1637	1245, 1173	485	437
HLL[10], ehvet	3475	1616	1242, 1169	-	-
RuLL[10] Ru(vet)	3435	1639	1244, 1171	548	475
HLL[11], ehbzc	3462	1605	1240, 1167	-	-
RuLL[11] Ru(bzc)	3416	1617	1243, 1169	475	436

Table 5.4: Selected infrared spectra bands of unsymmetrical tetradentate cobalt(II), nickel(II), copper(II) and zinc(II) complexes

Complexes	ν(OH)/ν(H_2O)	ν(C=N)	ν(C-O)	ν(M-N)	ν(M-O)
CoLL[2] Co(ata)	3422	1598	1250, 1190	504	427
CoLL[3] Co(bta)	3406	1598	1248, 1190	545	438
NiLL[2] Ni(ata)	3346	1584	1242, 1180	502	465
NiLL[3] Ni(bta)	3389	1597	1248, 1190	545	438
CuLL[2] Cu(ata)	3399	1588	1241, 1180	522	466
CuLL[3] Cu(bta)	3405	1592	1240, 1180	537	467
ZnLL[2] Zn(ata)	3417	1588	1266, 1242	536	436
ZnLL[3] Zn(bta)	3398	1601	1287, 1240	506	435

5.4.1 Infrared spectra of unsymmetrical tetradentate ruthenium(III) complexes with [HLL^2, ehata] and [HLL^3, ehbta] ligands

The significant FT-IR stretching frequencies of the compounds are presented in Table 5.3, spectra in Figures 5.1 and 5.2. The observed vibrations were classified by those originating from the ligands and those arising from the bounds formed between metal ion and the ligand coordinating sites by means of comparison with previous reports in the literature on similar complexes [191, 203, 220]. The IR spectra of the free ligands: [*N*-(2',4'-dihydroxyphenyl-ethylidene)-*N'*-(1,3-butanedioneethylidene)ethylenediamine [H_2LL^2, ehata], *N*-(2',4'-dihydro-xyphenylethylidene)-*N'*-(1,3-butanedionephenylidene)ethylenediamine [HLL^3, ehbta] showed bands in the region 3419 - 3430, 1605 - 1612 and 1171 - 1288 cm^{-1} assignable to ν(OH), ν(C=N), ν(C-O) respectively in view of previous report [191, 209, 220].

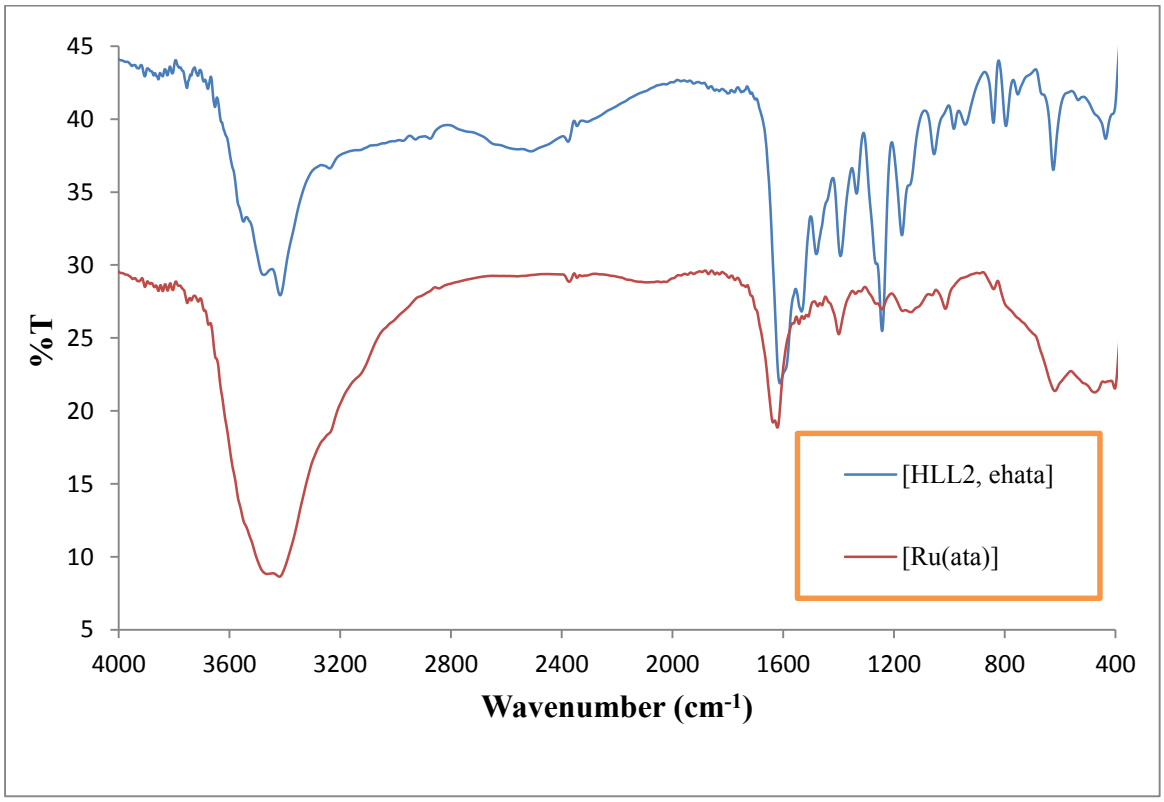


Figure 5.1: FTIR spectra of unsymmetrical ligand [HLL^2, ehata] and its complex [Ru(ata)]

The free ligands (H_2LL^2, H_2LL^3) showed very strong vibrations in the 1605 - 1612 cm^{-1} region in the individual IR spectra which are representative of the azomethine ν(C=N) group (Table 5.3). In the Ru(III) complexes, this absorption were shifted to the 1621 - 1623 cm^{-1} region signifying the coordination of the Schiff bases via the nitrogen atom [130, 209, 221] and this shifting of the wavenumber is expected due to bond formation between the nitrogen molecules of the azomethine group and the ruthenium ion, causing a reduction of the electron density in the azomethine link [133, 209]. A medium band conforming to phenolic oxygen ν(C–O) was identified at 1171 - 1288 cm^{-1} for the free ligands. Upon chelation, the band was moved to a lower frequency in the region 1138 - 1258 cm^{-1} for the unsymmetrical ruthenium(III) Schiff base complexes [130, 222].

This indicates the enolisation of >C=O followed by deprotonation and complexation of ligands with metal and the destruction of keto group apparently viz., enolisation and ketolisation bonding of the ligand through the subsequent enolate and ketolate oxygen. This is further confirmed by the disappearance of the ν(OH) in the range 3475 - 3479 cm^{-1} in the complexes. The presence of coordinated water in the complexes in the regions 3419-3430 and 851 - 861 cm^{-1} can be due to ν(O–H) stretching and ν(O–H) rocking vibrations further confirming the existence of non-ligand responsible for the rocking mode of water [116, 220, 223]. The observed bands in the region 519 - 522 cm^{-1} and 415 - 428 cm^{-1}, are assigned to ν(Ru–N) and ν(Ru–O) vibrations respectively [200, 224].

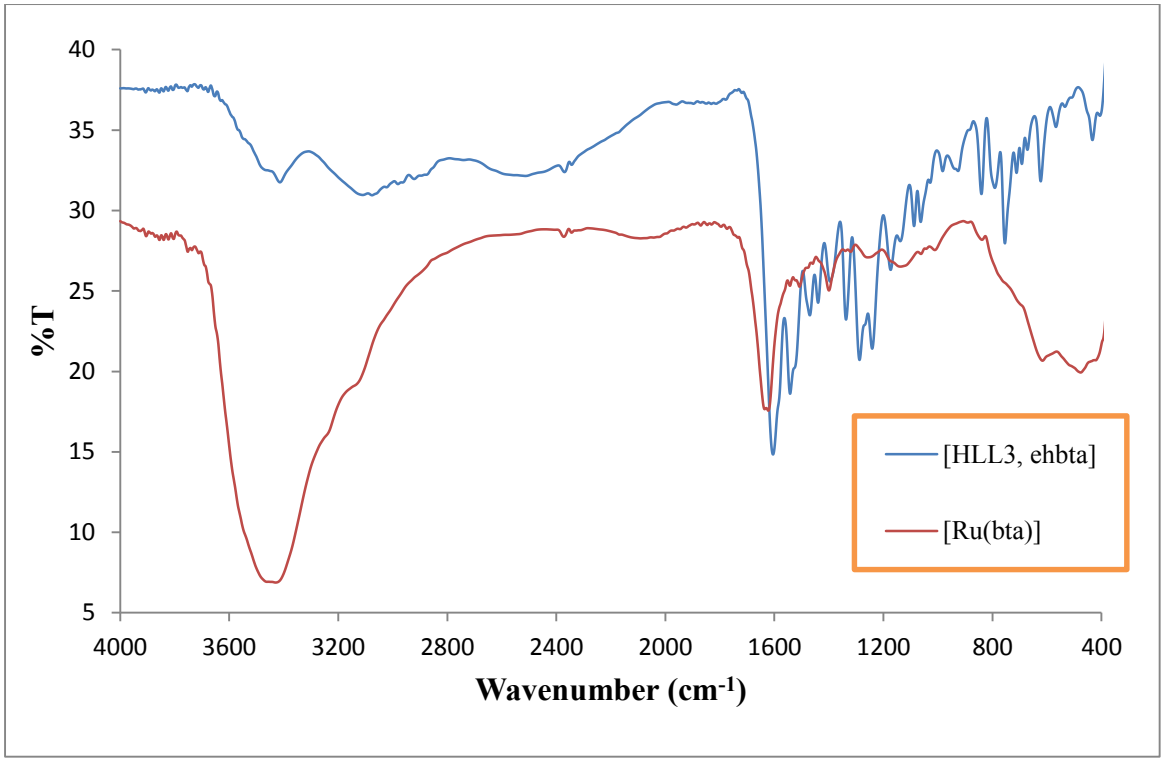


Figure 5.2: FTIR spectra of unsymmetrical ligand [HLL^3, ehbta] and its complex [Ru(bta)]

5.4.2 Infrared spectra of *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedione ethylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The relevant FTIR data for the ligand and metal complexes are given in Table 5.4 and the spectra presented in Figures 5.3 and 5.4. The infrared spectrum of the *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedioneethylidene)ethylenediamine [HLL^2, ehata] ligand shows characteristic bands at 3475 cm^{-1} has been attributed to the phenolic hydroxyl group. The characteristics absorption at 1612 cm^{-1} and 1243, 1271 cm^{-1} can be assigned to ν(C=N) and ν(C-O) respectively [100, 105]. The band at 3475 cm^{-1} was absent in the spectra of the complexes. This is a suggestion of the deprotonation and participation of the phenolic hydroxyl group in bond formation with the metal ion. The broad band at ~3400 cm^{-1} in the spectra of the Schiff base metal complexes is assigned to the ν(OH) frequency of the

coordinated H_2O [191]. This is supported by the upward shift in the phenolic ν(C-O) [116] to the extent of 23-30 cm^{-1} [228].

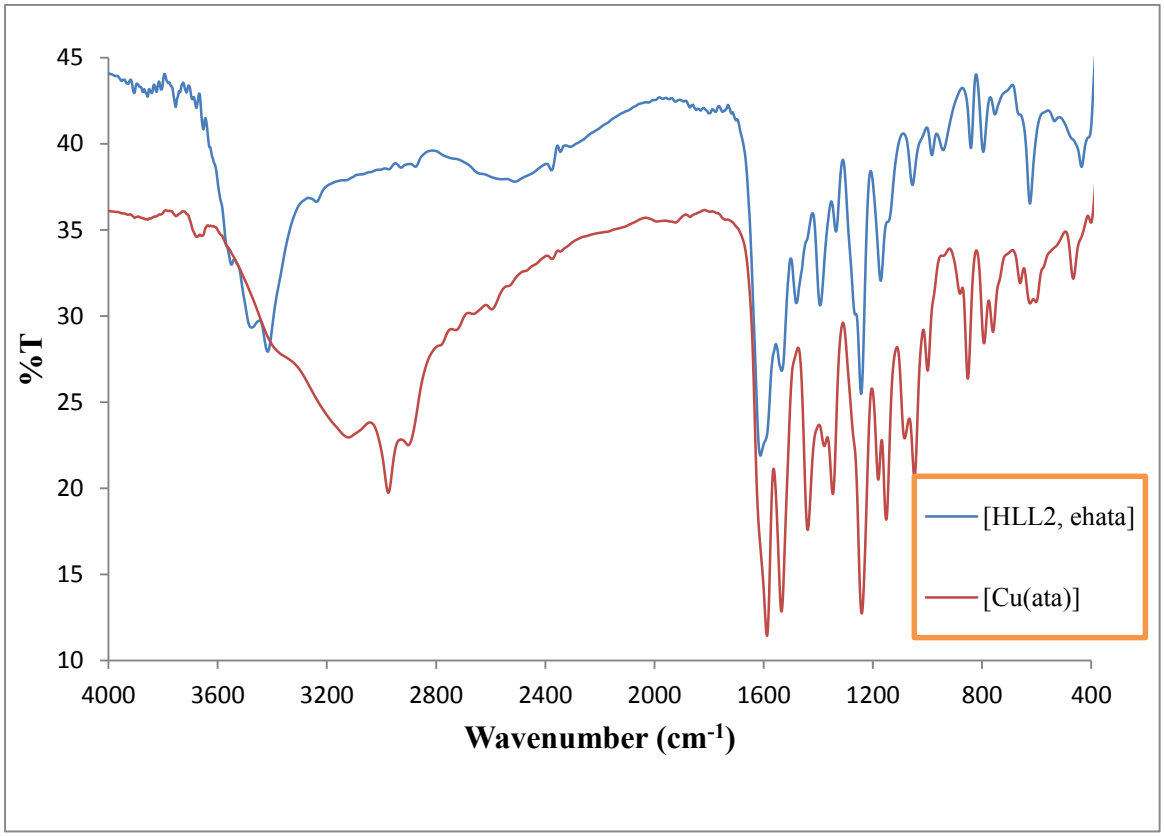


Figure 5.3: FTIR spectra of unsymmetrical ligand [HLL^2, ehata] and its complex [Cu(ata)]

The shift additionally confirms the participation of the phenolic oxygen leading to the formation of C-O-M bond. The metal complexes show a broad band at ~3400 cm^{-1} and a new band at ~860 cm^{-1} may be assigned to the stretching vibration and out of plane bending of coordinated water molecules to the complexes [191]. The strong vibration observed at 1612 cm^{-1} in the spectra of the free Schiff base ligand is characteristic of the azomethine ν(C=N) stretching vibration band. Upon complexation, this vibration underwent a shift to a lower frequency 1584 - 1598 cm^{-1}, indicating the bonding of unsaturated nitrogen of the azomethine

group of the HLL^2 to the metal ions [239]. This shift can further be explained by the donation of electrons from nitrogen to the empty d-orbitals of the metal atoms [228].

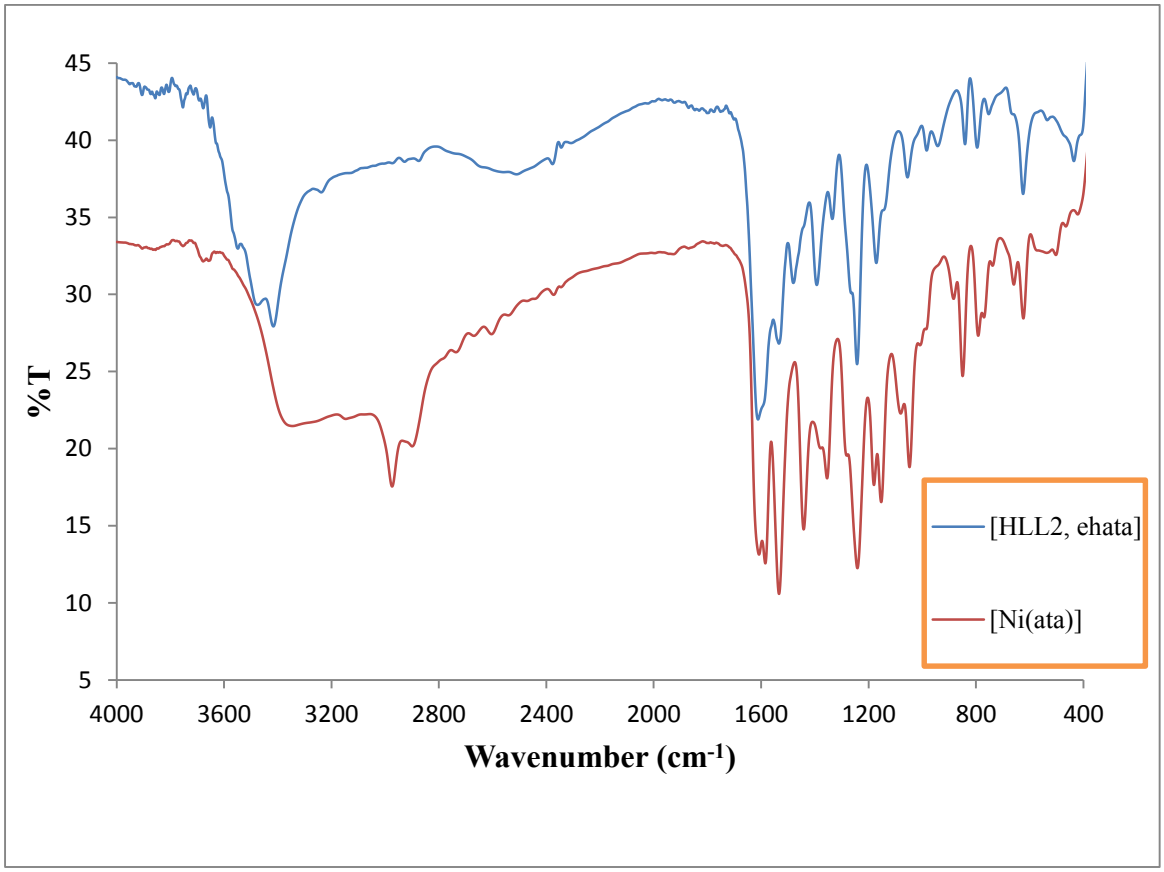


Figure 5.4: FTIR spectra of unsymmetrical ligand [HLL^2, ehata] and its complex [Ni(ata)]

Furthermore, some new bands have been observed between 502 - 536 cm^{-1} and are attributed to ν(M-N=C) vibrations: 502 cm^{-1} ν(Ni-N); 504 cm^{-1} ν(Co-N); 522 cm^{-1} ν(Cu-N); 536 cm^{-1} ν(Zn-N) bonds. Those within the band of 427 - 466 cm^{-1} are assigned to ν(M-O) vibrations: 465 cm^{-1} ν(Ni-O); 427 cm^{-1} ν(Co-O); 466 cm^{-1} ν(Cu-O); 436 cm^{-1} ν(Zn-O) bonds [200]. The IR spectra information supports the suggestion of coordination of the imino nitrogen and phenolic oxygen atoms to the Zn^{2+}, Cu^{2+}, Ni^{2+} and Co^{2+} ions.

5.4.3 Infrared spectra of *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedione phenylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The IR spectrum of the free ligand [HLL^3, ehbta] was studied and assigned on the basis of careful comparison with the complexes spectra with that of the free ligand and listed in Table 5.4 and the spectra presented in Figures 5.5 and 5.6. The infrared spectrum of the *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedionephenylidene)ethylenediamine [HLL^3, ehbta] ligand show characteristic bands at 3479 cm^{-1} attributed to the phenolic hydroxyl group, the characteristics absorption at 3076 and 2981, 1605, 1543 and 1470, 1288 and 1241 cm^{-1} can be assigned to $\nu(CH_3/CH_2)$, ν(C=N), ν(C=C), ν(C-O) respectively [100, 117]. The absence of a strong broad band in the 3479 cm^{-1} region, which is observed in the metal complexes spectra, is an indication of deprotonation of the intramolecular hydrogen bonded OH group on complexation and subsequent coordination of phenolic oxygen to the metal ion [209].

This further supports the upward shift in phenolic oxygen ν(C-O) [116] to an extent of 48-61 cm^{-1} [114]. This shift also confirms the participation of the enolic oxygen in C-O-M bond [200]. The strong band observed at 1605 cm^{-1} in the spectra of the free Schiff base [HLL^3, ehbta] is a characteristic of the azomethine ν(C=N) stretching vibration band, this vibration underwent a bathochromic shift to a lower frequency 1598-1601 cm^{-1} (Table 4.4), upon complexation indicating the bonding of nitrogen atom of the azomethine group of the free ligand to the metal ions [100, 239]. The shift can be explained by the donation of electrons from nitrogen to the empty d-orbitals of the metal atom [228]. The metal complexes show broad bands in the region 3389-3406 cm^{-1} and new bands at ~860 cm^{-1} assigned to the O-H stretching vibration and out of plane bending of coordinated water molecules to the

complexes [117, 191, 223]. The ring skeletal vibrations (C=C) were consistent in all derivatives and upon complexation, they were not altered.

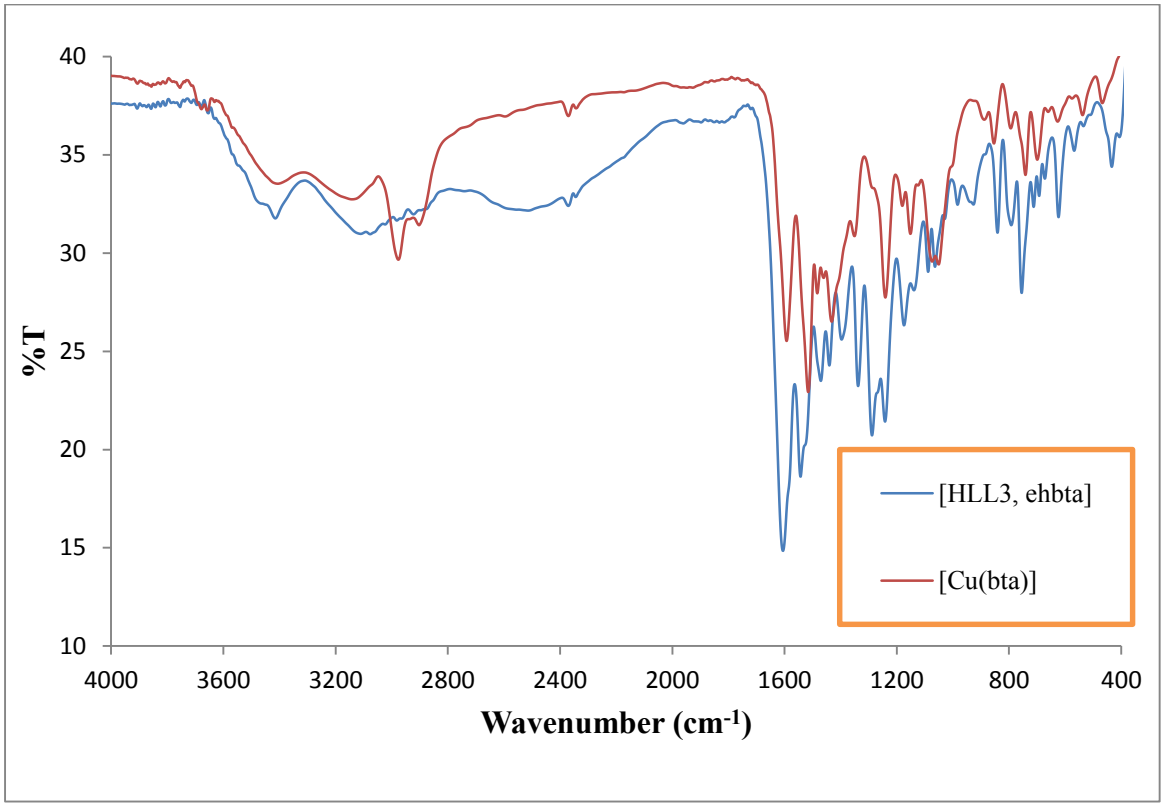


Figure 5.5: FTIR spectra of unsymmetrical ligand [HLL^3, ehbta] and its complex [Cu(bta)]

In the lower frequency region, some new bands have been observed between 506 - 569 cm^{-1} are attributed to ν(M-N) vibrations: 559 cm^{-1} ν(Ni-N); 545 cm^{-1} ν(Co-N); 537 cm^{-1} ν(Cu-N); 506 cm^{-1} ν(Zn-N) bonds and the bands in the region 435 - 467 cm^{-1} are assigned to ν(M-O) vibrations: 458 cm^{-1} ν(Ni-O); 438 cm^{-1} ν(Co-O); 467 cm^{-1} ν(Cu-O); 435 cm^{-1} ν(Zn-O) bonds [114, 200]. The IR spectra data supports the coordination of the imino nitrogen and phenolic oxygen atoms to the transition metal ions.

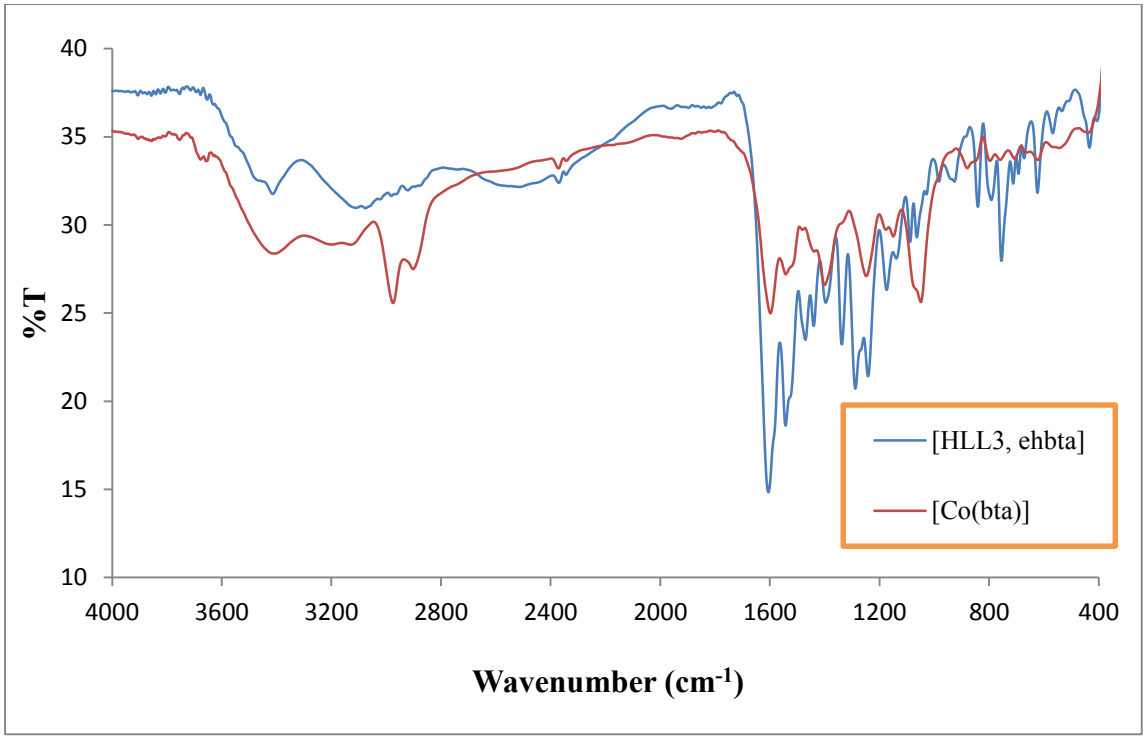


Figure 5.6: FTIR spectra of unsymmetrical ligand [HLL^3, ehbta] and its complex [Co(bta)]

5.5 Electronic spectra of the compounds

The electronic spectra of the metal complexes were measured in DMF in the range of 200 - 1000 nm (50 000 - 10 000 cm^{-1}) at room temperature. The results of the solution spectra are presented in Tables 5.5 - 5.6 and Figures 5.7 - 5.10.

5.5.1 Electronic spectra of unsymmetrical tetradentate ruthenium(III) complexes with [HLL^2, ehata] and [HLL^3, ehbta] ligands

The spectra of the complexes showed three to four bands in the region 280 - 618 nm (35 715 - 16 181 cm^{-1}) as listed in Table 5.5 and in Figures 5.7 and 5.8. Ruthenium(III) ground state is $^2T_{2g}$ and the first excited doublet levels in the order of increasing energy are $^2A_{2g}$ and $^2T_{1g}$, which arise from $t_{2g}^4 e_g^1$ configuration. The spectral profiles below 400 nm (25 000 cm^{-1}) are very similar, referred to as the ligand-centred transitions. These bands have

been characterized as π* ← π and π* ← n transitions of the benzene ring and the double bond of the azomethine group [133, 226].

Table 5.5: Electronic spectra data of unsymmetrical ruthenium(III) complexes

Complexes	Solvent	*d-d* transitions nm (cm^{-1})	C.T nm (cm^{-1})	Ligand nm (cm^{-1})
$RuLL^{2}$	DMF	618 (16 181)	506 (19 763)	280 (35 715) 316 (31 646) 393 (25 446)
$RuLL^{3}$	DMF	638 (15 674)	513 (19 493)	279 (35 843) 328 (30 488) 400 (25 000)
$RuLL^{4}$	DMF	506 (19 763)	391 (25 576)	311 (32 155) 343 (29 155) 381 (26 247)
$RuLL^{5}$	DMF	623 (16 051)	514 (19 455)	280 (35 715) 308 (32 468) 389 (25 707)
$RuLL^{6}$	DMF	635 (15 748)	517 (19 343)	282 (35 461) 326 (30 675) 384 (26 042) 401 (24 938)
$RuLL^{7}$	DMF	631 (15 848)	511 (19 570)	277 (36 101) 312 (32 052) 378 (26 455) 394 (25 381)
$RuLL^{8}$	DMF	613 (16 313)	452 (22 124) 525 (19 048)	281 (35 587) 310 (32 258) 391 (25 576)
$RuLL^{9}$	DMF	623 (16 051)	515 (19 418)	277 (36 101) 309 (32 363) 381 (26 247) 393 (25 446)
$RuLL^{10}$	DMF	623 (16 051)	510 (19 608)	277 (36 101) 311 (32 155) 380 (26 316) 393 (25 446)
$RuLL^{11}$	DMF	632 (15 823)	518 (19 305)	275 (31 364) 306 (32 680) 385 (25 974)

Table 5.6: Electronic spectra data of unsymmetrical tetradentate cobalt(II), nickel(II), copper(II) and zinc(II) complexes

Complexes	Solvent	*d-d* transitions nm (cm^{-1})	C.T nm (cm^{-1})	Ligand nm (cm^{-1})
$CoLL^2$	DMF	522 (18 116)	382 (26 316)	281 (35 587)
$CoLL^3$	DMF	406(24 631) 609 (16 420) 682 (14 663)	377 (26 525)	318 (31 447) 357 (28 011)
$NiLL^2$	DMF	436 (22 936) 564 (17 731)	394 (25 381)	288 (34 723) 308 (32 468)
$NiLL^3$	DMF	433 (23 095) 568 (17 606)	374 (26 738)	321 (31 056) 354 (28 249)
$CuLL^2$	DMF	447 (22 372)* 556 (17 668)	393 (25 446)	309 (32 363) 360 (27 778)
$CuLL^3$	DMF	401 (24 938) 558 (17 921)	380 (26 316)	322 (31 056) 362 (27 625)
$ZnLL^2$	DMF		411 (24 331)	319 (31 348) 361 (27 701)
$ZnLL^3$	DMF		413 (24 213)	320 (31 250) 368 (27 174) 379 (26 385)

* = shoulder

Within d^5 system, such as the ruthenium(III) metal ion possessing relatively high oxidizing properties, the charge transfer bands of the type $L_{\pi y} \rightarrow T_{2g}$ are noticeable in the low energy region, which obscures the weaker bands due to d-d transitions [227]. Consequently, it becomes difficult to assign categorically the bands of ruthenium(III) complexes that appear in the visible region [73, 227]. However, the extinction coefficient for the band in the 618 – 638

nm (16 180 - 15 674 cm^{-1}) region was observed to be very low as equated to that of charge transfer band.

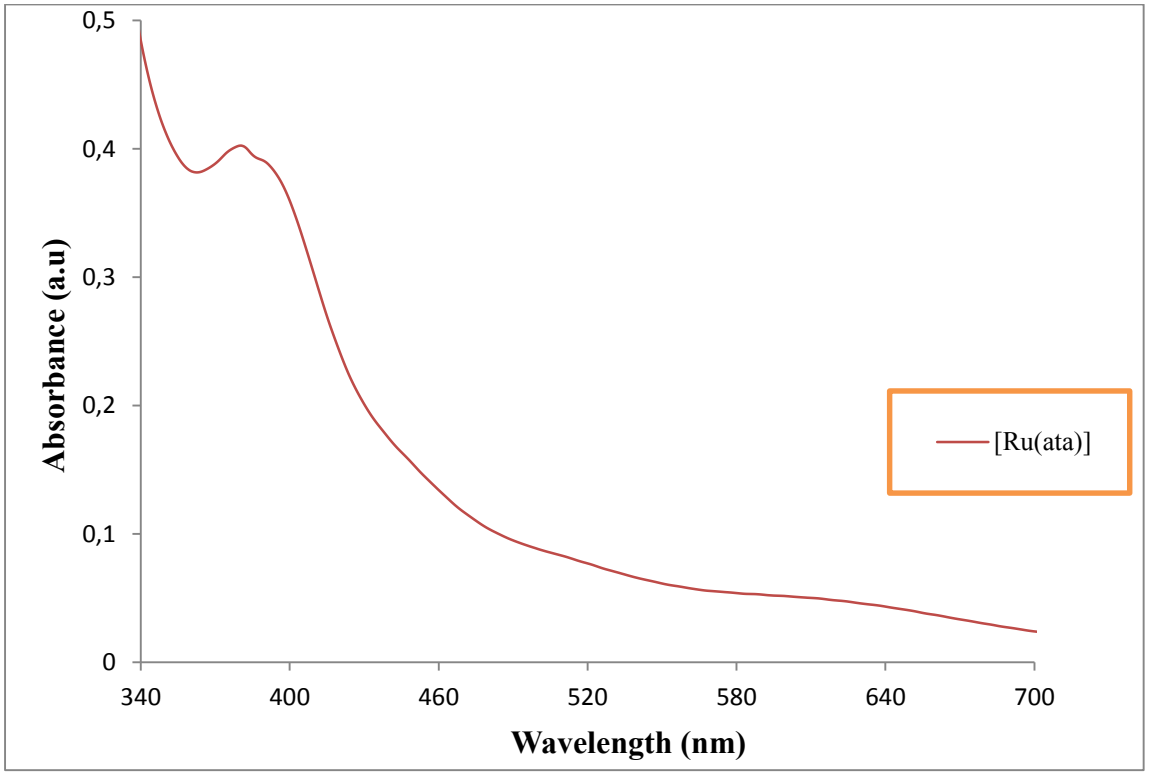


Figure 5.7: Electronic spectrum of unsymmetrical tetradentate [Ru(ata)] complexes

Hence, the band around 618 - 638 nm (16 180 - 15 674 cm^{-1}) were apportioned to $^2T_{2g}$ → $^2A_{2g}$ transition which is in conformity with assignment made for similar octahedral ruthenium(III) complexes [209, 226-228]. The electronic spectra of the complexes are typical of an octahedral environment around ruthenium(III) ions. The other band in the region 506 - 513 nm (19 763 - 19 493 cm^{-1}) has been assigned to charge transfer transition [209].

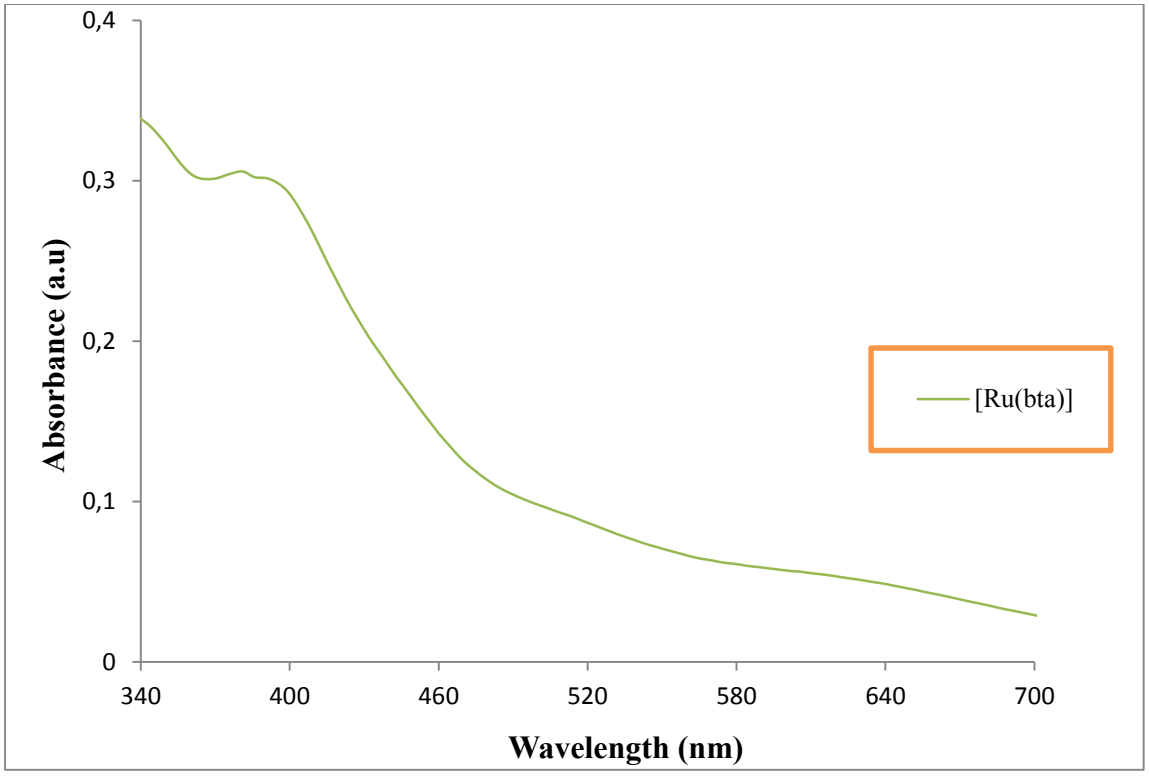


Figure 5.8: Electronic spectrum of unsymmetrical tetradentate [Ru(bta)] complex

5.5.2 Electronic spectra of *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedione ethylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The UV-Vis spectra of [HLL^2, ehata] ligand showed two bands around 317 nm (31 546 cm^{-1}) and 381 nm (26 247 cm^{-1}) regions. The first is be attributed to π* ← π transition within the aromatic ring, while the second band is then due to π* ← n transition within >C=N group as shown in Figure 5.9 and presented in Table 5.6. Upon complexation, π* ← n transition of ligand shift to a longer wavelength, and this is due to metal to ligand charge transfer transitions (MLCT) [23].

The electronic spectrum of Co(II) complex of [HLL^2, ehata] shows a charge transfer band at 382 nm (26 316 cm^{-1}). The d-d absorption at 522 nm (18 116 cm^{-1}) of less intensity,

indicating distorted tetrahedral environment of the ligand around the metal ion due to $^4A_2(F) \rightarrow {}^4T_1(P)$ transition [240]. Hence, [Co(ata)] complex can be assigned distorted tetrahedral geometry.

The electronic spectrum of [Ni(ata)] complex demonstrated a band at 25 381 cm^{-1} attributable to charge-transfer transitions L → M (LMCT) and two absorption bands at 22 936 and 17 731 cm^{-1} which might be allocated to two spin allowed transitions, $^1A_{1g} \rightarrow {}^1A_{2g}$, $^1A_{1g} \rightarrow {}^1B_{1g}$, respectively characteristic of square planar geometry around Ni(II) ion [147]. The observed electronic transitions and reddish-brown colour of the complex further confirms square-planar geometry for [Ni(ata)] complex [200].

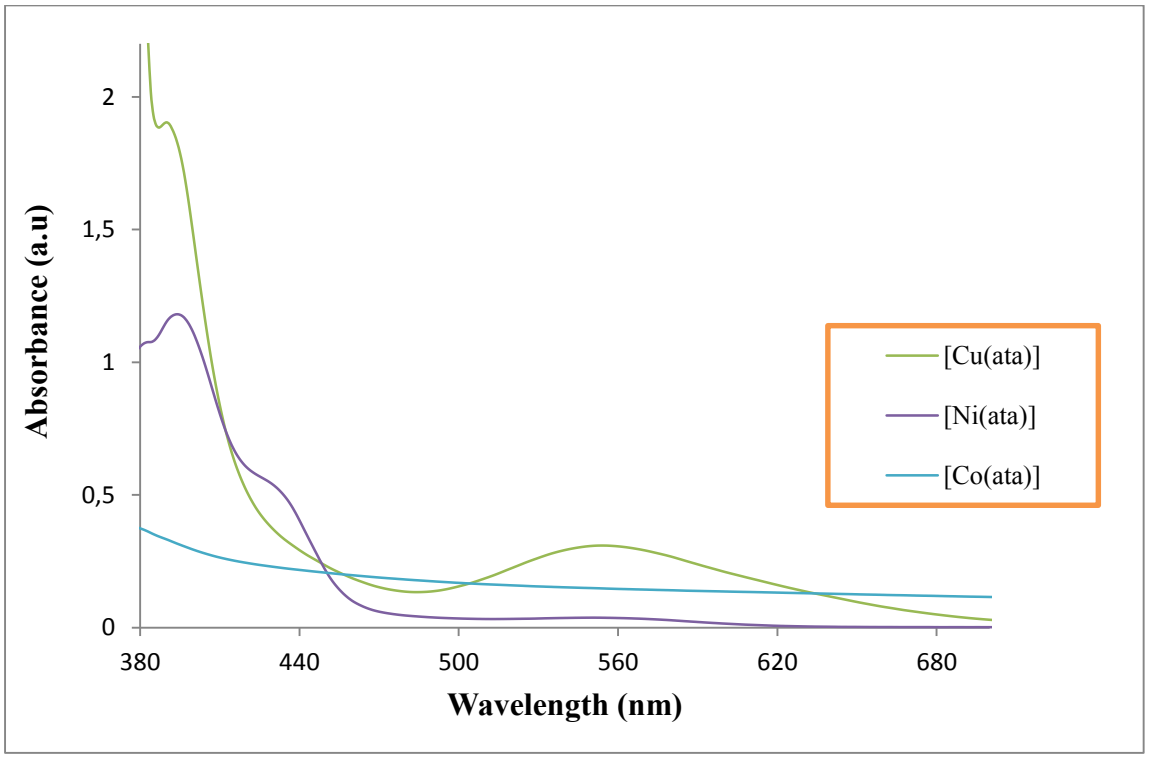


Figure 5.9: Electronic spectra of unsymmetrical tetradentate Cu(ata), Ni(ata) and Co(ata) complexes

Cu(II) complex of [HLL^2, ehata] displayed band at 393 nm (25 446 cm^{-1}), and can be attributed to charge transfer, the spectrum also displayed *d–d* electronic transition at 556 nm (17 668 cm^{-1}) which is assignable to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition, the absorption prefer square-planar geometry for the Cu(II) ion [100, 241]. The high-energy band in the region 411 nm (24 331 cm^{-1}) in the Zn(ata) complex are attributed to charge-transfer transition L → M (LMCT), as d-d transition is not expected for Zn complexes [191].

5.5.3 Electronic spectra of *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedione phenylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The electronic spectra of the [HLL^3, ehbta] ligand and its metal complexes were recorded in DMF at room temperature. The nature of the ligand field around the metal ion has been deduced from the electronic spectra. The bands 329, 339 and 378 nm are attributable to intraligand $\pi^* \leftarrow \pi$ and $\pi^* \leftarrow$ n transitions as presented in Table 5.6 and Figure 5.10. In the electronic spectra of the complexes, the intraligand transitions are slightly shifted as a result of coordination in the region at 374 - 380 nm (26 738 - 26 316 cm^{-1}).

The [Co(bta)] complex exhibits three spin allowed transitions at 406 nm (24 631 cm^{-1}), 609 nm (16 420 cm^{-1}) and 682 nm (14 663 cm^{-1}) assignable to $^4T_{1g}$ (F) → ^4T1_g (P) (ν_3), $^4T_{1g}$ (F) → $^4A_{2g}$ (F) (ν_2) and $^4T_{1g}$ (F) → $^4T_{2g}$ (F) (ν_1) transitions respectively, suggestive of an octahedral environment around the cobalt ion [203, 228].

The electronic spectrum of [Ni(bta)] complex exhibited two absorption bands at 23 095 and 17 606 cm^{-1} and may be allocated to two spin allowed transitions, $^1A_{1g} \rightarrow {}^1A_{2g}$, $^1A_{1g} \rightarrow {}^1B_{1g}$, typical characteristic of square planar geometry around Ni(II) ion [242].

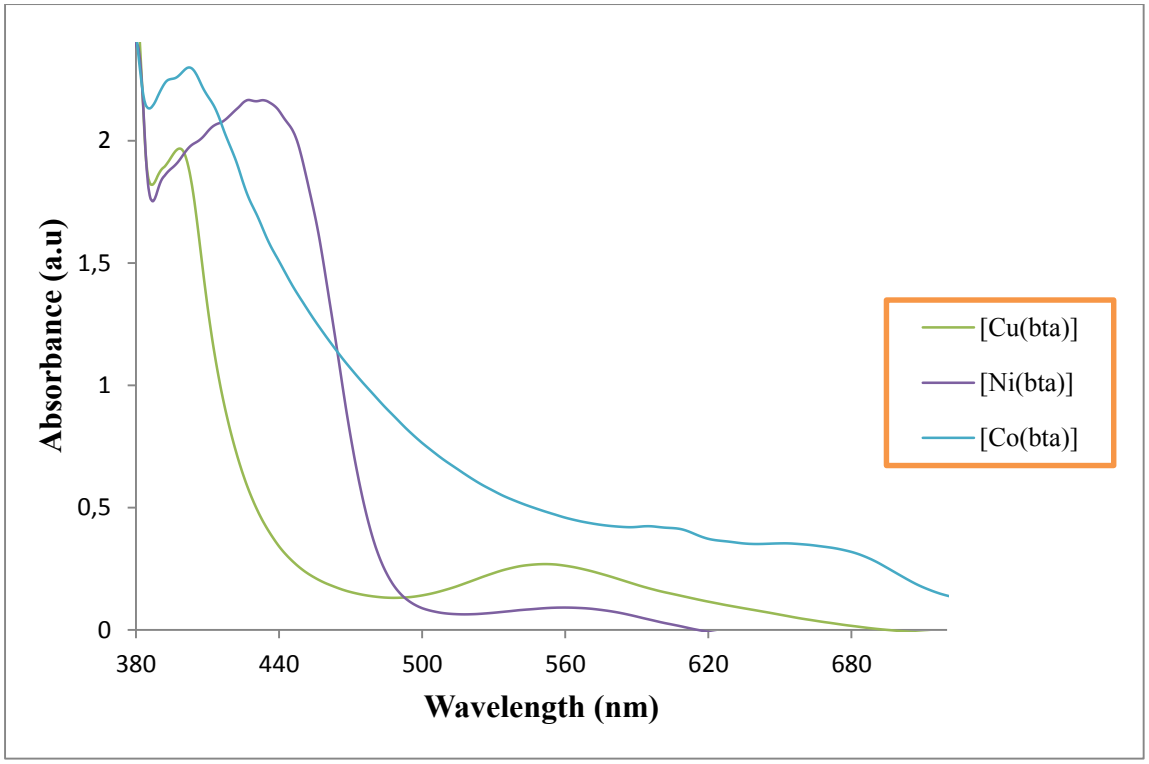


Figure 5.10: Electronic spectra of unsymmetrical tetradentate Cu(bta), Ni(bta) and Co(bta) complexes

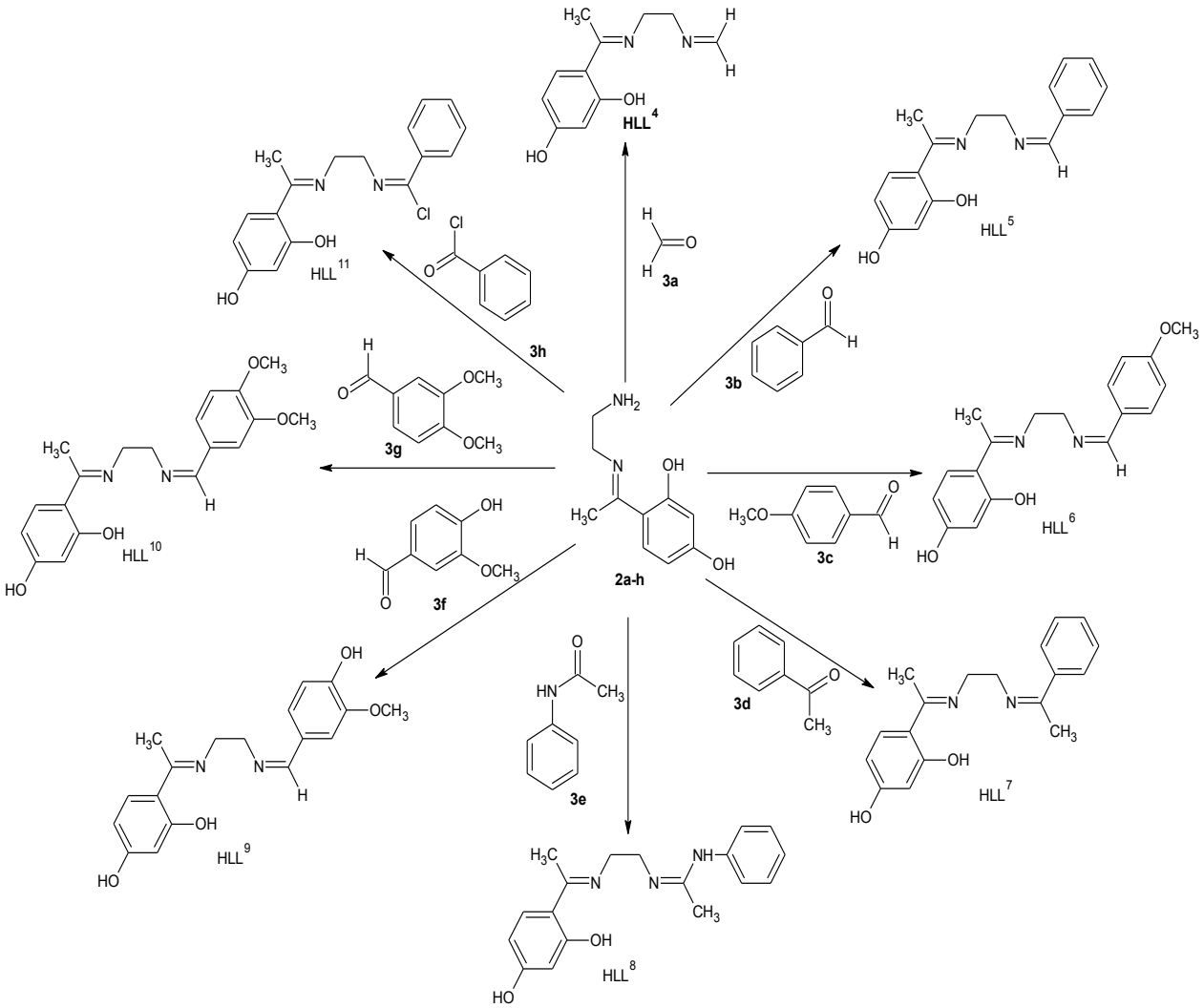
The spectra of [Cu(bta)] complexes show two bands in the visible region at about 558 nm (17 921 cm^{-1}) and 401 nm (24 938 cm^{-1}), this can attributed to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions respectively. The broad band centred at 558 nm (17 921 cm^{-1}) prefer the square-planar geometry around the Cu(II) ion [234]. The [Zn(bta)] complex is diamagnetic as expected and its geometry is most probably similar to the Ni(II) and Cu(II) complexes of the [HLL^3, ehbta] ligand.

5.6 Unsymmetrical tridentate Schiff bases and its metal complexes

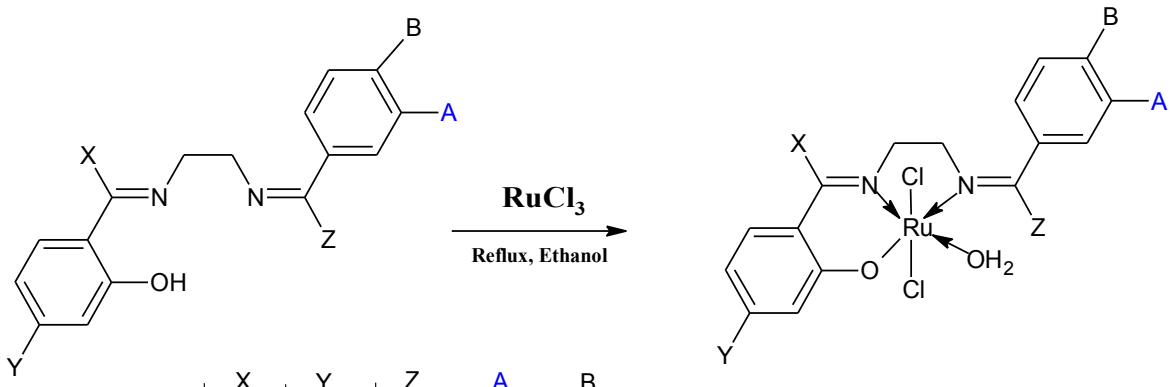
The unsymmetrical tridentate Schiff base ligands [HLL^4, ehmta] – [HLL^{11}, ehbzc] contained ethanimidoylbenzene-1,3-diol (2a-h) and substituted aromatic aldehydes (3a-h) as shown in Scheme 5.2 and isolated as golden yellow to darkish yellow solid with the

exception of [HLL[9], ehvan] that is brownish-red in good yield and decomposed at temperature greater than 209 ^{o}C. Its metal complexes were obtained by refluxing together the appropriate unsymmetrical tridentate Schiff base with ruthenium(III) chloride in absolute ethanol as shown in the Scheme 5.3. The compounds were isolated and in good yield.

The analytical data, percentage yields, colour and melting points of the complexes are presented in Table 5.1. Complexes RuLL[4] – RuLL[11] were found to be brownish-green to darkish-green with associated Cl atoms and H_2O molecule giving rise to a distorted octahedral complexes.



Scheme 5.2: Preparation of unsymmetrical tridentate Schiff base ligands



	X	Y	Z	A	B
I	H	H	-	-	-
II	Me	OH	H	H	H
III	Me	OH	H	H	OCH_3
IV	Me	OH	Me	H	H
V	Me	OH	H	OCH_3	OH
VI	Me	OH	H	OCH_3	OCH_3
VII	Me	OH	Me (NH)	H	H
VII I	Me	OH	Cl	H	H

Scheme 5.3: Preparation of unsymmetrical tridentate Schiff base Ru(III) complexes

5.7 Molar conductivity measurements of unsymmetrical tridentate ruthenium(III) complexes with [HLL^4, ehmta] – [HLL^{11}, ehbzc] ligands

The conductivity values ($\Lambda\mu$) of the metal complexes solution in DMF for ~ 10^{-3} M are presented in Table 5.1 gave conductance values ranging from 30.1 – 47.4 μScm^{-1}: 41.2 μScm^{-1} [RuLL^4]; 32.0 μScm^{-1} [RuLL^5]; 45.2 μScm^{-1} [RuLL^6]; 34.7 μScm^{-1} [RuLL^7]; 31.8 μScm^{-1} [RuLL^8]; 30.5 μScm^{-1} [RuLL^9]; 30.1 μScm^{-1} [RuLL^{10}]; 38.8 μScm^{-1} [RuLL^{11}] and this supports non-electrolyte nature of the complexes at room temperature [217].

5.8 Infrared spectra of unsymmetrical tridentate ruthenium(III) complexes with [HLL^4, ehmta] – [HLL^{11}, ehbzc] ligands

Relevant IR data of the free ligands and tridentate Ru(III) compounds are presented in Table 5.3 and the spectra in Figures 5.11 - 5.13. The Schiff base ligands showed the broad bands in the 3473 - 3477 cm^{-1} range which is attributable to the ν(OH) cm^{-1} vibration, C=N, azomethine group (1608 - 1619 cm^{-1}), -C-O group ranging from 1254 - 1288 cm^{-1}.

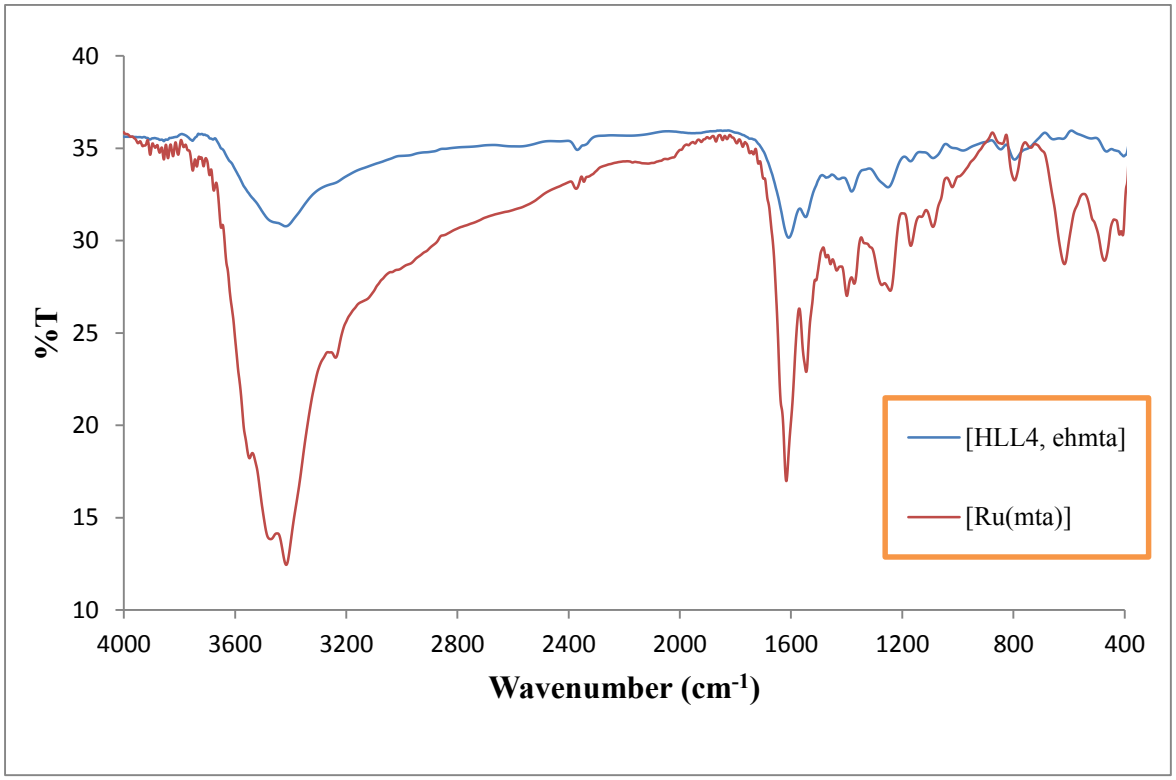


Figure 5.11: FTIR spectra of unsymmetrical ligand [HLL^4, ehmta] and its complex [Ru(mta)]

In the Ru(III) compounds, the stretching vibrations due to the OH modes were not observed, suggesting the deprotonation of hydroxyl proton by Ru^{3+} ion leading to covalent ν(Ru-O) bonding with the ligands [243]. This was further supported by the strong band

observed in the free Schiff bases in the range 1242 - 1288 cm^{-1} assigned to phenolic ν(C–O) stretching. In all the Ru(III) complexes, this band shifted to a greater frequency range 1169 - 1284 cm^{-1} indicating the involvement of the phenolic oxygen in bond formation [222, 244].

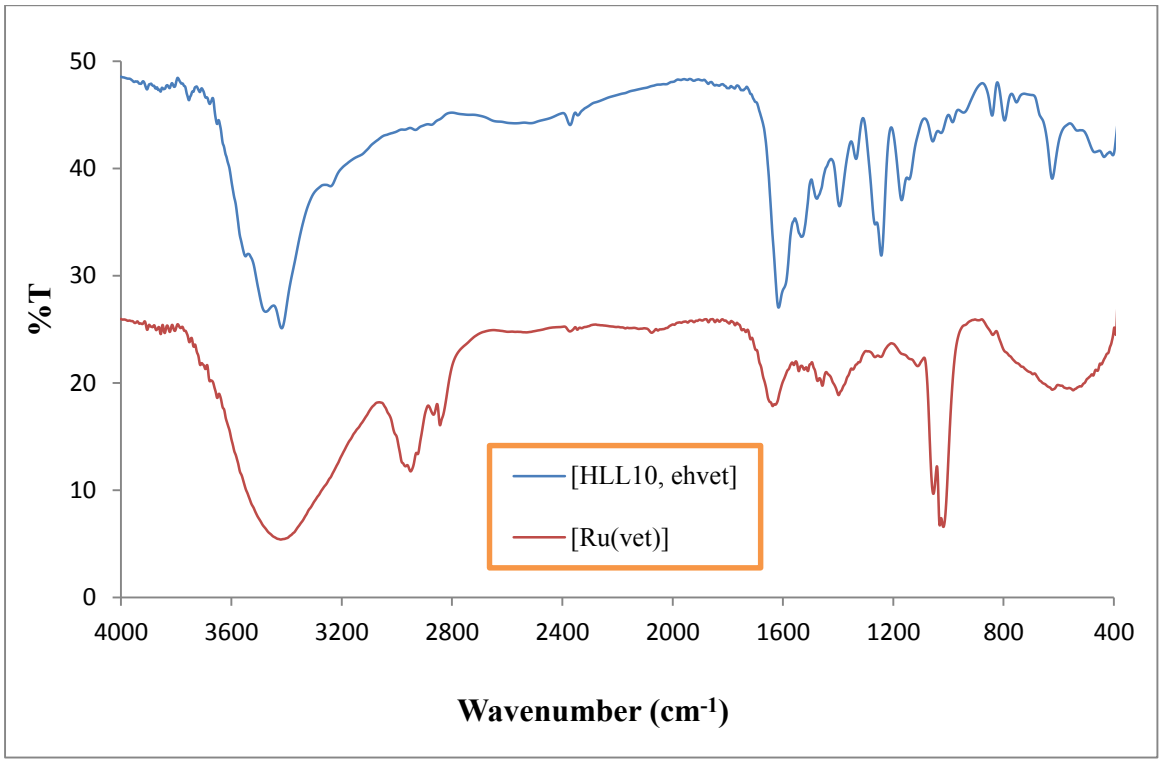


Figure 5.12: FTIR spectra of unsymmetrical ligand [HLL^{10}, ehvet] and its complex [Ru(vet)]

The presence of coordinated water in the tridentate Ru(III) complexes is confirmed by the stretching vibrations in the regions 3416 - 3449 and 854 - 810 cm^{-1}, assigned to ν(O-H) stretching and ν(O-H) rocking vibrations [116, 236]. The ν(CH=N) of the Ru(III) compounds showed a strong vibrations in the region 1617 - 1639 cm^{-1} [131, 126]. The shifting of this vibrations to higher vibrational frequency by 12 - 23 cm^{-1} denotes the nitrogen atom of the azomethine group coordination to the Ru(III) ion [196].

The bonding of the Ru^{3+} ions to the tridentate ligands through the (>C=N) nitrogen and phenolic oxygen atoms is further established through the evidence of new bands in the 475 - 548 and 418 - 476 cm^{-1} range due to the ν(Ru-N) and ν(Ru-O) vibrations, respectively [196, 243].

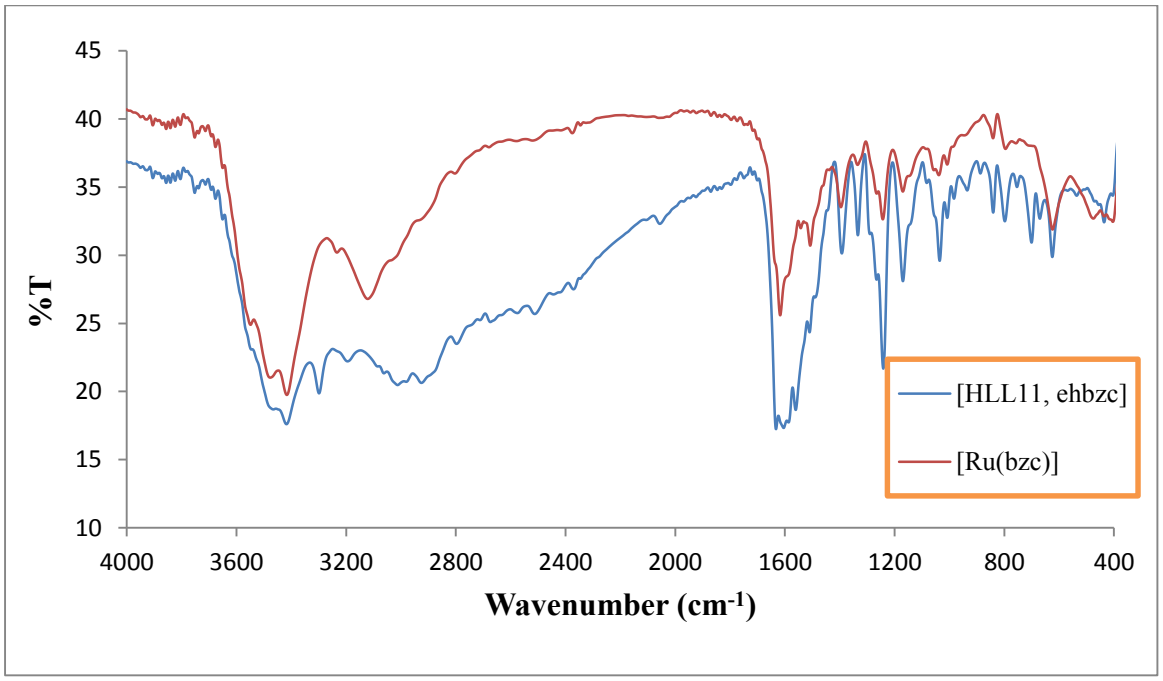
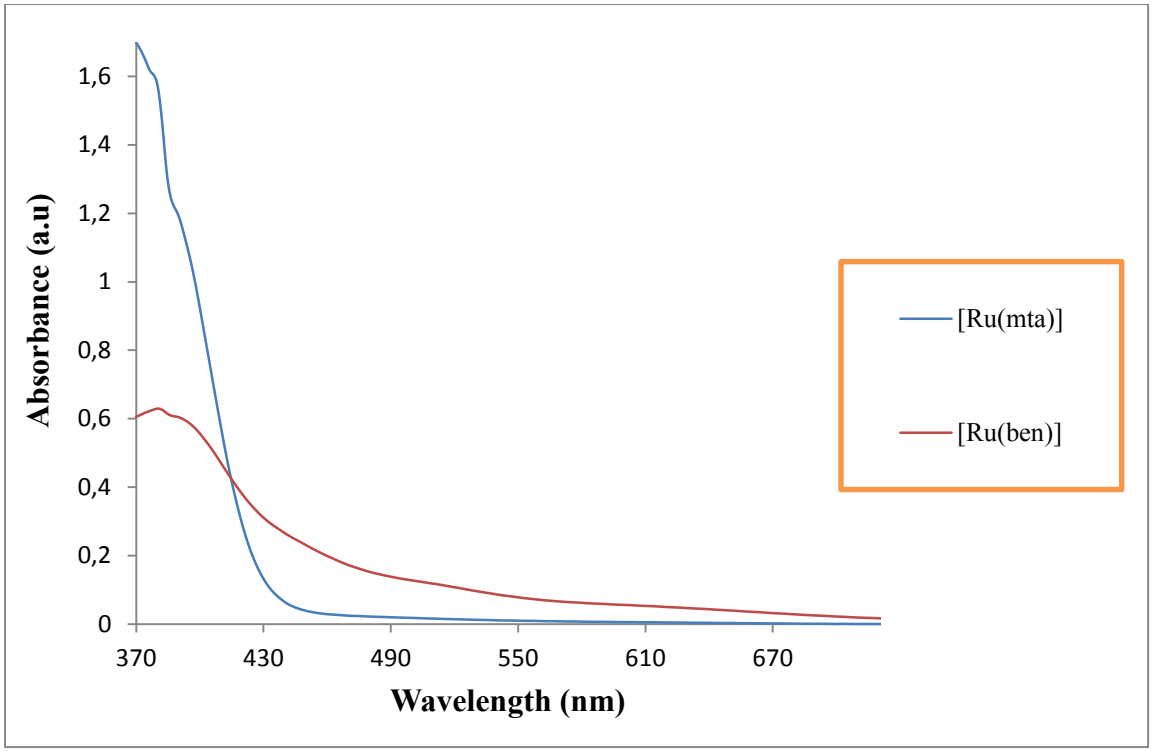


Figure 5.13: FTIR spectra of unsymmetrical ligand [HLL^{11}, ehbzc] and its complex [Ru(bzc)]

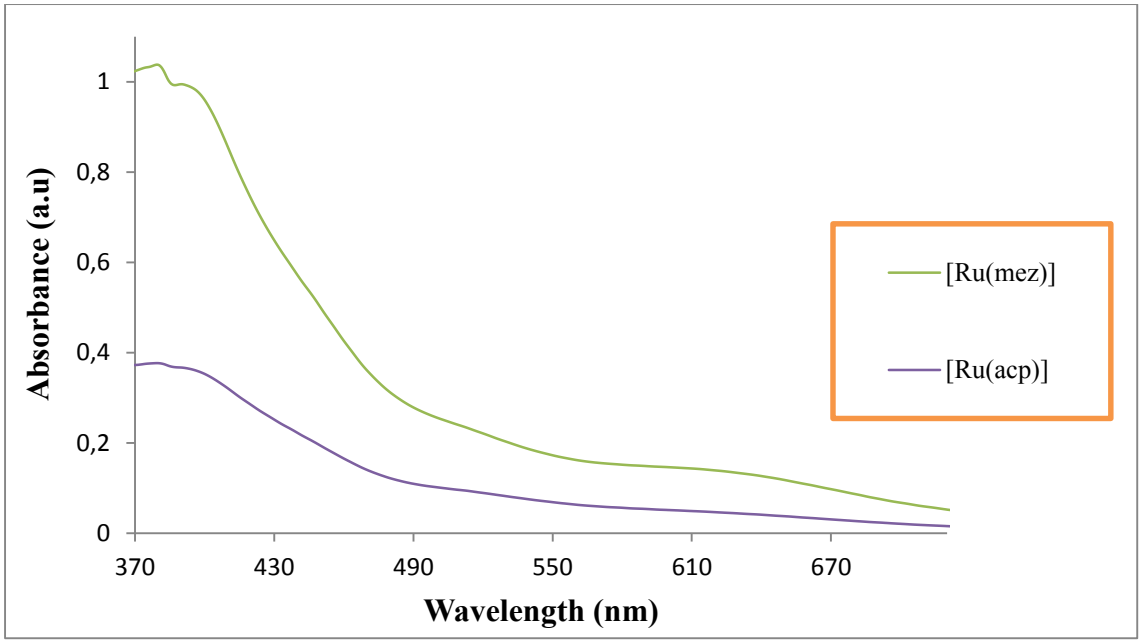
5.9 Electronic spectra of unsymmetrical tridentate ruthenium(III) complexes with [HLL^4, ehmta] – [HLL^{11}, ehbzc] ligands

The electronic spectra of tridentate Ru(III) complexes in DMF within the range of 200 - 1000 nm (50 000 - 10 000 cm^{-1}) displayed four to five bands within the region 277 - 635 nm (36 101 - 15 748 cm^{-1}) (Table 5.5 and Figures 5.14 - 5.17) Ruthenium(III) ground state is $^2T_{2g}$ and the first excited doublet levels in the direction of increasing energy are $^2A_{2g}$ and $^2T_{1g}$, which arise from $t_{2g}^4 e_g^1$ configuration [245].



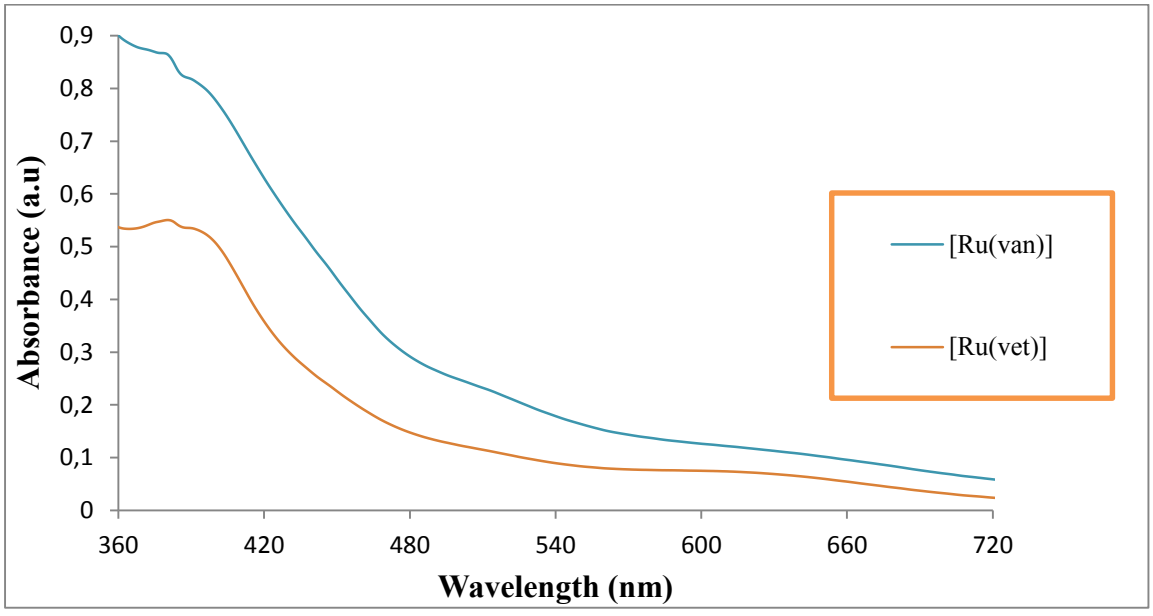
Figures 5.14: Electronic spectra of unsymmetrical tridentate [Ru(mta)] - [Ru(ben)] complexes

Ligand-centred transitions has ranged from 277 - 401 nm (36 101 - 24 938 cm^{-1}) in the spectra sketches, and these bands are attributable to $\pi^* \leftarrow \pi$ and $\pi^* \leftarrow$ n transitions of the aryl ring and the double bond of the >C=N- group [133, 196]. Ruthenium(III) ion, a member of d^5 system has relatively high oxidizing properties and large crystal field parameter, the bands charge transfer of the sort $L_{\pi y} \rightarrow T_{2g}$ are noticeable in the low energy level, and this obscures the weaker bands due to *d-d* transitions [133, 196]. The band in the 506 - 635 nm (19 763 - 15 748 cm^{-1}) region: 506 nm [$RuLL^4$]; 623 nm [$RuLL^5$]; 635 nm [$RuLL^6$]; 631 nm [$RuLL^7$]; 613 nm [$RuLL^8$]; 623 nm [$RuLL^9$]; 623 nm [$RuLL^{10}$]; 632 nm [$RuLL^{11}$] have been assigned to the $^2T_{2g} \rightarrow {}^2A_{2g}$ transition, and this is in conformity with assignments made for the similar ruthenium(III) complexes [76].



Figures 5.15: Electronic spectra of unsymmetrical tridentate [Ru(mez)] - [Ru(acp)] complexes

Absorption in the 391 - 517 nm (25 576 - 19 343 cm^{-1}) region: 391 nm [$RuLL^4$]; 514 nm [$RuLL^5$]; 517 nm [$RuLL^6$]; 511 nm [$RuLL^7$]; 452, 525 nm [$RuLL^8$]; 515 nm [$RuLL^9$]; 510 nm [$RuLL^{10}$]; 518 nm [$RuLL^{11}$] are due to the charge transfer transitions [198].



Figures 5.16: Electronic spectra of unsymmetrical tridentate [Ru(van)] - [Ru(vet)] complexes

The design of the absorption spectra for the tridentate Ru(III) complexes confirms the existence of an octahedral environment around the ruthenium(III) ion with comparable observation to that of other ruthenium octahedral complexes [196].

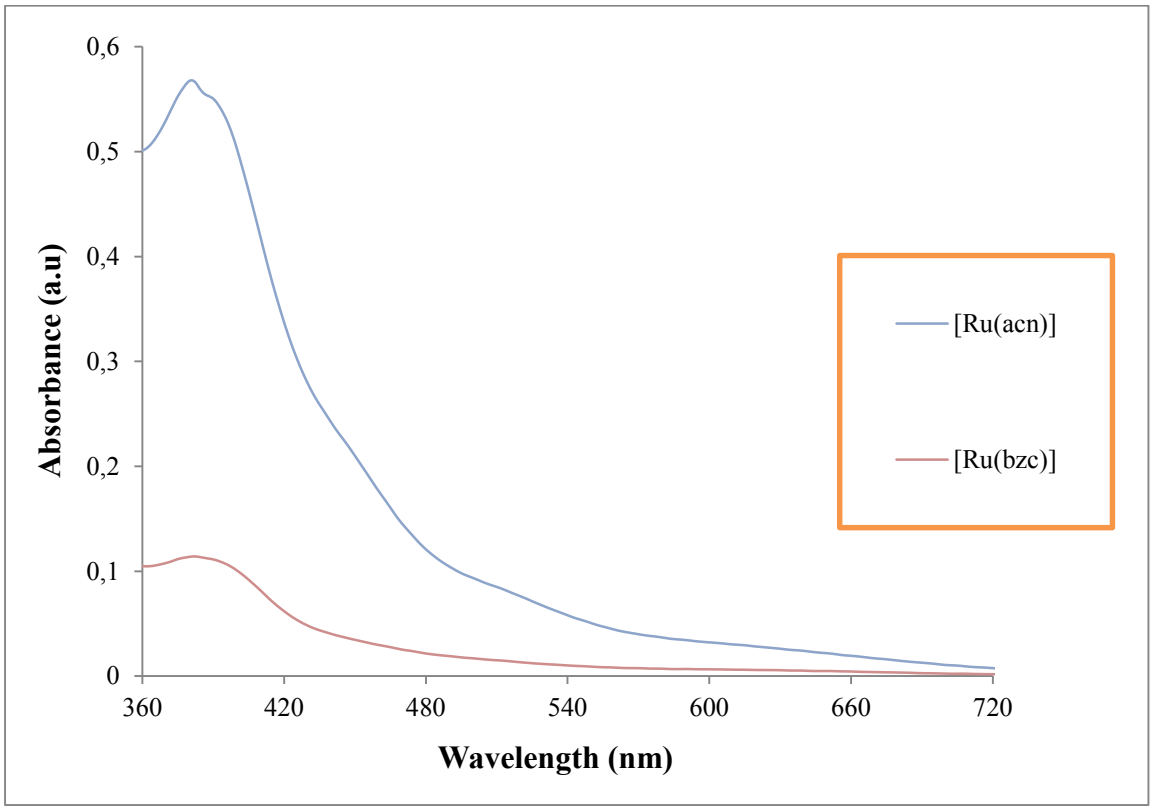


Figure 5.17: Electronic spectra of unsymmetrical tridentate [Ru(acn)] - [Ru(bzc)] complexes

5.10 Summary of the Chapter

Ru(III), Co(II), Ni(II), Cu(II) and Zn(II) complexes of unsymmetrical tetradentate [HLL^2, ehata] and [HLL^3, ehbta] ligands have been synthesized and characterized with a view to understand their physical and chemical properties. Elemental analysis further confirms the proposed structures of the complexes as given in Scheme 5.1. The analytical data show that the metal ligand stoichiometry in all these complexes is 1:1. All the complexes

are non-electrolytes in DMF solution. The spectra data show that the synthesized ligand binds with metal ions in tetradentate manner through nitrogen atom of the azomethine and oxygen atoms of hydroxyl group of the 2',4'-dihydroxyacetophenone beside the hydroxyl group of the carboxyl group of the acetylacetone/ 1-phenylbutane-1,3-dione, respectively.

Octahedral geometry was proposed for [Ru(ata)] and [Ru(bta)] complexes in which the coordination were completed by H_2O and a Cl^-. Electronic spectra revealed the unsymmetrical tetradentate Co(II) complexes to exhibit tetrahedral geometry, while square planar geometries were assigned for Ni(II) and Cu(II) complexes of the unsymmetrical tetradentate [HLL^2, ehata] and [HLL^3, ehbta] ligands. Eight unsymmetrical heterocyclic ruthenium(III)-tridentate Schiff base complexes formulated as [$Ru(L)Cl_2(H_2O)$] (where L = unsymmetrical tridentate ONN Schiff base ligand) were synthesized and characterized using spectroscopic and analytical techniques. The microanalyses were in good agreement with the proposed structures. The absorption spectra revealed that the geometry around the Ru^{3+} ion in the monomeric unsymmetrical tridentate ruthenium(III) complexes is octahedral, in which the ligands act as tridentate chelating ligands, coordinating through azomethine nitrogen atoms and a phenol oxygen atom.

Chapter Six

In vitro evaluation of Antibacterial, Antioxidant and Antiproliferative activity of the Schiff bases and corresponding metal complexes

Part of Chapter Six has been published as:

Ejidike, I. P.; Ajibade, P. A. Synthesis and *in vitro* anticancer, antibacterial, and antioxidant studies of unsymmetrical Schiff base derivatives of 4-[(1*E*)-*N*-(2-aminoethyl)ethanimidoyl] benzene-1,3-diol. *Research on Chemical Intermediates*, **2016**, pp 1-13, doi: 10.1007/s11164-016-2479-x.

CHAPTER SIX

IN VITRO BIOLOGICAL STUDIES

6.0 Background to the Chapter

Schiff bases are considered a very important class of organic ligands possessing diverse applications [34, 103, 196]. In biological processes, inorganic compounds play critical roles and it has been established that many organic compounds used in medicine are activated or bio-transformed by metal ions metabolism [126]. In recent times, transition metal complexes of Schiff base ligands have gained considerable attention, not only due to their spectroscopic properties and applications [233] but also due to their remarkable antifungal, antibacterial and antitumor activities [66, 217].

Biological reactions which are essential to life processes usually involve transition metals; these metals usually coordinate with O- or N-terminals from proteins in a variety of modes and play a vital crucial role in the conformation and function of biological macromolecules [203]. Schiff base-transition metal complexes obtained from heterocyclic molecules have received attention from many researchers regarding the development of bioinorganic compounds for biological application [233].

Metal-based antioxidants have gained attention recently for their capacity to protect organisms and cells from damage induced by oxidative stress or scavenge free radicals [139]. Interest in ruthenium is related to the promising results that have been obtained in the inorganic and organometallic fields exhibiting a cytotoxicity properties is comparable to or even better than that of cisplatin [72]. The Schiff base of ruthenium complexes is one of the

compounds that have attracted great attention, and some of its complexes have exhibited interesting properties such as intracellular accumulation and antiproliferative properties [196].

The chapter describes the results obtained from the *in vitro* antibacterial, antioxidant and antiproliferative screening of the synthesized complexes of ruthenium(III), nickel(II), zinc(II), cobalt(II) and copper(II) complexes with the symmetrical and unsymmetrical Schiff bases. Also, ruthenium(III) complexes of symmetrical and unsymmetrical Schiff bases were screened for their antioxidant and antiproliferative activity, while nickel(II), zinc(II), cobalt(II) and copper(II) complexes were evaluated for their antibacterial and free radical scavenging properties alongside the symmetrical and unsymmetrical Schiff bases.

6.1 Antimicrobial activities of the Schiff base ligands and metal complexes

The antibacterial screening of the Schiff base ligands, nickel(II), zinc(II), cobalt(II) and copper(II) complexes were compared with standards antibiotics: Amoxicilin and ciprofloxacin against selected bacterial strains including: *Staphylococcus aureus* (ATCC 25923), *Streptococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702)- Gram-positive bacteria and *Escherichia coli* (ATCC 25922), *Shigella flexineri* (KZN) and *Pseudomonas aeruginosa* (ATCC 19582)- Gram-negative bacteria using disc diffusion method by utilizing DMSO as solvent. The compounds were screened at the concentrations 0.312 - 10 mg mL^{-1} in DMSO. The MIC results are summarized in Table 6.1.

6.1.1 *In vitro* antimicrobial studies of unsymmetrical tridentate Schiff base ligands

The antimicrobial activity of tridentate ligands were compared with standard drug amoxicillin and ciprofloxacin. The MIC results are summarized in Table 6.1. A close survey of the results indicates that the derivatives of tridentate ligands [HLL^4, ehmta] – [HLL^{11}, ehbzc] exhibited varied range of minimum inhibition depending upon the substituent on the ring structure. The compounds have shown moderate to good biological activity against the bacterial strains. Compound [HLL^9, ehvan] have shown excellent activity against both gram(+) and gram(-) microbial strains as compared to standard drug amoxicillin and ciprofloxacin.

Table 6.1: Antibacterial activities of ligands and their complexes showing their minimum inhibitory concentration (MIC) (mg/mL) against microorganisms

Ligands/ Complexes	**Gram (+) bacteria**			**Gram (-) bacteria**		
	S. *aureus*	*B. cereus*	*S. faecalis*	*P. aeruginosa*	*S. flexineri*	*E. coli*
Tetradentate Schiff bases and their metal complexes						
[HLL^1, ehopd]	>10	>10	>10	>10	>10	>10
$CuLL^1$ [Cu(opd)]	5	10	5	5	10	5
$NiLL^1$ [Ni(opd)]	10	5	10	10	>10	>10
$CoLL^1$ [Co(opd)]	5	10	10	>10	10	10
$ZnLL^1$ [Zn(opd)]	10	>10	10	>10	>10	>10
[HLL^2, ehata]	>10	10	>10	>10	>10	>10
$CuLL^2$ [Cu(ata)]	5	2.5	5	>10	10	5
$NiLL^2$ [Ni(ata)]	>10	10	5	10	>10	>10
$CoLL^2$ [Co(ata)]	10	5	>10	>10	>10	10
$ZnLL^2$ [Zn(ata)]	>10	10	>10	>10	>10	10
[HLL^3, ehbta]	>10	>10	10	>10	>10	>10
$CuLL^3$ [Cu(bta)]	10	5	2.5	>10	5	10
$NiLL^3$ [Ni(bta)]	>10	10	5	>10	10	10
$CoLL^3$ [Co(bta)]	>10	10	>10	>10	>10	>10
$ZnLL^3$ [Zn(bta)]	>10	>10	10	>10	>10	>10

Tridentate Schiff bases						
[HLL[4], ehmta]	5	NA	NA	NA	10	NA
[HLL[5], ehben]	5	NA	10	5	5	NA
[HLL[6], ehmez]	5	10	NA	NA	10	NA
[HLL[7], ehacp]	NA	5	NA	NA	5	NA
[HLL[8], ehvap]	10	NA	NA	5	NA	NA
[HLL[9], ehvan]	5	1.25	5	5	1.25	2.5
[HLL[10], ehvet]	2.5	5	10	NA	5	NA
[HLL[11], ehbzc]	2.5	NA	10	NA	5	5
Amoxicillin[a]	1.250	0.312	1.250	1.250	1.250	1.250
Ciprofloxacin[a]	0.312	0.312	0.312	0.312	0.312	0.312

[a] Standards, NA = No activity

Compounds [HLL[10], ehvet] and [HLL[11], ehbzc] were observed to have excellent action against *S. aureus* at MIC value of 2.5 mg/mL. The compounds showed a minimum inhibition within the considered range of concentrations against *S. flexineri* except for [HLL[8], ehvap], being inactive. Furthermore, [HLL[9], ehvan] and [HLL[10], ehvet] compounds exhibited an inhibition against *E. coli* with MIC values of 2.5 and 5 mg/mL respectively. Presence of different functional groups on the benzene ring brings about selectivity in the inhibitory activity against bacteria strains with enhanced antibacterial activity against *S. aureus, S. flexineri, B. cereusa* and *E. coli*.

The development of hydrogen bond via the azomethine nitrogen atom with the active centres of cell components may affect the regular cell process, thereby bringing about interference with the cell wall synthesis [116]. Furthermore, the variation in the efficacy of the compounds [HLL[4], ehmta] – [HLL[11], ehbzc] against several bacteria depends either on the microorganisms cells impermeability or differences in ribosomes of bacteriological cells [120].

6.1.2 *In vitro* antimicrobial studies of tetradentate Schiff base ligands and metal complexes

6.1.2.1 Antibacterial screening of *N,N′*-bis(2',4'-dihydroxyphenylethylidene) ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The antimicrobial actions of *N,N′*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine [HLL^1, ehopd] and its metal complexes indicate that the complexes hold greater growth inhibition potential than the free Schiff base ligand (Table 6.1) but less potent than standard drugs; amoxicillin and ciprofloxacin. The metal complexes showed appreciable antibacterial potentials against S. *aureus, B. cereus, S. faecalis*, *E. coli.* The biological activity of the synthesized compounds was in the order: [Cu(opd)] > [Co(opd)] > [Ni(opd)] > [Zn(opd)] > [HLL^1, ehopd]. The compounds showed efficacy variation which is dependent of the cell microbes' impermeability or differences in the cells ribosomes [246]. The metal ions affect the growth, morphology and metabolism of microorganism, through functional disturbance.

Toxicity activity of metal complex mechanism is increased, and this could be elucidated on Overtone's theory of cell penetrability in which lipid materials that are soluble are allowed to pass through cell lipid membrane as a significant feature of antimicrobial activity [247] and Tweedy chelation theory [248] that supports the reduction of metal ion polarity to an extent with view of ligand orbital and metal ion positive charge partial sharing, enhancement in the complex lipophilicity [249-250] as a result of π-electrons delocalization within the chelate ring, disturbing the respiratory activity of the cell and restricts further organism growth [120].

6.1.2.2 Antibacterial screening of *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedioneethylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

It is evident that the growth inhibition by metal complexes in most cases is higher than that of free ligand *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedione ethylidene)ethylenediamine [HLL^2, ehata]. Hence, such enhanced action of the metal complexes could be due to the lipophilic nature of the metal ions in the complexes [116]. It also suggests that the complexes possess antibacterial activity inhibiting multiplication process of the micro-organisms by shedding their active sites [240]. The ligand shows no antibacterial activity against all tested bacteria strains. [Co(ata)] complex shows slight effect *against S. faecalis, B. cereus, E. coli*, while [Ni(ata)] complex shows low antibacterial activity against all the stains with the exception of *S. aureus, P. aeruginosa,* and *S. flexineri*.

The [Cu(ata)] complex exhibited low to higher bactericidal activities than other complexes against *S. faecalis, B. cereus, S. aureus, S. flexineri* and *E. coli.* The bioactivity of the ligand and its complexes is observed to be in the order: [Cu(ata)] > [Co(ata)] > [Ni(ata)] > [Zn(ata)] > [HLL^2, ehata]. The differences observed in the antimicrobial activities may be due to the nature of the metal ions electronic configuration and the cell membrane of the microorganisms.

6.1.2.3 Antibacterial screening of *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedionephenylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The results of *in vitro* antibacterial activity of the Schiff base *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedionephenylidene)ethylenediamine [HLL^3, ehbta] and [Co(bta)], [Ni(bta)], [Cu(bta)] and [Zn(bta)] complexes are presented in Tables 6.1. In

general, metal complexes have been reported to be more effective than the free ligands and the same was observe in this study. The [Cu(bta)] complex was found to show bactericidal activity *against B. cereus, S. aureus* and *S. flexineri* and bacteriostatic against *S. faecalis, P. aeruginosa, E. coli*. A fairly moderate effectiveness is shown by [Ni(bta)] complex, which acts within the MIC range of 5 - 10 mg/mL towards *B. cereus, S. aureus, S. flexineri* and *E. coli*.

The [Co(bta)] and [Zn(bta)] complexes show less activity towards *B. cereus* and *S. aureus* respectively but had no activity against *S. faecalis, P. aeruginosa, S. flexineri, E. coli* organisms within the limit of concentration considered. However, these compounds could be active against the organisms at concentrations greater than 10 mg/mL (MIC > 10 mg/mL). The *in vitro* antibacterial activity results (Table 6.1) revealed that [HLL^3, ehbta] was bacteriostatic against all bacterial strains except *S. aureus*. The activity order of the synthesized compounds is as follows: [Cu(bta)] > [Ni(bta)] > [Co(bta)] > [Zn(bta)] > [HLL^3, ehbta]. The higher activity of the metal complexes may be due to the effect of metal ions on the normal cell membrane. The metal ions affect the growth, morphology and metabolism of microorganism, through functional disturbance, protein denaturation or destruction of the cell membranes [240, 246-247].

The variation in the activities of different complexes against different organisms depend either on the impermeability of cells of the microbes or difference in ribosomes of microbial cells [120]. The higher antibacterial activity of the Schiff base metal complexes than the free Schiff base ligands can be ascribed to the coordination of the Schiff base to metal ion which renders the microorganisms inactive and inhibits their growth [240].

6.2 The Antioxidant assay of the Schiff base ligands and metal complexes

Oxidative reactions of biological molecules have been revealed to induce a variety of pathological events such as cellular injury and ageing process, these damaging events are caused by free radicals [251-252]. Therefore to prevent the free radical damage in the body, it is important to administer drugs that may be rich in antioxidants. The antioxidant activity of organic ligand and their metal complexes have been investigated using the *in vitro* method [147, 253].

However, the antioxidant mechanisms of the complexes have not been exactly explained so far [145]. The antioxidant assay study was carried out using different concentrations of the test samples (Schiff base ligands and the metal complexes) with DPPH and ABTS radicals, while ascorbic acid, rutin and butylated hydroxytoluene (BHT) were used as standards in order to establish some structure antioxidant-activity relationship.

Table 6.2: Antioxidant potentials of Schiff base ligands, metal complexes and standard drugs on DPPH* and ABTS* free radicals

Ligands/ Complexes	**DPPH radical scavenging activity**		**ABTS radical scavenging activity**	
	IC_{50} (μM)	R^2	IC_{50} (μM)	R^2
Tetradentate Schiff bases and their metal complexes				
[HLL^1, ehopd]	5.99±1.37	0.953	1.81±1.59	0.843
[HLL^2, ehata]	6.47±2.96	0.909	1.86±2.28	0.745
[HLL^3, ehbta]	4.24±1.23	0.988	1.98±1.55	0.876
$RuLL^1$ [Ru(opd)]	1.83±0.23	0.997	2.11±1.40	0.807
$RuLL^2$ [Ru(ata)]	1.58±0.50	0.990	5.68±1.17	0.734
$RuLL^3$ [Ru(bta)]	1.62±0.21	0.990	3.35±2.14	0.933
$CuLL^1$ [Cu(opd)]	2.14±1.39	0.979	1.92±1.11	0.920
$NiLL^1$ [Ni(opd)]	2.69±1.99	0.990	2.80±1.31	0.888
$CoLL^1$ [Co(opd)]	2.47±1.53	0.991	2.47±0.86	0.889

ZnLL[1] [Zn(opd)]	3.34±1.80	0.962	3.59±1.90	0.874
CuLL[2] [Cu(ata)]	2.31±1.54	0.992	2.15±1.85	0.992
NiLL[2] [Ni(ata)]	2.34±0.85	0.977	3.66±1.16	0.975
CoLL[2] [Co(ata)]	3.18±0.96	0.968	1.83±1.08	0.986
ZnLL[2] [Zn(ata)]				
CuLL[3] [Cu(bta)]	2.08±0.47	0.996	2.11±1.60	0.925
NiLL[3] [Ni(bta)]	2.52±1.15	0.995	3.14±1.78	0.840
CoLL[3] [Co(bta)]	3.04±0.59	0.985	3.37±0.78	0.975
ZnLL[3] [Zn(bta)]	3.64±1.65	0.992	2.77±0.74	0.964
Tridentate Schiff bases and their metal complexes				
[HLL[4], ehmta]	2.63±0.79	0.974		
RuLL[4] [Ru(mta)]	2.86±0.57	0.971	2.98±1.44	0.878
[HLL[5], ehben]	3.05±0.54	0.962		
RuLL[5] [Ru(ben)]	1.52±0.36	0.936	3.28±1.26	0.967
[HLL[6], ehmez]	2.99±0.93	0.988		
RuLL[6] [Ru(mez)]	1.55±0.54	0.973	3.29±0.94	0.917
[HLL[7], ehacp]	3.00±0.52	0.992		
RuLL[7] [Ru(acp)]	1.50±0.40	0.960	3.54±1.31	0.812
[HLL[8], ehacn]	3.07±0.63	0.957		
RuLL[8] [Ru(acn)]	1.60±0.68	0.965	3.30±0.89	0.959
[HLL[9], ehvan]	3.85±0.83	0.989		
RuLL[9] [Ru(van)]	1.54±0.44	0.974	4.27±1.17	0.808
[HLL[10], ehvet]	3.17±0.63	0.987		
RuLL[10] [Ru(vet)]	1.63±1.05	0.991	3.30±1.48	0.877
[HLL[11], ehbzc]	3.13±0.55	0.985		
RuLL[11] [Ru(bzc)]	1.51±0.50	0.963	3.24±0.93	0.855
Vit. C*	1.92±1.07	0.978	-	-
Rutin*	2.52±1.60	0.798	2.83±1.84	0.983
BHT*	-	-	1.64±1.54	0.919

* Standards

6.2.1 DPPH Radical Scavenging Assay

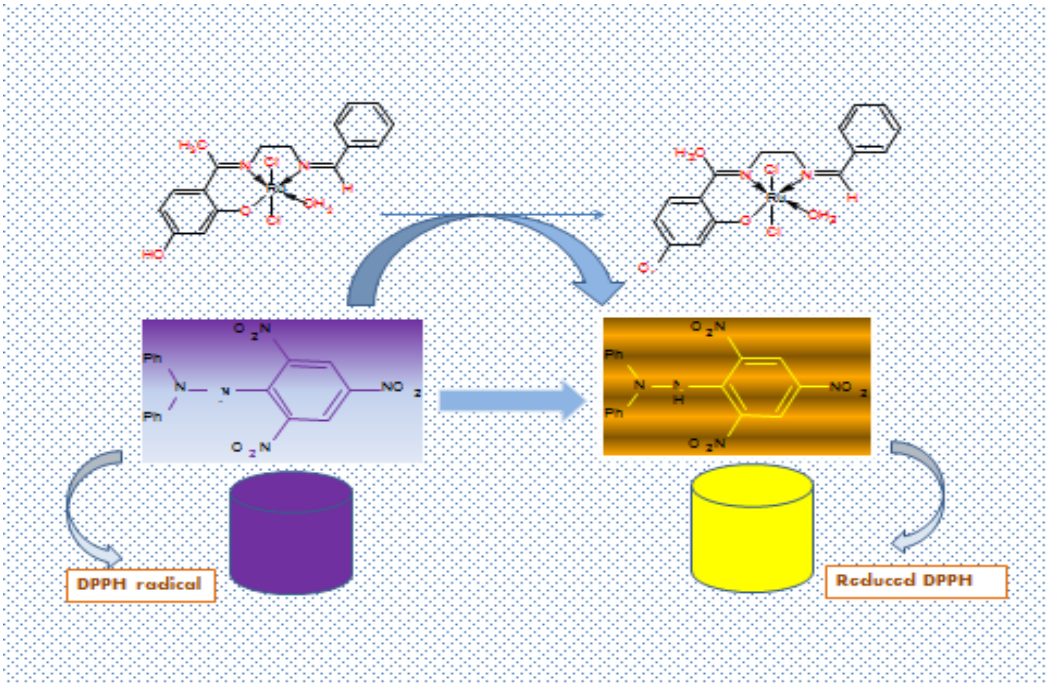
2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a compound that is used widely to examine the ability of a given sample to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods [67]. The result of the DPPH radical scavenging ability of the isolated Schiff bases and their complexes are presented Table 6.2.

Principle

The scavenging reaction between (DPPH) and an antioxidant (H-D) can be written as [233]:

(DPPH) + (H-D) ⟶ DPPH-H + (D)

(Purple) (Yellow)



Scheme 6.1: Conversion of DPPH* (purple) to its corresponding hydrazine form (yellow) due to proton transfer by the addition of metal-Schiff base complexes

6.2.1.1 Symmetrical and unsymmetrical tetradentate Schiff bases DPPH radical scavenging assay

[HLL^1, ehopd], [HLL^2, ehata] and [HLL^3, ehbta] ligand scavenging activities were studied and compared with the standard (ascorbic acid and rutin) as presented in Figure 6.1. The DPPH radical scavenging ability in all the concentrations was found to be low, as compared to the standard agents. Compound [HLL^3, ehbta] gave the highest percent inhibition at all concentrations amongst the isolated samples, while at the lowest

concentration (100 µg/ mL), [HLL^3, ehbta] still exhibited better inhibition than the standard drug: Rutin hydrate.

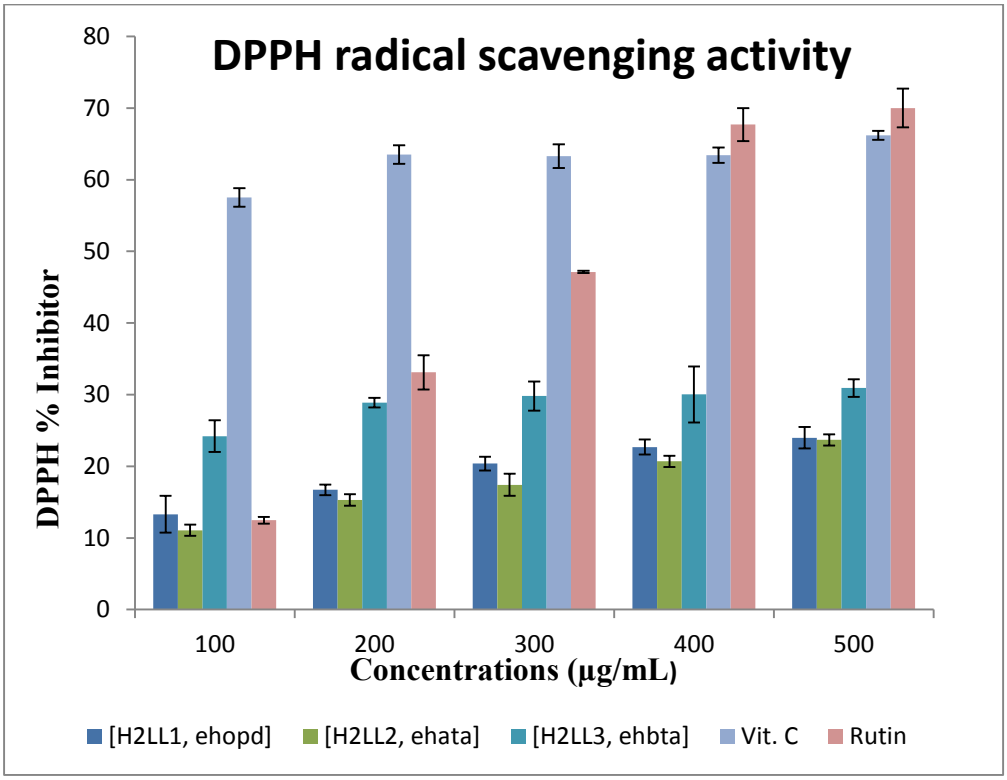


Figure 6.1: DPPH scavenging activities of symmetrical and unsymmetrical tetradentate Schiff bases

IC_{50} value of the tetradentate ligands on DPPH radical ranged from 4.24±1.23 – 6.49±2.96 µM and, the standards: Vit. C and rutin showed their IC_{50} values at 1.92±1.07 µM and 2.52±1.60 µM respectively as listed in Table 6.2 and follows the order: [HLL^3, ehbta] > [HLL^1, ehopd] > [HLL^2, ehata].

6.2.1.2 DPPH scavenging activity of symmetrical and unsymmetrical tetradentate Ru(III) Schiff base complexes

The Ru(III) complexes of tetradentate Schiff bases exhibited significantly high DPPH radical scavenging ability in all the concentrations, that is, chelated Ru(III)-tetradentate Schiff base complexes were more effective radical scavengers than the corresponding ligands: *N,N′*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine [HLL^1, ehopd], *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedioneethylidene)ethylenediamine [HLL^2, ehata], and *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedionephenylidene)ethylenediamine [HLL^3, ehbta] Schiff bases, this could be attributed to the acquisition of additional superoxide dismutating centres [254].

However, the isolated complexes showed enhanced activity in comparison to the standards (ascorbic acid and rutin), with [Ru(ata)] showing significantly higher scavenging ability. IC_{50} value of [Ru(opd)], [Ru(ata)] and [Ru(bta)] complexes are 1.83±0.23, 1.58±0.50 and 1.62±0.21 μM respectively as shown in Table 6.2 and Figure 6.2. The DPPH radical scavenging ability of the complexes can be ranked in the order: [Ru(ata)] > [Ru(bta)] > [Ru(opd)]. Therefore, the scavenging effects of the free ligands are lower than their corresponding Ru(III) complexes which is related to the chelation of the organic molecules with the metal ions.

Also, the change of the violet colour of DPPH radical into yellow colour upon addition of Ru(III) compounds indicates proton from the test samples were transferred to DPPH, converting it into the corresponding hydrazine form [151] and thus, these compounds could be a promising therapeutic agent to treat stress induced pathological conditions such aging, cancer, and cardiovascular and neurodegenerative diseases.

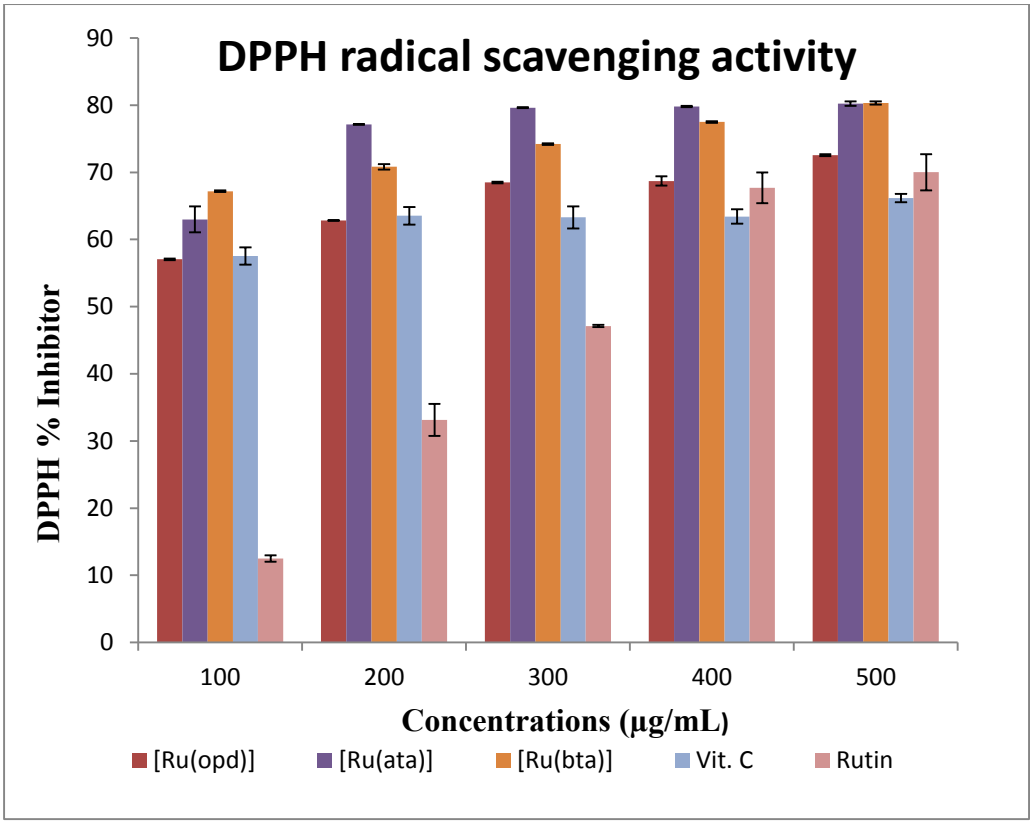


Figure 6.2: DPPH scavenging activities of symmetrical and unsymmetrical tetradentate Ru(III) Schiff base complexes

6.2.1.3 Free Scavenging assay of *N,N'*-bis(2',4'-dihydroxyphenylethylidene) ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The DPPH scavenging effect is based on the absorbance decrease of alcoholic DPPH solution in the existence of proton releasing spicy [255]. Scavenging activities of [HLL^1, ehopd], metal complexes; ascorbic acid (Vit. C), rutin as standards are shown in Figure 6.3. The ligand *N,N'*-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine [HLL^1, ehopd] displayed trivial DPPH activity, nevertheless upon coordination with Zn^{2+}, Cu^{2+}, Co^{2+}, and Ni^{2+} ions, scavenging properties were significantly improved.

The [Cu(opd] complex possessing strong electron donating power displayed strong DPPH activity than complexes of cobalt, nickel and zinc. IC_{50} and its corresponding R^2 (correlation coefficient) values for the tested compounds are listed in Table 6.2. IC_{50} value of the free ligand is 5.99±1.37 µM whereas, [Cu(opd)], [Co(opd)], [Ni(opd)] and [Zn(opd)] revealed IC_{50} values of 2.14±1.39, 2.47±1.53, 2.69±1.99 and 3.34±1.80 µM respectively.

Furthermore, the IC_{50} value of [Cu(opd)] was found to be higher than rutin (standard) but lower than that of Vit. C with IC_{50} value of: 1.92±1.07 µM. However, antioxidant activities of the complexes are moderate to strong free radical inhibitors or scavenger for treating pathological impairment connected with radical-generation.

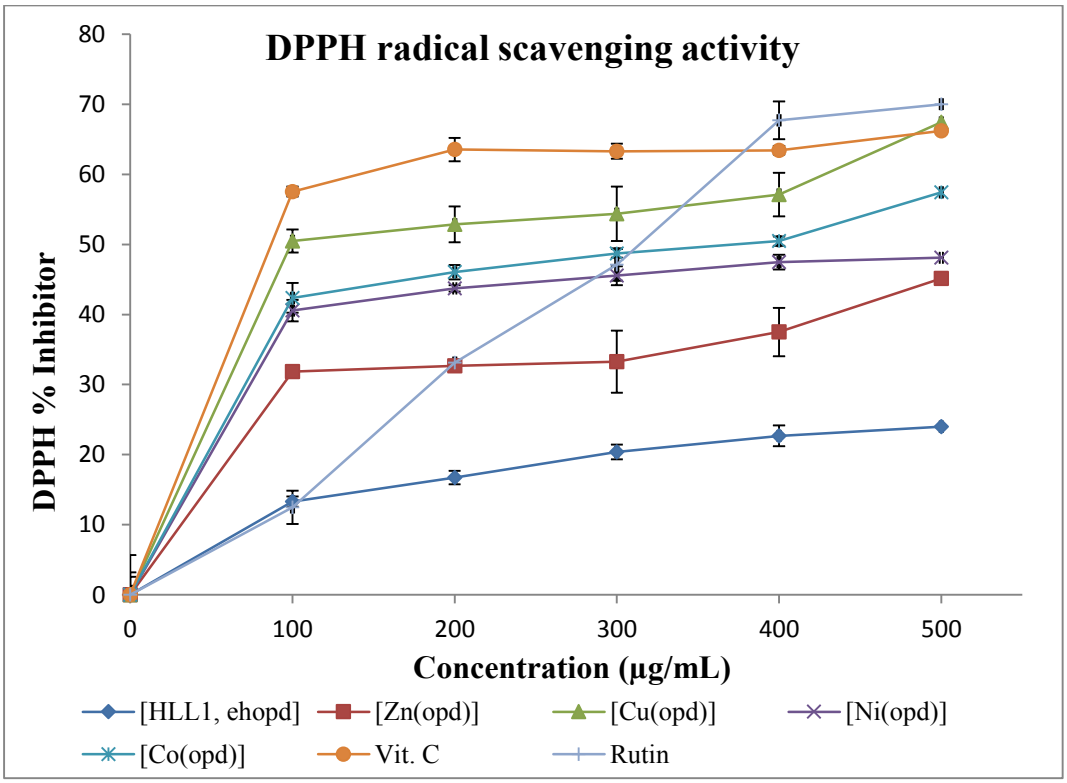


Figure 6.3: DPPH scavenging activities of symmetrical tetradentate complexes of Co(II), Ni(II), Cu(II) and Zn(II) with [HLL^1, ehopd] ligand

6.2.1.4 DPPH scavenging activity of *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedioneethylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a steady organic radical compound and its oxidative assay is used extensively in the quantification of radical scavenging capacity or hydrogen donors ability of samples. The antioxidant potentials of Schiff base *N*-(2',4'-dihydroxyphenylethylidene)-*N′*-(1,3-butanedioneethylidene)ethylenediamine [HLL^2, ehata], [Zn(ata)], [Ni(ata)], [Cu(ata)] and [Co(ata)] metal complexes together with the standards were measured on the basis of the scavenging impact of the stable DPPH free radical activity [256].

The examined changes in the scavenging ability of the test samples on the basis of percent inhibition are presented in Figure 6.4. The DPPH scavenging capability of Schiff base metal complexes were significantly higher than the free ligand [HLL^2, ehata], indicating that the complexes are better free radical scavenger and exhibit better antioxidant property than [HLL^2, ehata] but lower activity when compared to the standard drugs: ascorbic acid (Vit. C) and rutin.

Radical scavenging activity of metal complexes as well as the standards were amplified in a dose-dependent mode, and the antioxidant property of Schiff base [HLL^2, ehata], increased significantly after coordination to the metal ions (Figure 6.4). IC_{50} value of the tested compounds is presented in Table 6.2 alongside with the correlation coefficient (R^2) values. The [Cu(ata)] and [Ni(ata)] complexes possess higher antioxidant potential (IC_{50}) than rutin but lower than Vit. C.

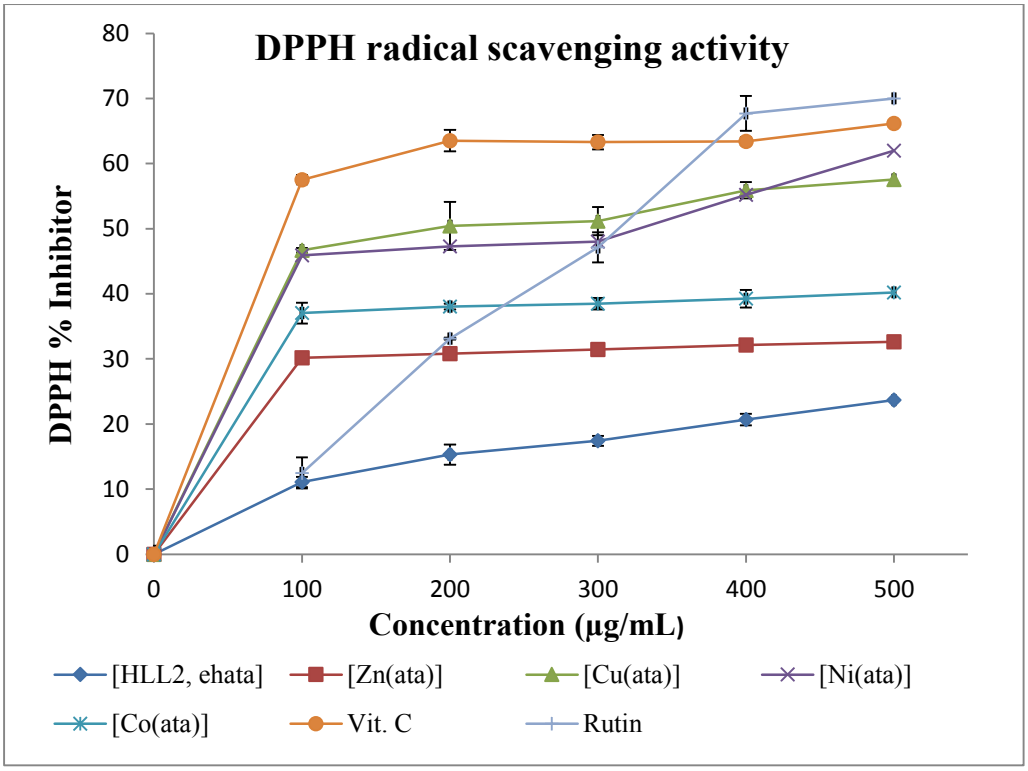


Figure 6.4: DPPH scavenging abilities of unsymmetrical tetradentate complexes of Co(II), Ni(II), Cu(II) and Zn(II) with [HLL^2, ehata] ligand

The order of scavenging capability of the compounds can be given as Vit. C > [Cu(ata)] > [Ni(ata)] > [Co(ata)] > Rutin > [Zn(ata)] > [HLL^2, ehata] with IC_{50} values as 1.92±1.07 > 2.31±1.54 > 2.34±0.85 > 3.18±0.96 > 2.52±1.60 > 3.93±1.64 > 6.47±2.96 µM. The oxidizing potentials of the samples are linked with the capability of the compounds to exert actions by infringing the free radical chain via hydrogen atom donation [257]. Therefore, the results obtained from this study provide linkage to the use of the synthesised compounds in the treatment of pathological diseases arising from oxidative stress.

6.2.1.5 DPPH scavenging activity of *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedionephenylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

Scavenging activity of a chemical/ or compound on DPPH radical as a fast and reliable parameter to measure the *in vitro* antioxidant activity of such sample have been used by diverse researchers [140]. This assay is based on the measurement of the decrease in molar absorptivity of DPPH at 517 nm after reaction with the test compound. The effect of the antioxidant on DPPH radical scavenging was due to the hydrogen donating ability or radical scavenging activity of the samples [258]. Antioxidants react with DPPH, a stable free radical and thus reduced, as a result the absorbance's decreased from the DPPH radical to the DPPH-H form.

The degree of decolouration indicates the scavenging potential of the antioxidant compounds or samples in terms of hydrogen donating ability [258]. Figure 6.5 shows the dose-response curve of DPPH radical scavenging activity of the Schiff base [HLL^3, ehbta] and [Zn(bta)], [Ni(bta)], [Cu(bta)] and [Co(bta)] complexes, compared with rutin and ascorbic acid. It was observed that metal(II) complexes had higher activity than that of the free ligand [HLL^3, ehbta]. At the lowest concentration (100 µg/mL) the antioxidant activity of the free ligand was found to be 24.20% but, upon complexation, it increased significantly within the range 29.80 - 45.01% from [Zn(bta)] to [Cu(bta)] (Figure 6.5). The increased antioxidant activity of these complexes can be attributed to the electron withdrawing effect of the metal ions which facilitates the release of hydrogen to reduce the DPPH radical [139].

These proton release were very pronounced in [Cu(bta)] with an IC_{50} value of 2.08±0.47 µM followed by [Ni(bta)] [2.52±1.15 µM], [Co(bta)] [3.04±0.59 µM] and [Zn(bta)] [3.64±1.65 µM]. The DPPH radical scavenging ability of the test samples can be

ranked in the order (Table 6.2): Vit. C > [Cu(bta)] > [Ni(bta)] = Rutin > [Co(bta)] > [Zn(bta)] > [HLL^3, ehbta]. The enhanced inhibition displayed on this type of radical by the test samples shows that the compounds are capable of donating electrons to neutralise the effect of the free radical and thus, could be a promising therapeutic agent for the treatment of pathological diseases and conditions as a results of radicals or stress.

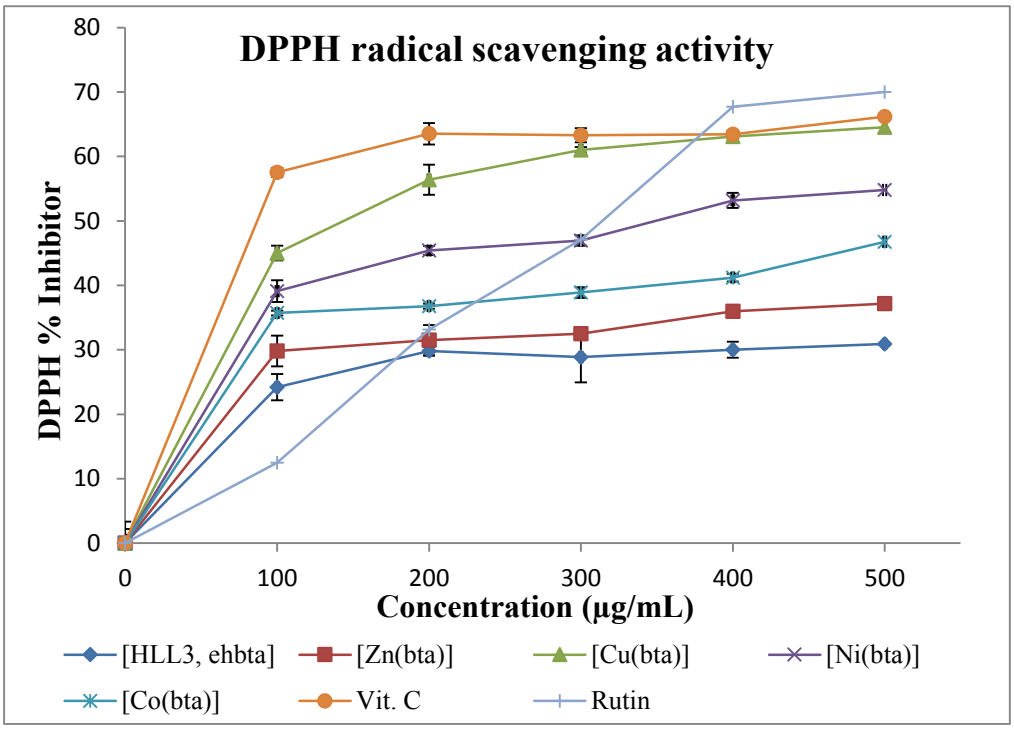


Figure 6.5: DPPH scavenging abilities of unsymmetrical tetradentate complexes of Co(II), Ni(II), Cu(II) and Zn(II) with [HLL^3, ehbta] ligand

6.2.1.6 (DPPH) Free radical scavenging assay of unsymmetrical tridentate Schiff bases

The antioxidant activities of the newly synthesised compounds were evaluated on the basis of their DPPH radical scavenging activities [256]. The changes or trend of inhibition in the DPPH radical scavenging ability as percentage inhibition of the compounds [HLL^4, ehmta] - [HLL^{11}, ehbzc] is presented in Fig 6.6 and are concentration dependent. The results

of percentage inhibition of the samples as expressed in IC_{50} value (the extent of antioxidant required to reduce the original DPPH concentration by 50%).

It is observable that at higher concentration (500 µg/mL), [HLL^4, ehmta] exhibited the highest inhibition of DPPH radical while [HLL^9, ehvan] has the lowest free radical scavenging potential among the synthesised compounds. It is worthy of note that the compounds exhibited higher inhibition of DPPH at the lowest concentration (100 µg/mL) than the rutin (standard) while at 200 µg/mL all other compounds showed higher percentage inhibition than the rutin (standard) except for [HLL^9, ehvan].

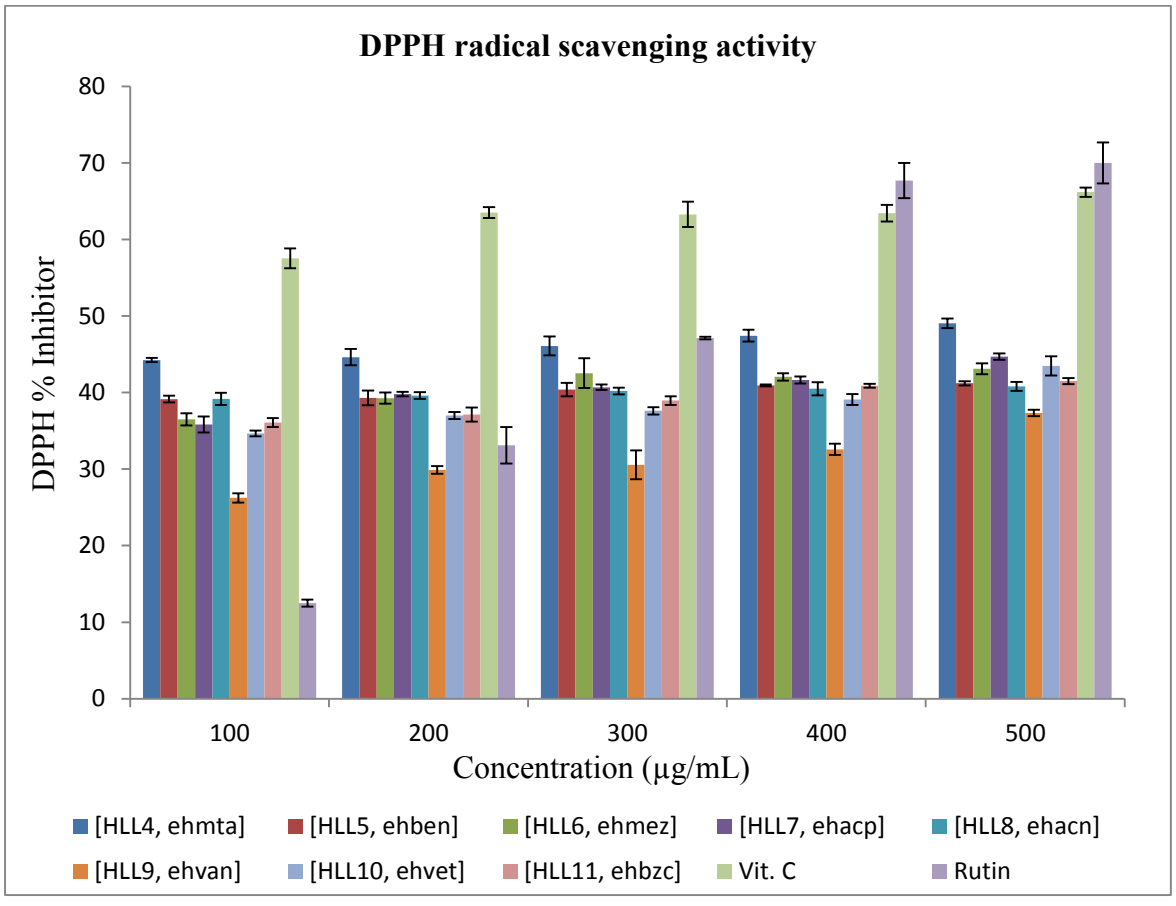


Figure 6.6: DPPH scavenging activities of unsymmetrical tridentate Schiff bases

IC_{50} and the corresponding R^2 (correlation coefficient) standards of the tested compounds are enumerated in Table 6.2, and ranged from 2.63±0.79 - 3.85±0.83 μM. The order of antioxidant activity in the series of this derivatives can be given as HLL^4 > HLL^6 > HLL^7 > HLL^5 > HLL^8 > HLL^{11} > HLL^{10} > HLL^9. The identified differential scavenging actions of the synthesised compounds against the DPPH radical could be because of the existence of different substituent on the ring structure [259], but the reaction of DPPH compounds containing hydroxyl groups such as phenols are reversible, resulting in low readings for antioxidant activity [260].

6.2.1.7 (DPPH) Free radical scavenging activity assay of unsymmetrical tridentate Ru(III) complexes with [HLL^4, ehmta] - [HLL^{11}, ehbzc] ligand

The DPPH scavenging effect is established on the absorbance decrease of methanolic DPPH solution in the presence of proton releasing spicy [255]. Activities of tridentate Ru(III) complexes solution are displayed in Figure 6.7. The tridentate Schiff bases *viz*. [HLL^4, ehmta] - [HLL^{11}, ehbzc] displayed trivial DPPH activity, however upon coordination with Ru^{3+} ion, scavenging properties were significantly improved, thereby making the Ru(III)-tridentate Schiff base complexes [$RuLL^4$, Ru(mta)] - [$RuLL^{11}$, Ru(bzc)] more effective DPPH radical scavenger than the corresponding free Schiff bases.

The displayed DPPH activities by the tested samples possess strong electron donating power as compared to those of the standards (ascorbic acid and rutin). IC_{50} and its corresponding R^2 (correlation coefficient) values for the tested compounds are listed in Table 6.2. Ru(III)-tridentate Schiff base complexes, alongside with Vit. C and rutin DPPH

scavenging capability can be ranked in the order: Ru(acp) > Ru(bzc) > Ru(ben) > Ru(van) > Ru(mez) > Ru(acn) > Ru(vet) > Vit. C > rutin > Ru(mat).

Compounds [RuLL^{5}, Ru(ben)]- [RuLL^{11}, Ru(bzc)] with an IC_{50} values ranging from 1.50±0.40 - 1.63±1.05 µM respectively exhibited higher activity against DPPH than the commercially available Vit. C and rutin drugs, however, [RuLL^{4}, Ru(mta)] showed the lowest activity of all investigated samples with an IC_{50} value of 2.86±0.57 µM. In addition, the isolated Ru(III)-tridentate Schiff base complexes were found effective as DPPH scavengers at different concentrations, thereby making them potential compounds for developing anti-stress inducing agents [126, 252].

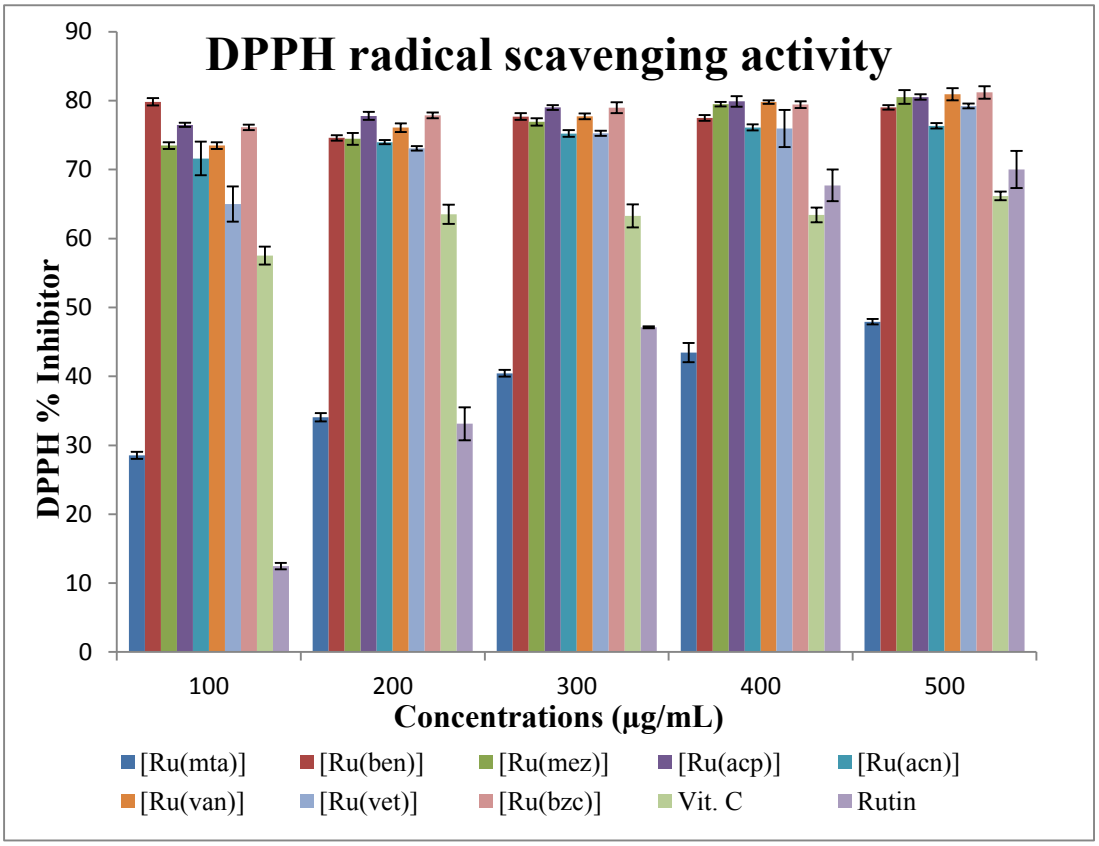


Figure 6.7: DPPH scavenging activities of unsymmetrical tridentate Ru(III) Schiff base complexes

6.2.2 ABTS: 2,2'-azino-bis-(-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

ABTS [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation ($ABTS^{+}$) are produced by the oxidation of ABTS with potassium persulfate and thus, are reduced in the presence of hydrogen-donating antioxidants [254]. This has been the basis of one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of solutions of pure substances and aqueous extracts [261, 262]. The method described gives a measure of the antioxidant activity of the range of test samples determined by the decolourization of the $ABTS^{+}$, through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734 nm [263]. Antioxidants inhibit the oxidation of ABTS by electron transfer radical scavenging

6.2.2.1 Symmetrical and unsymmetrical tetradentate Schiff bases ABTS radical scavenging assay

[HLL^{1}, ehopd], [HLL^{2}, ehata] and [HLL^{3}, ehbta] ligand scavenging activities of ABTS radical are presented in Figure 6.8. The ABTS radical scavenging ability in all the concentrations was found to be high in comparison to the standard agents. Compound [HLL^{2}, ehata] gave the highest percent inhibition amongst the isolated samples at the lowest concentration (100 µg/ mL), and all the compounds had better inhibition (IC_{50}) than the standard drug: rutin hydrate.

IC_{50} value of the tetradentate ligands on ABTS radical ranged from 1.86±2.28 – 1.98±1.55 µM and, the standards: Rutin and BHT showed their IC_{50} values at 2.83±1.84 µM

and 1.64±1.54 µM respectively as listed in Table 6.2 and follows the order: [BHT] > [HLL^2, ehata] > [HLL^1, ehopd] > [HLL^3, ehbta] > [Rutin].

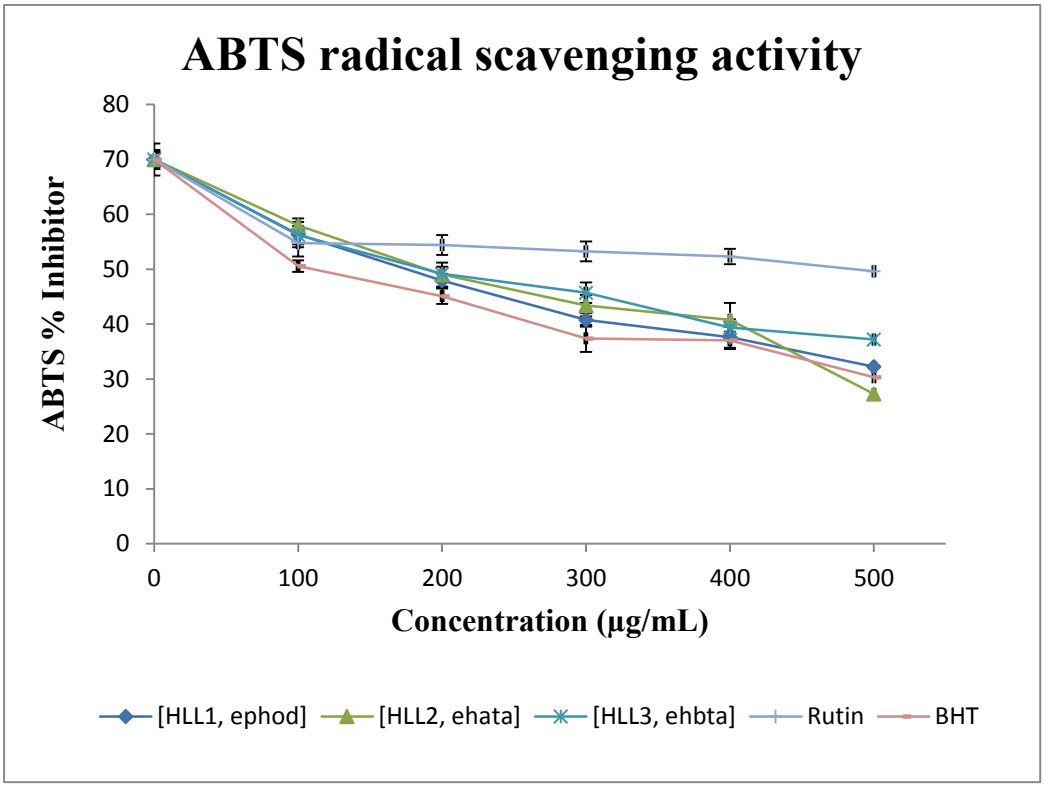


Figure 6.8: ABTS scavenging activities of symmetrical and unsymmetrical tetradentate Schiff bases

6.2.2.2 ABTS scavenging activity of symmetrical and unsymmetrical tetradentate Ru(III) Schiff base complexes

The tetradentate Ru(III) complexes: [Ru(opd)], [Ru(ata)] and [Ru(bta)] exhibited low to moderate scavenging ability on the ABTS radical (Figure 6.9) and showed comparable or slightly less activity to that of Rutin and BHT (standard drugs). At concentration of 100 µg/mL, the percentage inhibition was 60.2, 66.4, 59.8, 54.8 and 50.6% for [Ru(opd)], [Ru(ata)], [Ru(bta)], Rutin and BHT respectively.

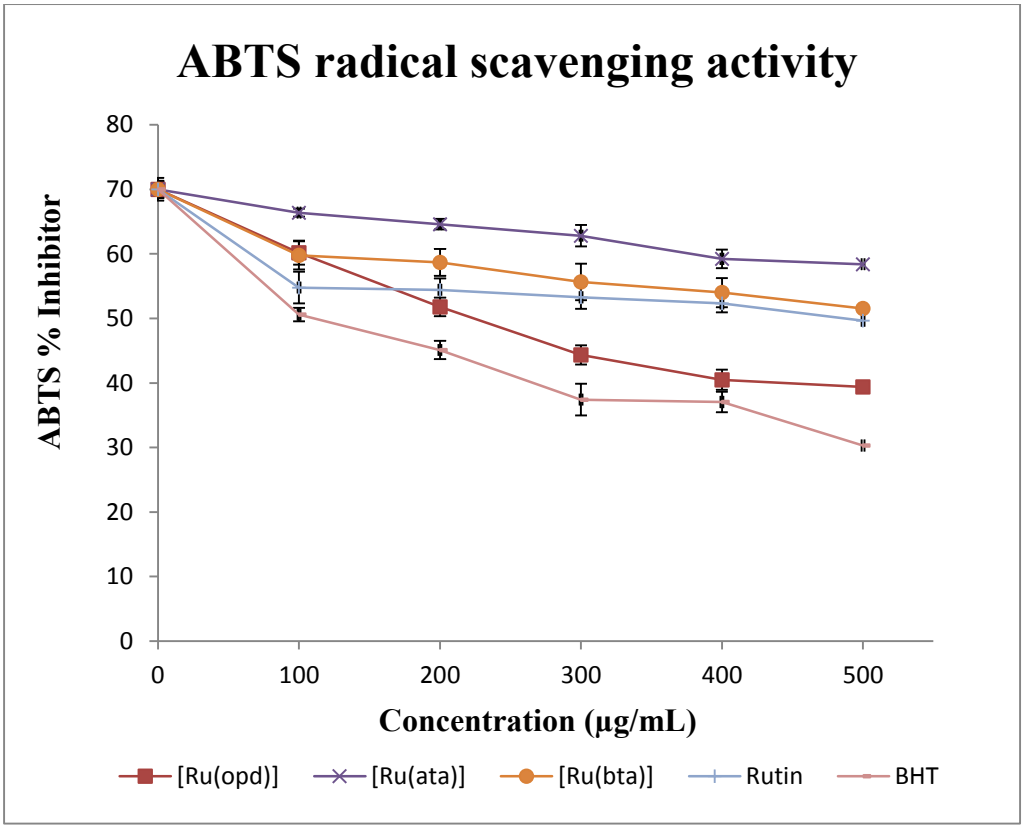


Figure 6.9: ABTS scavenging activities of symmetrical and unsymmetrical tetradentate Ru(III) Schiff base complexes

Nevertheless, the ABTS activities of the Ru(III) complexes were significantly enhanced than its corresponding free tetradentate Schiff base ligands. It was observed that the lowest concentration of the Ru(III) complexes were more effective in quenching ATBS radicals in the system. The IC_{50} values at 2.11±1.40, 5.68±1.17 and 3.35±2.14 are for [Ru(opd)], [Ru(ata)] and [Ru(bta)] respectively (Table 6.2). Furthermore, the synthesized compounds scavenged the ABTS radical in a concentration-dependent pattern with the order: [Ru(opd)] > [Ru(ata)] > [Ru(bta)].

6.2.2.3 ABTS Scavenging assay of *N,N′*-bis(2',4'-dihydroxyphenylethylidene) ethylenediamine complex of Co(II), Ni(II), Cu(II) and Zn(II)

To further confirm the synthesized [HLL^1, ehopd] and Opd–M(II) complexes anti-radical potential, the ABTS assay was examined. This assay measures radical scavenging by electron donation. The outcomes of the compounds on ABTS are presented in Table 6.2. At 734 nm, the absorbance of active ABTS* solution [57] obviously declined upon the addition of different concentrations of *N,N′*-bis(2',4'-dihydroxyphenylethylidene) ethylenediamine [HLL^1, ehopd] and Opd–M(II) complex, the same trend was also observed for the standard drugs: Butylated hydroxytoluene (BHT) and rutin hydrate with the percentage inhibition displayed in Figure 6.10.

The effectiveness of the tested samples in quenching $ATBS^+$ in the system was observed to be high at the lowest concentration of 100 µg/ mL with both the ligand and the metal complexes exhibiting higher ABTS % inhibition than the standards. However, [Cu(opd)] complex showed significantly higher ABTS scavenging activity with an IC_{50} value of 1.92±1.11 µM as listed in Table 6.2 while complexes of [Co(opd)], [Ni(opd)] and [Zn(opd)] gave an IC_{50} value of 2.47±0.86, 2.80±1.31, 3.59±1.90 µM respectively.

The scavenging activity pattern of the complexes on ABTS radicals was observed to be in the order: [Cu(opd)] > [Co(opd)] > [Ni(opd)] > [Zn(opd)]. Therefore, the study showed that the synthesised compounds may be useful in developing therapeutic agent for averting cell oxidative damage, as various free radicals are generated in the system leads to cancer, aging and cardiovascular diseases [264].

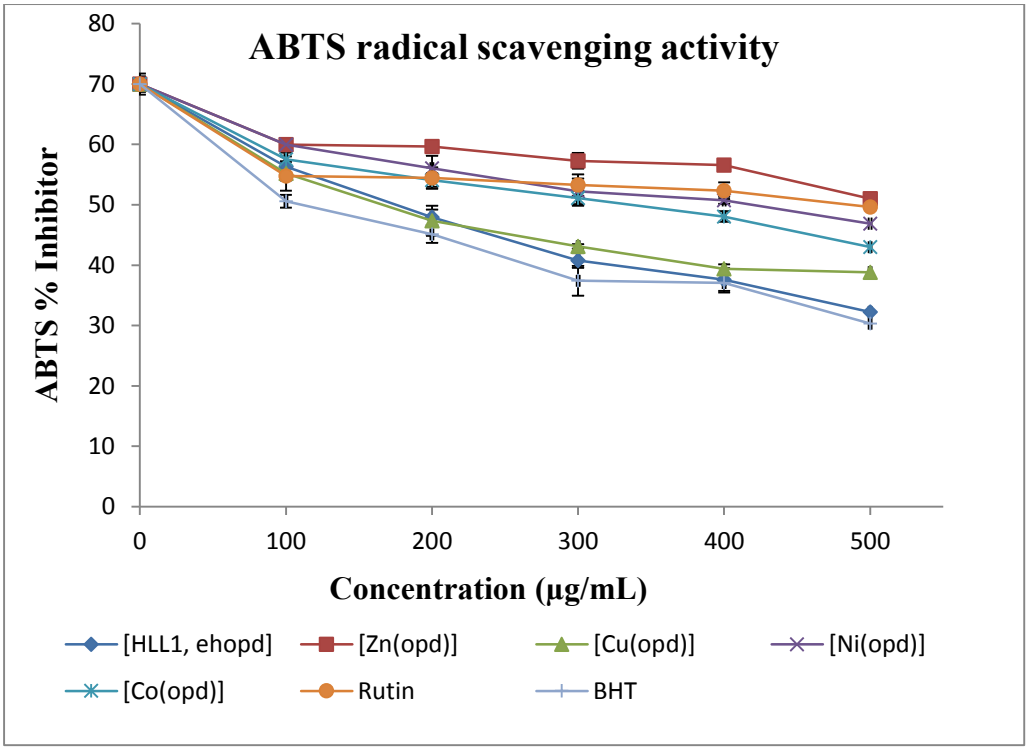


Figure 6.10: ABTS scavenging activities of symmetrical tetradentate complexes of Co(II), Ni(II), Cu(II) and Zn(II) with [HLL[1], ehopd] ligand

6.2.2.4 ABTS scavenging activity of *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedioneethylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

An imperative characteristic of antioxidants is the proton radical scavenging. A well-known protonated radical like 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), possess typical absorbance maxima at 734 nm which decreases with the rummaging of the proton radicals [36]. The ligand, Zn(II), Ni(II), Cu(II) and Co(II) metal complexes were moderate and effective scroungers of the ABTS radical as displayed in Figure 6.11 and this action was comparable to those of rutin and BHT (standard drugs).

Lowest concentrations of the test samples were more efficient in quenching $ABTS^{+}$ radicals in the system. The scavenging of the $ABTS^{+}$ radical by the *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedione ethylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II) [HLL^2, ehata] and its metal complexes was found to possess moderate to high activities as compared to that of rutin and BHT. [Co(ata)] exhibited the highest activity with an IC_{50} of about 1.83±1.08 µM amongst the isolated metal complexes (Table 6.2). The ABTS radical scavenging ability of the test samples can be ranked in the order [BHT] > [Co(ata)] > [HLL^2, ehata] > [Cu(ata)] > [Rutin] > [Zn(ata)] > [Ni(ata)]. The scavenging of the ABTS radical via the compounds indicates their potentials as chemotherapeutic agents for radical chains terminator.

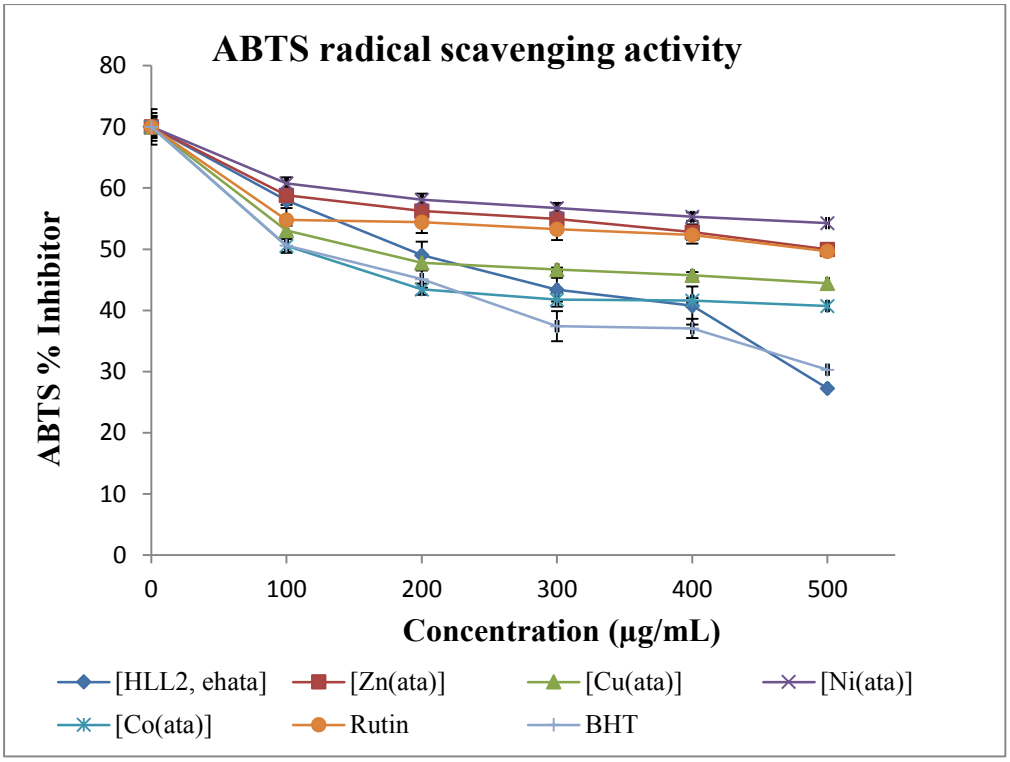


Figure 6.11: ABTS scavenging abilities of unsymmetrical tetradentate complexes of Co(II), Ni(II), Cu(II) and Zn(II) with [HLL^2, ehata] ligand

6.2.2.5 ABTS scavenging activity of *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedionephenylidene)ethylenediamine complexes of Co(II), Ni(II), Cu(II) and Zn(II)

The [HLL^3, ehbta] together with its complexes were screened for free radical scavenging activity by ABTS method [213]. The percentage inhibition results of free radical scavenging activity of the test samples are shown in Figure 6.12. The coordination of metal ions with *N*-(2',4'-dihydroxyphenylethylidene)-*N'*-(1,3-butanedionephenylidene) ethylene-diamine [HLL^3, ehbta] enhanced the activities in comparison to those of the standards drugs used (rutin and BHT). The test samples exhibited moderate to high percentage inhibition scavenging activity than rutin and BHT at the lowest concentration (100 µg/ mL).

IC_{50} values of the compounds are presented in Table 6.2 with [Cu(bta)] possessing the highest potency (IC_{50} = 2.11+1.69 µM) followed by the zinc, nickel and cobalt complexes. The ABTS radical scavenging ability of the test samples can be ranked in the order: BHT > [HLL^3, ehbta] > [Cu(bta)] > [Zn(bta)] > [Rutin] > [Ni(bta)] > [Co(bta)]. Furthermore, the synthesized compounds scavenged the ABTS radical in a concentration-dependent manner.

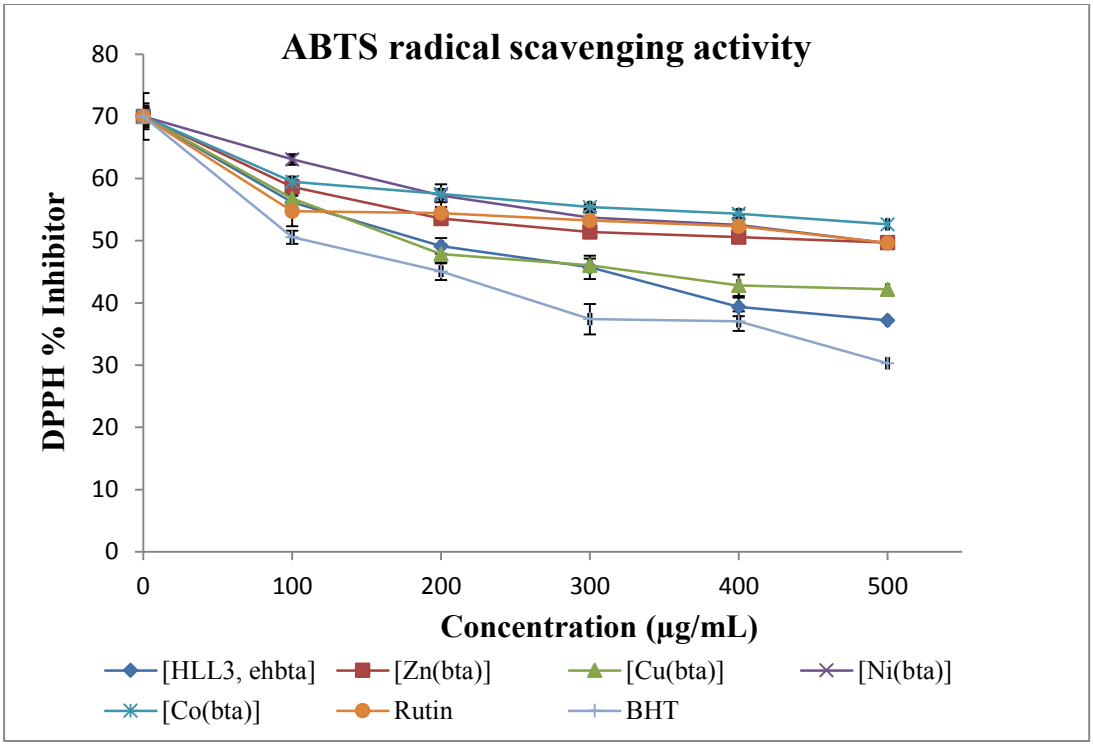


Figure 6.12: ABTS scavenging activities of unsymmetrical tetradentate complexes of Co(II), Ni(II), Cu(II) and Zn(II) with [HLL^3, ehbta] ligand

6.2.2.6 ABTS radical scavenging assay of unsymmetrical tridentate Ru(III) complexes with [HLL^4, ehmta] - [HLL^{11}, ehbzc] ligand

The antioxidant potentials of the heterocyclic Ru(III) complexes was further confirmed by examining their ABTS capability. The outcome of the ONN-Ru(III) complexes on ABTS are presented in Table 6.2. At 734 nm, the absorbance of active ABTS* solution [264] obviously declined upon the addition of different concentrations of heterocyclic Ru(III) complexes. The same trend was also observed for the standard drugs with the percentage ATBS inhibition displayed in Figure 6.13.

The effectiveness of the tested samples with highest inhibition in quenching $ATBS^{+}$ in the system was identified at the lowest concentration 100 µg/mL with the metal complexes exhibiting higher % inhibition than the standards. The [Ru(van)] complex showed the best inhibition at all the examined concentrations. However, compound [Ru(mta)] showed significantly higher ABTS scavenging capability with an IC_{50} value of 2.98±1.44 µM while complexes of other Ru(III) compounds gave an IC_{50} value ranging from 3.24±0.93 - 4.27±1.17 µM respectively.

The scavenging activity pattern of the complexes on ABTS radicals is in the order of: [Ru(van)] < [Ru(acp)] < [Ru(acn)] = [Ru(vet)] < [Ru(mez)] < [Ru(ben)] < [Ru(bzc)] < [Ru(mta)]. Conclusively, the heterocyclic tridentate Ru(III) complexes had a moderate potential to scavenge ABTS radicals, hence, making the compounds worthwhile therapeutic agent for developing compounds useful in averting cell oxidative damage, as various free radicals are generated in the system leading to cancer, aging and cardiovascular diseases [233, 264].

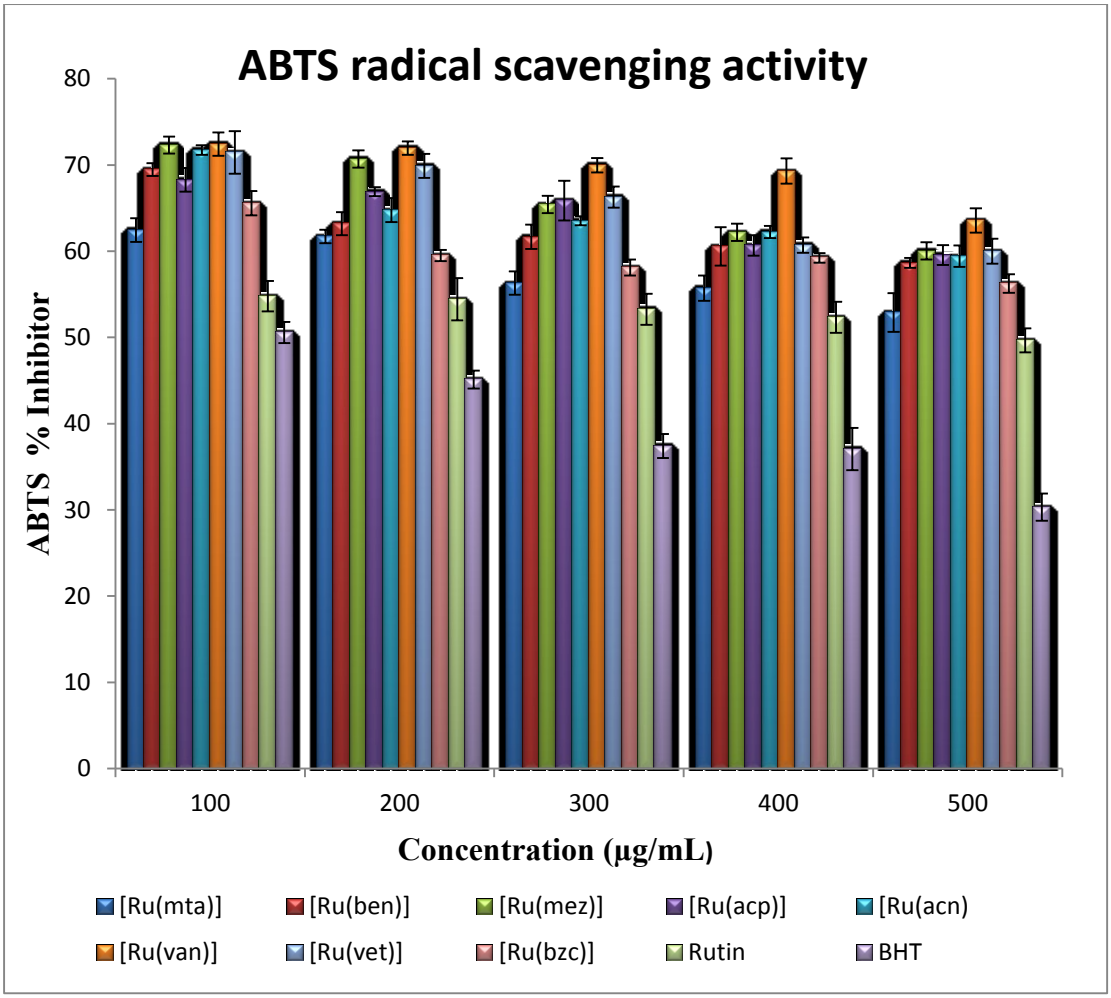


Figure 6.13: ABTS scavenging activities of unsymmetrical tridentate Ru(III) Schiff base complexes

6.3 *In vitro* antiproliferative evaluation

The *in vitro* antiproliferative activities of the isolated compounds and parthenolide (at various concentrations ranging from 0.01 to 100 µM) were screened against three cancer cell lines: Human renal cancer cell (TK-10), human melanoma cancer cell (UACC-62) and human breast cancer cell (MCF-7) using the SRB assay. The SRB assay adopted for the *in vitro* cell lines screening was carried out at the CSIR in accordance with the protocol of the Drug Evaluation Branch, NCI.

Table 6.3: Antiproliferative screening activities of ligands and complexes showing the IC_{50} values (μM) against human cell lines

Ligands/ Complexes	**Molecular formula**	**Anticancer activity IC_{50} (μM) 48 h**		
		TK-10	**UACC-62**	**MCF-7**
[HLL^1, ehopd]	$C_{18}H_{20}N_2O_4$	6.01±2.24	9.03±0.52	4.31±2.53
[HLL^9, ehvan]	$C_{18}H_{20}N_2O_4$	24.12±3.13	10.31±0.59	4.10±1.32
[HLL^{11}, ehbzc]	$C_{17}H_{17}ClN_2O_2$	4.48±2.49	8.48±0.94	4.01±2.26
$RuLL^1$ [Ru(opd)]	$C_{18}H_{24}N_2O_7RuCl$	10.66±1.99	7.03±1.22	4.69±1.11
$RuLL^2$ [Ru(ata)]	$C_{15}H_{23}N_2O_6RuCl$	13.41± 2.93	5.28±0.95	3.58±1.62
$RuLL^3$ [Ru(bta)]	$C_{20}H_{25}N_2O_6RuCl$	29.55±1.92	6.28±0.80	3.29±1.05
$RuLL^5$ [Ru(ben)]	$C_{17}H_{21}N_2O_4RuCl_2$	10.34± 1.35	6.63±1.92	3.63±1.92
$RuLL^6$ [Ru(mez)]	$C_{18}H_{23}N_2O_5RuCl_2$	14.47± 0.98	6.27±0.89	3.99±1.45
$RuLL^7$ [Ru(acp)]	$C_{18}H_{23}N_2O_4RuCl_2$	11.85±4.50	4.88±0.53	3.79±3.03
$RuLL^8$ [Ru(acn)]	$C_{18}H_{24}N_3O_4RuCl_2$	9.06±1.18	6.44±0.38	3.57±1.09
$RuLL^9$ [Ru(van)]	$C_{18}H_{23}N_2O_6RuCl_2$	41.09±4.44	6.31±1.47	4.88±1.28
$RuLL^{11}$ [Ru(bzc)]	$C_{17}H_{20}N_2O_4RuCl_3$	13.10±2.81	5.14±1.09	3.43±1.48
Parthenolide*	$C_{15}H_{20}O_3$	0.50±1.43	0.89±2.18	0.44±2.02

*Standard drug; Cells were treated with various concentrations of compounds required to inhibit 50% of the culture growth when exposed for 48 h (IC_{50} values was obtained). Each value represents the mean ± SD of three independent experiments.

6.3.1 Antiproliferative screening of symmetrical and unsymmetrical Schiff base ligands

[HLL^1, ehopd], [HLL^9, ehvan], [HLL^{11}, ehbzc] were selected among the isolated Schiff bases and tested for their anticancer activity *in vitro* against human cancer cell line: TK-10, UACC-62 and MCF-7 with respect to their aforementioned biological properties in this study. Figure 6.17 show the concentration-dependent inhibitory effect of the compounds as the percentage of cell survival at 48 h of exposure on culture medium. In the Figure 6.14, it was observed that the compounds showed major cytotoxic activities (~100 μM) in the survival range of 55 - 80 %.

The compound [HLL^{11}, ehbzc] showed a higher inhibition of cell proliferation at the concentration of 100 μM against human renal cancer cell (TK-10) and human melanoma cancer cell (UACC-62) with % cell viabilities of 58.52 and 75.92 than other Schiff base, while [HLL^{9}, ehvan] inhibited human breast cancer cell (MCF-7) efficiently with % cell viabilities of 53.76. Parthenolide showed strong level of antiproliferative effect against all cell lines, in line with previous reports [265, 266]. Table 6.3 shows the IC_{50} values obtained from non-linear regression analysis of dose response data for the compounds tested.

The results established that treatment of cell lines with compounds: [HLL^{1}, ehopd], [HLL^{9}, ehvan], [HLL^{11}, ehbzc] and parthenolide affected cell viability efficiently toward MCF-7 cells with IC_{50} values of 4.31±2.53, 4.10±1.32, 4.01±2.26 and 0.44±2.02 μM respectively (Figure 6.14 and Table 6.3). The nature of substituents: hydroxyl, methoxy, chloride and the bridging spacer ethylenediamine play critical roles in determining the cytotoxicity of [HLL^{1}, ehopd], [HLL^{9}, ehvan], [HLL^{11}, ehbzc] [267].

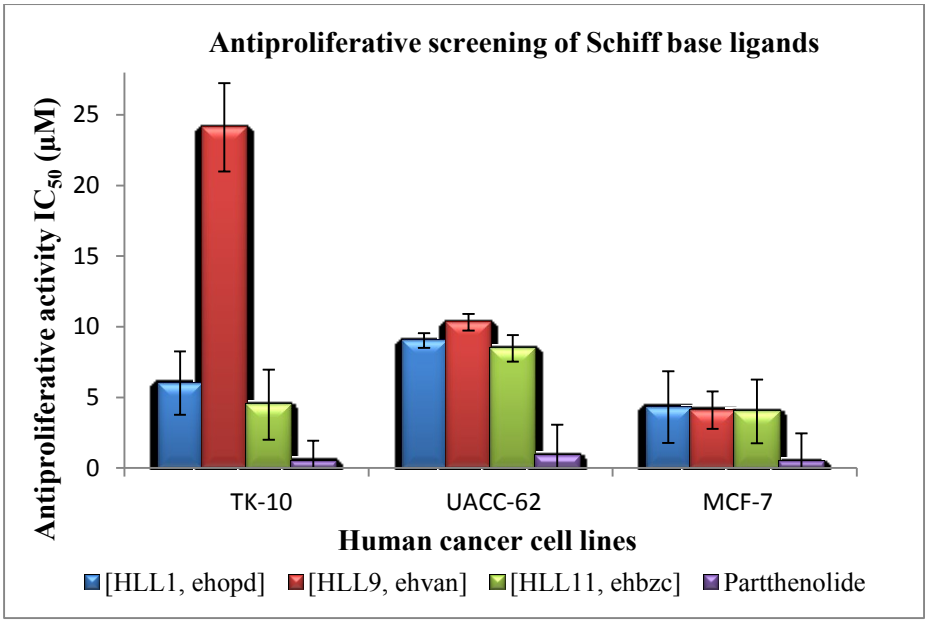


Figure 6.14: Antiproliferative activities of symmetrical and unsymmetrical Schiff bases against human cancer cell lines

6.3.2 Antiproliferative evaluation of symmetrical and unsymmetrical tetradentate Ru(III) compounds

The *in vitro* antiproliferative activities of the Ru(III) complexes and parthenolide (at various concentrations ranging from 0.01 to 100 µM) were evaluated against the three cancer cell lines using the SRB assay. Table 6.3 shows the IC_{50} values obtained from non-linear regression analysis of dose response data for the compounds tested. The [Ru(opd)], [Ru(ata)] and [Ru(bta)] complexes demonstrated low to moderate *in vitro* antiproliferative effect as compared to parthenolide (standard agent) against the selected tumor cell lines (Figure 6.15).

Parthenolide showed high levels of antiproliferative effect against all cell lines, in accordance with previous reports [265, 266]. The [Ru(opd)] displayed non-selective

antiproliferative activity against all tumor cells tested while [Ru(ata)] and [Ru(bta)] had a low antiproliferative effect with IC_{50} at (Z' factor > 0.5). The inhibition effects were enhanced by increasing concentration of the Ru(III) complexes: [Ru(opd)], [Ru(ata)] and [Ru(bta)] complexes showed higher inhibitions of cell proliferation at concentration of 100 μM against human breast cancer cell (MCF-7) than other cell lines with cell viabilities of 57.65%, 45.54% and 46.82% (Figure 6.17).

The IC_{50} results showed that [Ru(ata] exhibited better antiproliferative effect against all the selected tumor cell lines than [Ru(opd)] and [Ru(bta)] (Table 6.3), which is in agreement to their order of *in vitro* DPPH scavenging ability of the Ru(III) complexes. Towards human breast cancer cell (MCF-7), [Ru(bta)] showed better activity and follows the order: [Ru(bta)] > [Ru(ata)] > [Ru(opd)] and IC_{50} values 3.29±1.05, 3.58±1.62 and 4.69±1.11 respectively. Binding of the three N_2O_2 Schiff base Ru(III) complexes to biological targets other than DNA could be responsible for the observed antiproliferative activity of the complexes [196].

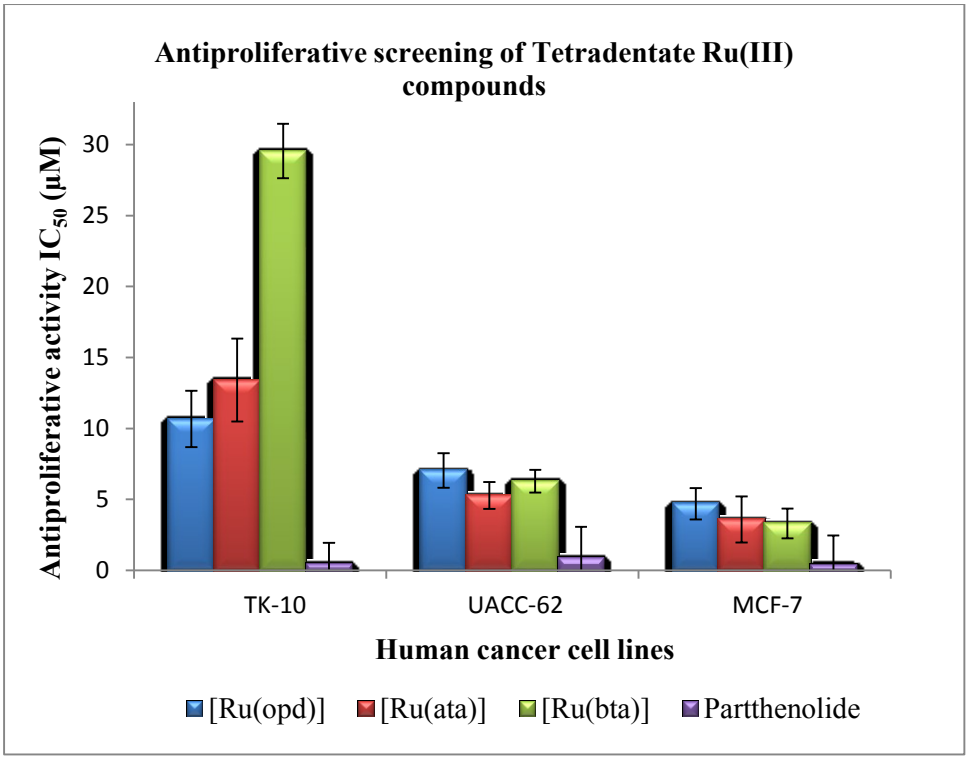


Figure 6.15: Antiproliferative activities of symmetrical and unsymmetrical tetradentate Ru(III) compounds against cancer cell lines

6.3.3 Antiproliferative activity of unsymmetrical tridentate ruthenium(III) complexes

The biochemical actions of Ru(III)-tridentate Schiff base complexes was analysed, in order to establish the structure-activity relationship of the isolated compounds with respect to various characteristic reactive atoms (functional groups). The tridentate Ru(III) compounds were subjected to cytotoxicity test using different sample concentrations towards human cancer cell lines namely: TK-10, UACC-62 and MCF-7 via the Sulforhodamine B (SRB) assay [196]. Parthenolide served as positive control.

The percentage cells viability were plotted as a function of tridentate ruthenium(III) complexes concentration as shown in Figure 6.17. The results obtained from this study

demonstrates that treatment of cells with different tridentate Ru(III) complexes concentrations, affected cell viability efficiently toward MCF-7 cell as [Ru(bzc)] takes the lead with cells viability of 45.87%, followed by [Ru(ben)] with 49.04% at the highest concentration (100 μM) and less active towards UACC-62 cell and inactive towards TK-10 cell. IC_{50} values are summarized in Table 6.3. Parthenolide exhibited strong intensities of antiproliferative activity against the studied cell lines, in accordance with previous reports [266, 268].

The tridentate Ru(III) compounds exhibited low-moderate to inactive *in vitro* antiproliferative activities against the three selected cell lines as compared to the standard drug Parthenolide. [Ru(ben)], [Ru(mez)], [Ru(acp)], [Ru(acn)], [Ru(van)] and [Ru(bzc)] induced strong efficient cell death with IC_{50} values of 3.63±1.92, 3.99±1.45, 3.79±3.03, 3.57±1.09, 4.88±1.28 and 3.43±1.48 μM respectively, towards MCF-7 cells than other investigated cell lines (Figure 6.16 and Table 6.3). In contrast, the Ru(III) compound showed weak activity against human renal cancer cell (TK-10), while compounds [Ru(acp)] and [Ru(bzc)] induced moderate cell death on human melanoma cancer cell (UACC-62) with IC_{50} values of 4.88±0.53 μM and 5.14±1.09 μM respectively.

In all, the orders of antiproliferation of the compounds are in the order: [Ru(bzc)] > [Ru(acn)] > [Ru(ben)] > [Ru(acp)] > [Ru(mez)] > [Ru(van)] and this activity could be based on the nature of substituents: Hydroxyl, alkyl and methoxy groups and ethylenediamine, acting as bridging spacers playing significant roles in antiproliferative of Ru(III)-tridentate Schiff base complexes. The antiproliferative evaluation data gave some insight into the structure-activity relationship, but the overall anticancer activity of metal complexes usually

depends on various factors including: Complexes/ compounds reactivity, intrinsic structure, cellular uptake potential, interaction of the cells [269].

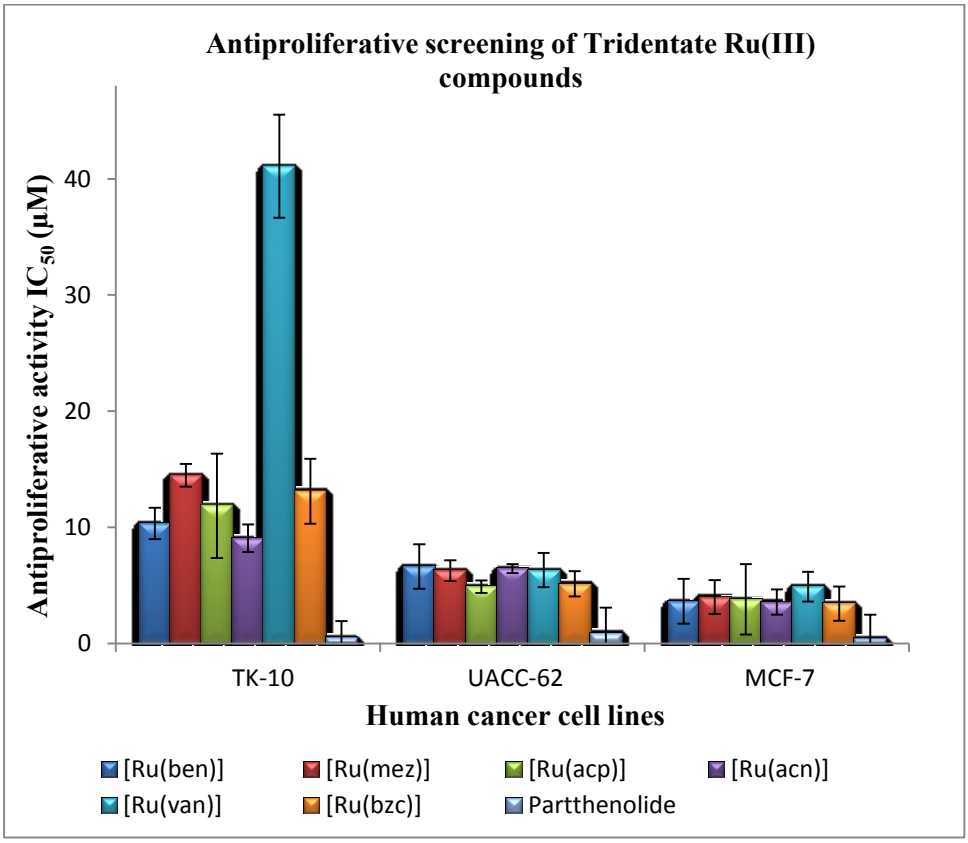


Figure 6.16: Antiproliferative activities of unsymmetrical tridentate Ru(III) compounds against cancer cell lines

6.4 Summary of the Chapter

It is evident that the microbial growth inhibition by metal complexes was in most cases higher than that of the free ligands. Tridentate Schiff base ligands derived from different aldehyde moieties exhibited better anti microbiological activity than the tetradentate

Schiff base ligands. The decreased activity of tetradentate Schiff bases may be due to steric effect which hinders the azomethine group (>C=N) interaction with the cells of the microbes. The same trend was also observed for their metal(II) complexes, where the unsymmetrical tetradentate complexes of Co(II), Ni(II), Cu(II) and Zn(II) with [HLL^2, ehata] ligand had the highest antibacterial activity compared to the unsymmetrical tetradentate complexes of Co(II), Ni(II), Cu(II) and Zn(II) with [HLL^3, ehbta] and [HLL^1, ehopd] ligands.

The antioxidant assay revealed the synthesized tetradentate and tridentate Schiff base Ru(III) complexes exhibited strong scavenging activities against DPPH and moderate ABTS radicals and same goes to complexes of Co(II), Ni(II), Cu(II) and Zn(II) with [HLL^1, ehopd], [HLL^2, ehata] and [HLL^3, ehbta] ligands. On the basis on the cancer growth inhibition assay, the IC_{50} results showed that the tetradentate Ru(III) complexes and tridentate Ru(III) complexes exhibited strong antiproliferative effect towards human breast cancer cell (MCF-7) but lesser activities on human melanoma cancer cell (UACC-62) and human renal cancer cell (TK-10).

Chapter Seven

Summary of Results, Conclusion and Future prospect

CHAPTER SEVEN

SUMMARY OF RESULTS, CONCLUSION AND FUTURE PROSPECT

7.1 Summary of Results

The study is mainly directed towards the synthesis, characterization and biological studies of symmetrical and unsymmetrical Schiff base complexes of ruthenium(III), zinc(II), cobalt(II), nickel(II), and copper(II). Symmetrical tetradentate Schiff bases were derived from 2',4'-dihydroxyacetophenone and ethylenediamine. The unsymmetrical tetradentate Schiff bases were formed in the condensation of 2',4'-dihydroxyacetophenone, ethylenediamine, and acetylacetone/ 1-phenylbutane-1,3-dione. While tridentate unsymmetrical Schiff bases were derived 2',4'-dihydroxyacetophenone, ethylenediamine, substituted aldehydes: Benzaldehyde, benzoyl chloride, methyladehyde, acetanilide, acetophenone, vanillin (4-hydroxy-3-methoxy benzaldehyde), 4-methoxybenzaldehyde, veratraldehyde (3,4-dimethoxybenzaldehyde).

Ruthenium(III), nickel(II), zinc(II), cobalt(II) and copper(II) complexes were prepared with the symmetrical and unsymmetrical Schiff bases. The synthesized complexes were characterized by chemical analysis (CHN analyses), molar conductivity, FT-IR and UV-Vis spectroscopy. The ruthenium(III) complexes of symmetrical and unsymmetrical Schiff bases were screened for their antioxidant and antiproliferative activity, while nickel(II), zinc(II), cobalt(II) and copper(II) complexes were evaluated for their antibacterial and free radical scavenging properties alongside the symmetrical and unsymmetrical Schiff bases.

The thesis is divided into six chapters. Chapter 1 gave an introduction on bioactive metal complexes derived from various organic ligands for therapeutic uses and that has led to recent advancement in bioinorganic chemistry. It also re-accounted the earliest use of transition metal complexes such as cisplatin and related platinum complexes in tumor treatment and leukaemia; development of the ruthenium-based anti-tumor complexes. The use of Schiff bases as models for biologically active compounds for application in metal activities regulation for biological, catalytic conversions. The chapter ended with the scope of the present investigation.

Chapter 2 presented a review on Schiff bases and metal complexes. Transition metal complexes of symmetrical and asymmetrical Schiff bases as antibacterial, antifungal, antioxidant, and anticancer agents were reviewed. The review examined the use of metal Schiff base complexes as promising alternative to cis-platin chemotherapy and as a model in biological, analytical and industrial applications. *In vitro* antifungal and antibacterial potentials of heterocyclic Schiff bases and their metal complexes were described. Antioxidants activities by different methods revealed that symmetrical and unsymmetrical Schiff base metal complexes can acts as free radical scavengers. *In vitro* anticancer and DNA interaction studies of Schiff base metal complexes revealed this class of compound as potential therapeutic agents for disease control.

In chapter 3, detailed experimental procedures on the synthesis of the Schiff base ligands in this research are presented. The ligands are sub-divided with codes: tetradentate ligands as: ehopd, ehata and ehbta; tridentate ligands as: ehmta, ehben, ehmez, ehacp, ehacn, ehvan, ehvet and ehbzc. Preparation of symmetrical and unsymmetrical tetradentate ruthenium(III), zinc(II), cobalt(II), nickel(II) and copper(II) complexes were highlighted in

this chapter. Further, the details regarding analytical and spectroscopic methods employed for the analysis of the Schiff base ligands and their complexes; the techniques employed for the antibacterial, antioxidant and antiproliferative studies were also highlighted.

Chapter 4 gave an insight into the synthesis and characterization of ruthenium(III), zinc(II), cobalt(II), nickel(II) and copper(II) complexes with symmetrical Schiff base [HLL^1, ehopd]. Elemental analyses gave the molecular formulae of the complexes as: $Ru(opd)Cl(H_2O)_3$, $Zn(opd)(H_2O)$, $Cu(opd)(H_2O)_2$, $Ni(opd)(H_2O)_2$ and $Co(opd)(H_2O)_2$. The analytical data show that the metal ligand stoichiometry in all these complexes is 1:1. All the complexes are non-electrolytes in DMF solution, indicating that the ligand is coordinated to the central metal(II) and ruthenium(III) ion in the complexes. The conductivity properties of the metal(II) complexes are in the order of [Co(opd)] > [Ni(opd)] > [Zn(opd)] > [Cu(opd)]. IR spectra indicated the hydroxyl involvement through phenolic oxygen and nitrogen atom of the azomethine (>C=N-) group are coordinated to the metal ions. Relevant information that relate to the coordinated water molecule and those that are held in the lattice was also obtain from the FTIR studies. Metal ions to ligand bonding were confirmed by the UV-Vis spectra and indicates tetrahedral geometry for [Co(opd)], square planar geometry is assigned for [Cu(opd)] and [Ni(opd)] complexes. The electronic spectra data for the [Ru(opd)] complex is a representative of an octahedral environment. The present studies indicated that [HLL^1, ehopd] acts as tetradentate ligand. The physico-chemical data for [Ru(opd)] complex suggested a mononuclear octahedral structure with chlorine and H_2O molecule.

Chapter 5 contained the synthesis and characterization of Schiff base-metal complexes with unsymmetrical tetradentate Schiff base [HLL^2, ehata] and [HLL^3, ehbta] and unsymmetrical tridentate Schiff base [HLL^4, ehmta], [HLL^5, ehben], [HLL^6, ehmez], [HLL^7,

ehacp], [HLL[8], ehacn], [HLL[9], ehvan], [HLL[10], ehvet] and [HLL[11], ehbzc]. Characterization of the synthesized complexes was done by different analytical and physico-chemical techniques. Molecular formulae for the unsymmetrical tetradentate ruthenium(III) complexes was suggested as $Ru(ata)Cl(H_2O)_3$ and $Ru(bta)Cl(H_2O)_3$. Molar conductivity measurements of unsymmetrical tetradentate metal(II) complexes with [HLL[2], ehata] ligand ranged from 3.22–4.27 μScm^{-1}, follows the order: [Zn(ata)] < [Co(ata)] < [Cu(ata)] < [Ni(ata)], while those of unsymmetrical tetradentate metal(II) complexes with [HLL[3], ehbta] ligand are in the order of: [Co(bta)] > [Ni(bta)] > [Zn(bta)] > [Cu(bta)].

The interaction of the unsymmetrical tridentate ligands with $RuCl_3 \cdot 3H_2O$ brought about neutral complexes of the type $[Ru(LL)_nCl_2(H_2O)_x]$ [where LL = ehmta, ehben, ehmez, ehacp, ehacn, ehvan, ehvet, ehbzc; n = 1 and x = 1 or 2]. Conductance measurements indicated that the complexes are non-electrolytes in DMF solution. IR spectra indicated the deprotonation and involvement of the phenolic oxygen atom and nitrogen atom of the azomethine (>C=N-) group being coordinated to the centre metal ion. Relevant information that relates the coordinated water molecule and the lattice water molecule were also obtained from the FTIR analysis. New bands were observed at the fingerprint region which are attributed to ν(M-N=C) vibrations and ν(M-O) vibrations. The IR spectra information supported the coordination of the imino nitrogen and phenolic oxygen atoms to the metal ions. The electronic spectra data of Cu(II) and Ni(II) complexes of unsymmetrical tetradentate Schiff base [HLL[2], ehata] and [HLL[3], ehbta], displayed both charge-transfer (LMCT) and d-d electronic transition that are characteristic of square planar geometry. The absorption spectra revealed that the geometry around the Ru^{3+} ion in the monomeric unsymmetrical tridentate/tetradentate ruthenium(III) complexes are octahedral.

Chapter 6 is divided into three parts. The first part dealt with the antibacterial activities of the synthesised compounds. The tetradentate and tridentate Schiff bases and their metal(II) were screened for antimicrobial potentials against three Gram-positive bacteria, viz. *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus cereus* and three Gram-negative bacteria viz. *Pseudomonas aeruginosa*, *Escherichia coli*, and *Shigella flexineri*. The enhanced biological action of the metal complexes than that of the Schiff base ligands was elucidated on the basis of Overtone's idea and Tweedy's chelation hypothesis [248]. Upon coordination, metal ion polarity is reduced to a more prominent degree because of the overlapping of the ligand orbital and incomplete sharing of positive charge of metal ion with donor groups [247]. Notably, the normal cell process may be affected by the formation of hydrogen bond via the azomethine nitrogen atom with the dynamic centres of cell constituents leading to interference with the cell wall synthesis [116, 225]. Furthermore, the delocalization of the π-electrons is increased over the entire chelate sphere and improves the lipophilicity of the complex. The size of the particle of the complexes, partly affect their antimicrobial activity because nanosized particles exhibit increasing antimicrobial activity [270].

The second aspect is the free radical scavenging (antioxidant) potential of the synthesised compounds. In general, the scavenging activity of the DPPH radical by some of the compounds especially the Ru(III) complexes were higher than that of ABTS radical. Some of the symmetrical and unsymmetrical metal(II) complexes also exhibited higher DPPH scavenging potential. Wang and co-workers [271] reported that some compounds which exhibited ABTS scavenging activity do not possess DPPH scavenging activity. Hence, this study shows the synthesized tetradentate and tridentate Ru(III)- Schiff base complexes exhibited strong scavenging activities against DPPH and moderate ABTS radicals and same

goes to complexes of Co(II), Ni(II), Cu(II) and Zn(II) with [HLL^1, ehopd], [HLL^2, ehata] and [HLL^3, ehbta] ligands. The complexes show higher scavenging effects than the ligands.

The third part of the chapter dealt with the antiproliferative activities of Ru(III) compounds with an investigation into the structure-activity relationship of the isolated ruthenium(III) compounds with respect to different characteristic reactive atoms (functional groups) of the ligands around the metal ion were made possible by subjecting the test samples to cytotoxicity test using different sample concentrations towards human cancer cell lines: TK-10, UACC-62 and MCF-7 via the Sulforhodamine B (SRB) assay. From the Figure 6.17, for the percentage cells viability against the compounds concentrations, it will be observed that inhibition of the growth of the tested cells were in a dose-dependent manner. This action supports the fact that the results obtained from the anticancer activity of the tested compounds increases with increase in its concentrations [195]. The IC_{50} results showed that the test compounds exhibited better antiproliferative effect against human breast cancer cell (MCF-7). In contrast, the tridentate and tetradentate Ru(III) compound were inactive towards human renal cancer cell (TK-10), but induced moderate efficient cell death on human melanoma cancer cell (UACC-62) with IC_{50} values ranging from: 5.28±0.95 - 7.03±1.22 μM and 4.88±0.53 - 6.63±1.92 μM for tetradentate Ru(III) complexes and tridentate Ru(III) complexes respectively. It is therefore noted that the significant anticancer activity of tridentate-Ru(III) and tetradentate-Ru(III) complexes against human breast cancer cell lines (MCF-7) could be explained as cellular damage.

7.2 Conclusion

The quest for novel chemotherapeutic agents to combat human diseases has continued to fascinate the attention of inorganic/ coordination chemists. Hence, the pressing need for drugs with new and distinct structure-activity relationship to deal with the development of therapeutic agents that exhibits higher biological activities. Numerous researches have proven that coordination of different organic donor ligands to metal ions exhibits broad range of activities than the corresponding ligands [20, 100, 112, 145, 147].

This study investigate the synthesis, characterization and biological potentials of symmetrical and unsymmetrical Schiff base-metal complexes, with the aim to contribute toward the search for novel metal complexes that exhibits diverse biological activities and disease control. The coordination of the phenolic oxygen and imino nitrogen atoms of the tetradentate and tridentate Schiff base ligands to the metal ions have been confirmed via FT-IR data comparison of the ligands with those of the ruthenium(III) and metal(II) complexes. The bonding of the Schiff bases through the (>C=N) nitrogen and phenolic oxygen atoms to the metal ions was further confirmed through the evidence of new bands due to the ν(M-N) and ν(M-O) vibrations respectively in the metal complexes spectra. The existence of coordinated water in the ruthenium(III) complexes which appeared in the regions 810-861 cm^{-1}, is due to ν(O–H) stretching and ν(O–H) rocking vibrations which additional confirmed the presence of non-ligand responsible for the rocking mode of water, giving rise to an octahedral environment. The molar conductivity ($\Lambda\mu$) value of all Ru(III), Zn(II), Cu(II), Ni(II) and Co(II) complexes in 10^{-3} $molL^{-1}$ DMF solution at room temperature, indicated the non-electrolytic character of the synthesized complexes.

The electronic spectra were employed to assign the stereochemistry of each metal(II) complexes. It indicated tetrahedral geometries for all complexes of Co(II) due to single *d-d* transition except for [Co(bta)] that exhibited three spin allowed transition assignable to octahedral environment. The electronic spectra's of all Ni(II) complexes exhibited a band attributable to charge-transfer transitions L → M (LMCT) and two absorption bands assigned to two spin allowed transitions, that is a typical of square planar geometry. The observed electronic transitions and reddish-brown colour of the complexes further confirms square planar geometry for nickel complexes. Copper(II) complexes in the visible region displayed two spectroscopic bands that prefer the square-planar geometry. In generally, Zn(II) complexes possess completely filled d^{10} electronic configuration. Therefore, does not exhibit *d-d* electronic configuration, rather charge transfer transition. All Zn(II) complexes exhibited a band ascribed to ligand to metal charge transfer transitions.

The electronic absorption spectra of Ru(III) complexes in DMF within the range of 200 – 900 nm displayed four to five bands. The ruthenium(III) metal ion which is in a d^5 system has relatively high oxidizing properties and large crystal field parameter, the charge transfer bands of the sort $L_{\pi y} \rightarrow T_{2g}$ are noticeable in the low energy region, which sheds the weaker bands due to *d-d* transitions. The configuration of the absorption spectra for the Ru(III) complexes agrees with the existence of octahedral environment around the ruthenium(III) ion.

The antimicrobial activity revealed that the tridentate Schiff bases especially [HLL^9, ehvan] exhibited excellent activity against Gram-positive and Gram-negative bacterial strains than the tetradentate Schiff bases as compared to standard drug: Amoxicillin and ciprofloxacin. However, the *in-vitro* antimicrobial evaluation of complexes against various

pathogenic microbial strains reveals that all the metal complexes exhibited higher antibacterial action than the free Schiff base ligands with Cu(II) complexes possessing high bacterial activities than the other complexes.

In addition, the compounds exhibited some antioxidant properties by scavenging free radicals. The results from DPPH, ABTS methods revealed that compounds are efficient towards donating electron or hydrogen atom, and subsequently react with free radicals or terminate chain reactions in dose-dependent patterns. It was observed from the results obtained that the Ru(III) and metal(II) complexes are effective towards DPPH radicals formation prevention, while the free Schiff bases are active against ABTS radicals and the lower IC_{50} values obtained in the antioxidant assays demonstrated that the synthesized compounds exhibited differential and selective effects to scavenge radicals. With this result, it shows that the compounds in this study can scavenge free radicals in systems, indicating that they may be useful for developing chemotherapeutic drugs that can be use to eliminate the pathological radical's related diseases from the system, some diseases induced by oxidative stress leading to aging, degenerative diseases and cancer.

The *in vitro* antiproliferative activities demonstrated that treatment with the Schiff bases affected cell viability efficiently toward MCF-7 cells. Symmetrical and unsymmetrical tetradentate Schiff base complexes of ruthenium(III) were also tested *in vitro* for 48 h and demonstrated low to moderate antiproliferative effect against selected tumor cell lines but the compounds showed a moderated effect towards the human breast cancer cell (MCF-7) with unsymmetrical tetradentate [Ru(bta)] complex exhibiting the highest antiproliferation on the MCF-7 cell lines. Hence, the binding of the N_2O_2 Schiff base Ru(III) complexes to biological targets other than DNA and alkyl substitution such as methyl and phenyl could have

increased the activity markedly and be responsible for the observed antiproliferative activity of the tetradentate complexes.

Unsymmetrical tridentate Schiff base complexes of ruthenium(III) were also tested *in vitro* for 48 h and demonstrated low to moderate antiproliferative effect against selected tumor cell lines but the compounds showed moderate effect towards the human breast cancer cell (MCF-7) with [Ru(bzc)] complex exhibiting the highest antiproliferation on the MCF-7 cell lines. This enhanced activity could be attributed to the nature of substituents: Hydroxyl, alkyl and methoxy groups and ethylenediamine, acting as bridging spacers play significant roles in antiproliferative of Ru(III)-tridentate Schiff base complexes. The antiproliferative results obtained from the studies gave some information into the structure-activity relationship, but the overall antiproliferative activity of metal complexes usually depends on various factors including: Complexes/compounds reactivity, intrinsic structure, cellular uptake potential, and interaction of the cells.

7.3 Future prospect

The present study describes a convenient, economic and user-friendly protocol for the successful synthesis of symmetrical and unsymmetrical tetradentate, tridentate Schiff base complexes of ruthenium(III) and metal(II) with moderate to high yield products. Hence, the preparation methodology will be useful for the synthesis of additional unsymmetrical Schiff bases with different substituents on the aromatic aldehydes and 2',4'-Dihydroxyacetophenone and their metal complexes followed by various biological activities exploration.

The outcomes from this research revealed that most of the complexes could serve as lead for the development of novel antibacterial, antioxidants and/ or cell antiproliferative agents. In order for proper insight into the structure-activity relationship of the compounds to be gained, further studies into the metal-DNA interaction, cellular uptake potential, DNA binding properties, oxidative cleavage studies should be carried out, *in vitro* and *in vivo* anticancer studies should be carried out on other carcinoma cell lines, Also, investigation into the possible side effects of the tested complexes on normal living cell. *In vitro* activity of the compounds by the hydroxyl radical scavenging, NO and reducing power methods should be examined, the antimicrobial activities against several other pathogenic microorganisms by Ru(III) complexes. Schiff base complexes have been found to exhibit catalytic activity in the existence of N-methylmorpholine-N-oxide as co-oxidant, hence, the need to test the ruthenium complexes for this property. There is need to obtain single crystals of the Schiff bases and their metal complexes for structural studies.

It is expected that progress could contribute further development of Schiff base metal complexes for chemotherapeutic application.

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Appendices

APPENDICES

1.0 Nomenclature of compounds reported in this project

The compounds synthesized in this research are enumerated with their formulae and codes.

1.1 Nomenclature and formulae for the Schiff bases

Ref. No.	Name of Ligand	Formulae	Code
Tetradentate ligands			
HLL[1]	N,N'-bis(2',4'-dihydroxyphenylethylidene)ethylenediamine	H_2dhae$_2$en	ehopd
HLL[2]	N-(2',4'-dihydroxyphenylethylidene)-N'-(1,3-butanedioneethylidene)ethylenediamine	H_2(dhae-bde)en	ehata
HLL[3]	N-(2',4'-dihydroxyphenylethylidene)-N'-(1,3-butanedionephenylidene)ethylenediamine	H_2(dhae-bdp)en	ehbta
Tridentate Ligands			
HLL[4]	N-(2',4'-dihydroxyphenylethylidene)-N'-(methylidene)ethylenediamine	H(dhae-mta)en	ehmta
HLL[5]	N-(2',4'-dihydroxyphenylethylidene)-N'-(benzylidene)ethylenediamine	H(dhae-ben)en	ehben
HLL[6]	N-(2',4'-dihydroxyphenylethylidene)-N'-(4-methoxybenzylidene)ethylenediamine	H(dhae-mez)en	ehmez
HLL[7]	N-(2',4'-dihydroxyphenylethylidene)-N'-(1-phenylethylidene)ethylenediamine	H(dhae-acp)en	ehacp
HLL[8]	N-(2',4'-dihydroxyphenylethylidene)-N'-(phenylethanimidamide)ethylenediamine	H(dhae-acn)en	ehacn
HLL[9]	N-(2',4'-dihydroxyphenylethylidene)-N'-(4-hydroxy-3-methoxybenzylidene)ethylenediamine	H(dhae-van)en	ehvan
HLL[10]	N-(2',4'-dihydroxyphenylethylidene)-N'-(3,4-dimethoxybenzylidene)ethylenediamine	H(dhae-vet)en	ehvet
HLL[11]	N-(2',4'-dihydroxyphenylethylidene)-N'-(benzenecarboximidoyl chloride)ethylenediamine	H(dhae-bzc)en	ehbzc

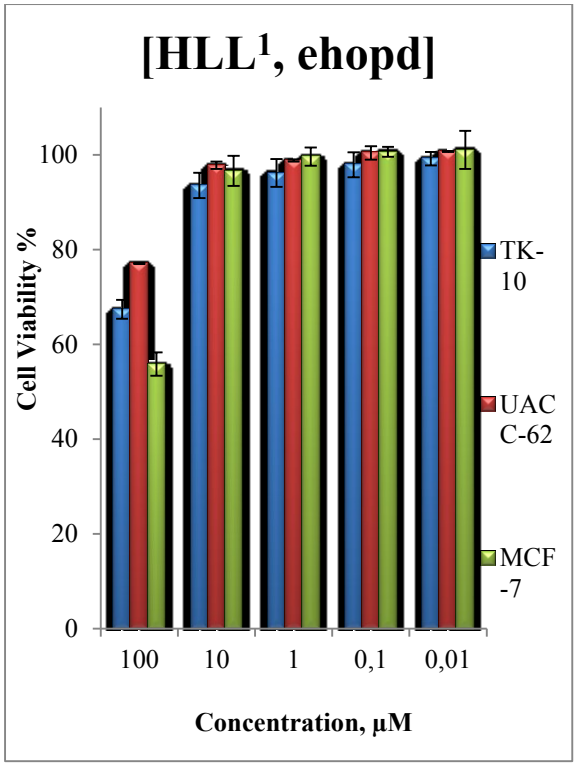
1.2 Nomenclature and formulae for the ruthenium(III) complexes

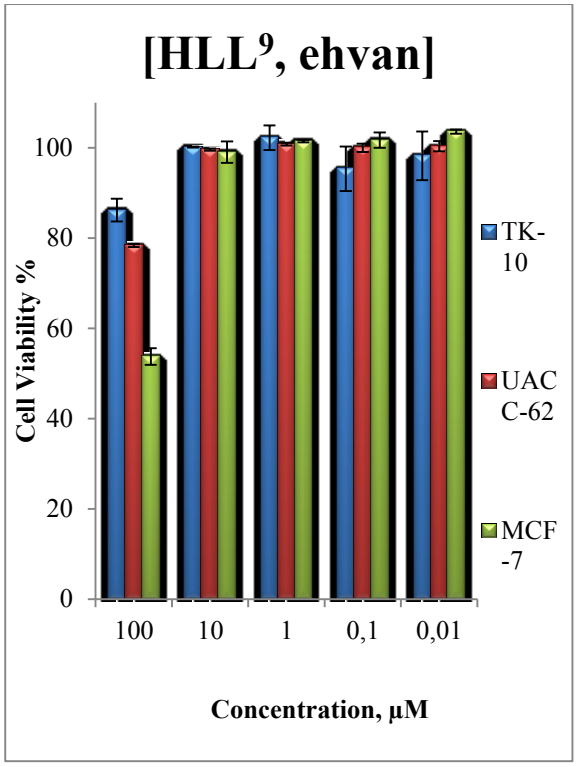
Ref. No.	Name of Ligand	Formulae	Code
Tetradentate ligands			
RuLL[1]	N,N'-bis(2',4'-dihydroxyphenylethylidene)ethylenediiminato ruthenium(III)	[Ru(dhae)$_2$en]	[Ru(opd)]
RuLL[2]	N-(2',4'-dihydroxyphenylethylidene)-N'-(1,3-butanedioneethylidene)ethylenediiminato ruthenium(III)	[Ru(dhae-bde)en]	[Ru(ata)]
RuLL[3]	N-(2',4'-dihydroxyphenylethylidene)-N'-(1,3-butanedionephenylidene)ethylenediiminato ruthenium(III)	[Ru(dhae-bdp)en]	[Ru(bta)]
Tridentate Ligands			
RuLL[4]	N-(2',4'-dihydroxyphenylethylidene)-N'-(methylidene)ethylenediiminato ruthenium(III)	[Ru(dhae-mth)en]	[Ru(mta)]
RuLL[5]	N-(2',4'-dihydroxyphenylethylidene)-N'-(benzylidene)ethylenediiminato ruthenium(III)	[Ru(dhae-ben)en]	[Ru(ben)]
RuLL[6]	N-(2',4'-dihydroxyphenylethylidene)-N'-(4-methoxybenzylidene)ethylenediiminato ruthenium(III)	[Ru(dhae-mez)en]	[Ru(mez)]
RuLL[7]	N-(2',4'-dihydroxyphenylethylidene)-N'-(1-phenylethylidene)ethylenediiminato ruthenium(III)	[Ru(dhae-acp)en]	[Ru(acp)]
RuLL[8]	N-(2',4'-dihydroxyphenylethylidene)-N'-(phenylethanimidamide)ethylenediiminato ruthenium(III)	[Ru(dhae-acn)en]	[Ru(acn)]
RuLL[9]	N-(2',4'-dihydroxyphenylethylidene)-N'-(4-hydroxy-3-methoxybenzylidene) ethylenediiminato ruthenium(III)	[Ru(dhae-van)en]	[Ru(van)]
RuLL[10]	N-(2',4'-dihydroxyphenylethylidene)-N'-(3,4-dimethoxybenzylidene)ethylenediiminato ruthenium(III)	[Ru(dhae-vet)en]	[Ru(vet)]
RuLL[11]	N-(2',4'-dihydroxyphenylethylidene)-N'-(1-chlorobenzenecarboximidoylidene) ethylenediiminato ruthenium(III)	[Ru(dhae-bzc)en]	[Ru(bzc)]

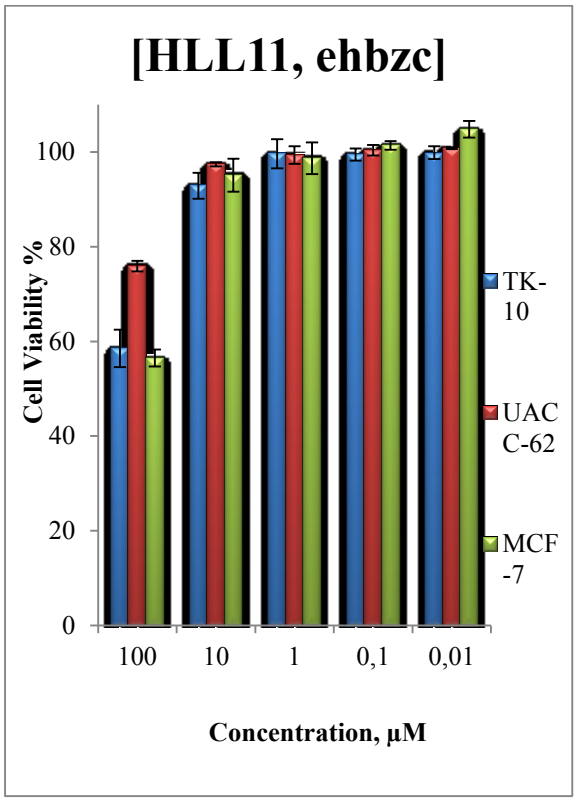
1.3 Nomenclature and formulae for the metal(II) complexes

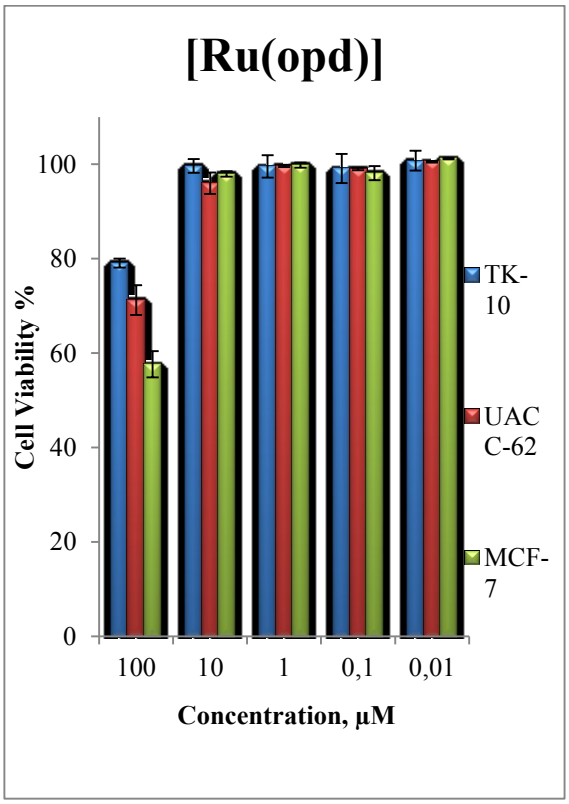
Ref. No.	Name of Ligand	Formulae	Code
Tetradentate ligands			
$CuLL^1$	N,N'-bis(2',4'-dihydroxyphenylethylidene)ethylenediiminato copper(II)	$[Cu(dhae)_2en]$	[Cu(opd)]
$CuLL^2$	N-(2',4'-dihydroxyphenylethylidene)-N'-(1,3-butanedioneethylidene)ethylenediiminato copper(II)	[Cu(dhae-bde)en]	[Cu(ata)]
$CuLL^3$	N-(2',4'-dihydroxyphenylethylidene)-N'-(1,3-butanedionephenylidene)ethylenediiminato copper(II)	[Cu(dhae-bdp)en]	[Cu(bta)]
$NiLL^1$	N,N'-bis(2',4'-dihydroxyphenylethylidene)ethylenediiminato nickel(II)	$[Ni(dhae)_2en]$	[Ni(opd)]
$NiLL^2$	N-(2',4'-dihydroxyphenylethylidene)-N'-(1,3-butanedioneethylidene)ethylenediiminato nickel(II)	[Ni(dhae-bde)en]	[Ni(ata)]
$NiLL^3$	N-(2',4'-dihydroxyphenylethylidene)-N'-(1,3-butanedionephenylidene)ethylenediiminato nickel(II)	[Ni(dhae-bdp)en]	[Ni(bta)]
$CoLL^1$	N,N'-bis(2',4'-dihydroxyphenylethylidene)ethylenediiminato cobalt(II)	$[Co(dhae)_2en]$	[Co(opd)]
$CoLL^2$	N-(2',4'-dihydroxyphenylethylidene)-N'-(1,3-butanedioneethylidene)ethylenediiminato cobalt(II)	[Co(dhae-bde)en]	[Co(ata)]
$CoLL^3$	N-(2',4'-dihydroxyphenylethylidene)-N'-(1,3-butanedionephenylidene)ethylenediiminato cobalt(II)	[Co(dhae-bdp)en]	[Co(bta)]
$ZnLL^1$	N,N'-bis(2',4'-dihydroxyphenylethylidene)ethylenediiminato zinc(II)	$[Zn(dhae)_2en]$	[Zn(opd)]
$ZnLL^2$	N-(2',4'-dihydroxyphenylethylidene)-N'-(1,3-butanedioneethylidene)ethylenediiminato zinc(II)	[Zn(dhae-bde)en]	[Zn(ata)]
$ZnLL^3$	N-(2',4'-dihydroxyphenylethylidene)-N'-(1,3-butanedionephenylidene)ethylenediiminato zinc(II)	[Zn(dhae-bdp)en]	[Zn(bta)]

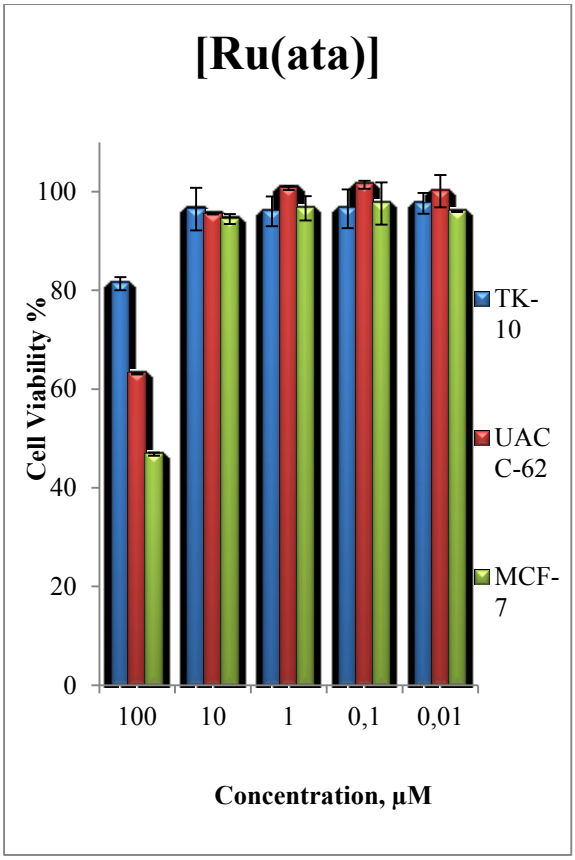
2.0 Antiproliferative screening (Cell viability vs Concentration) of Schiff base ligands and metal complexes against cancer cell lines







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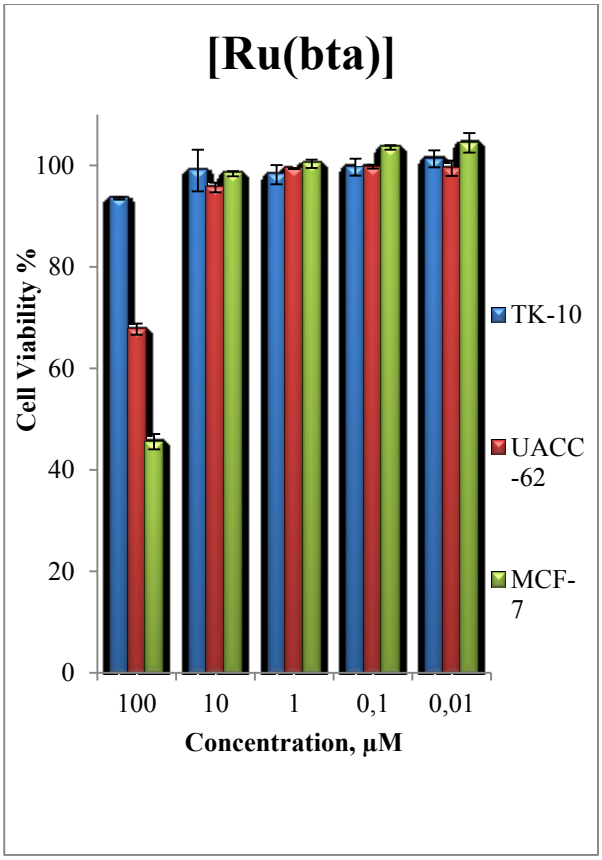
60
40
20
0
100
10
1
0,1
0,01
Concentration, µM
TK-10
UACC-62
MCF-7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40
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Concentration, µM
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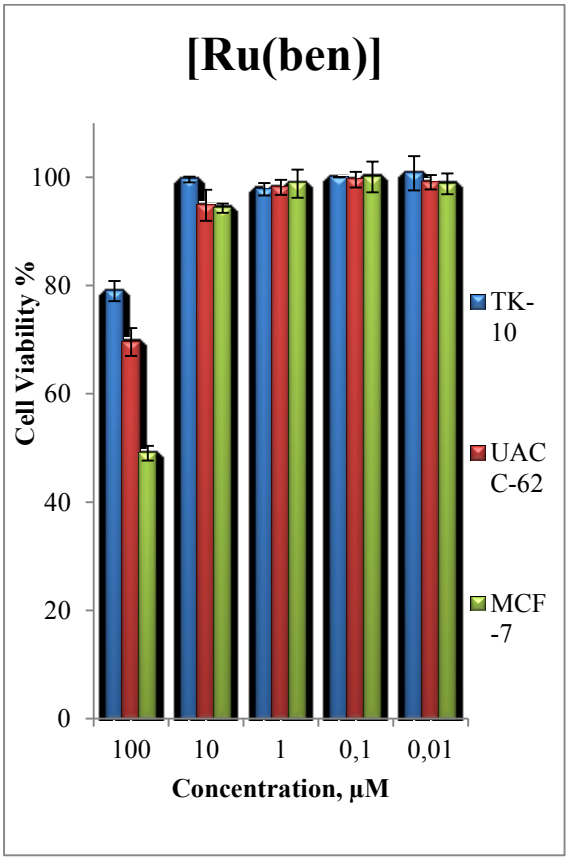
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Concentration, µM
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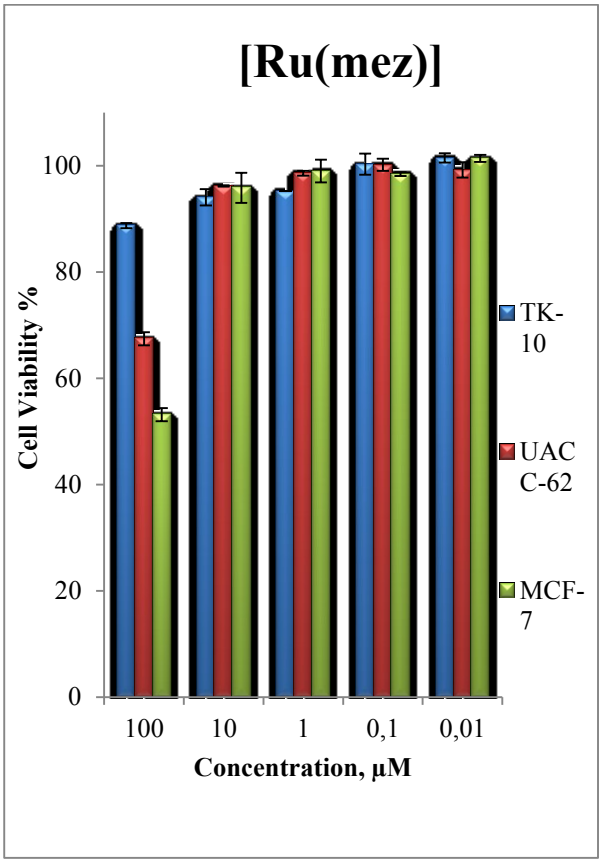
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Concentration, µM
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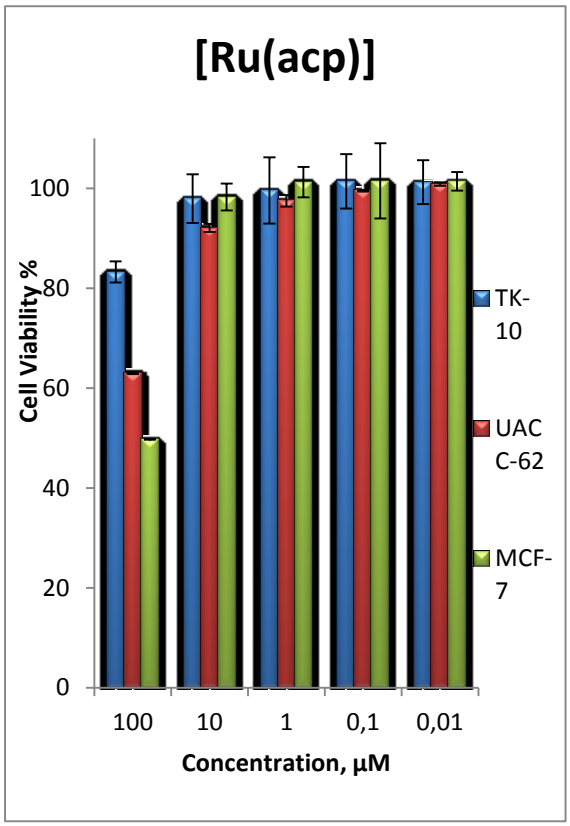
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Concentration, µM
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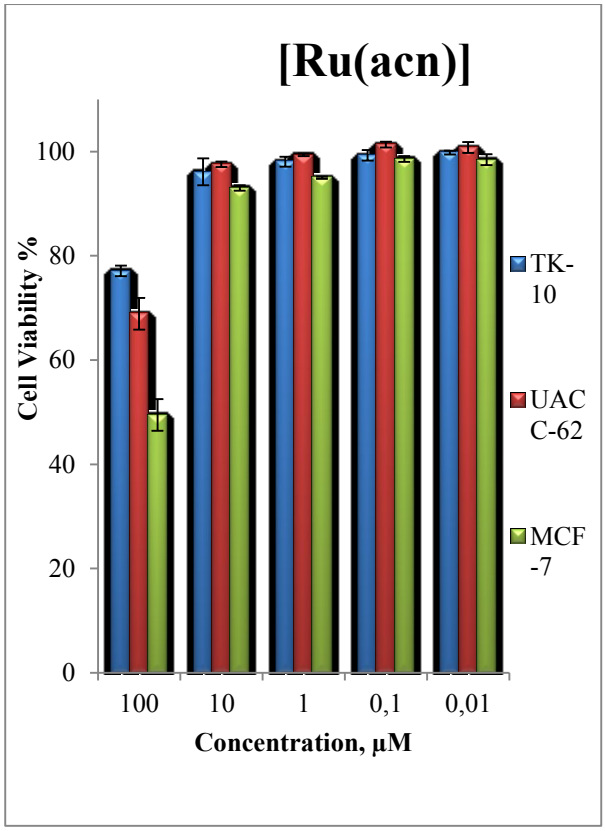
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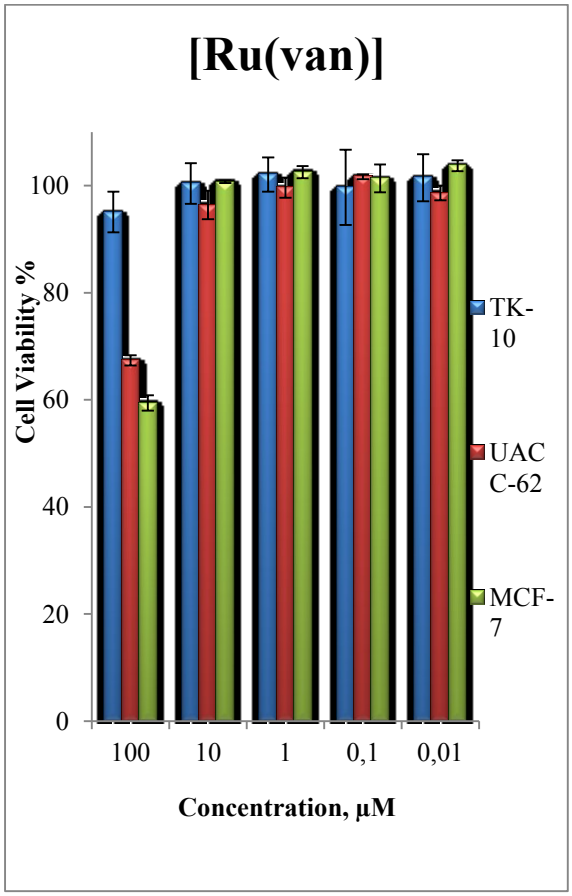
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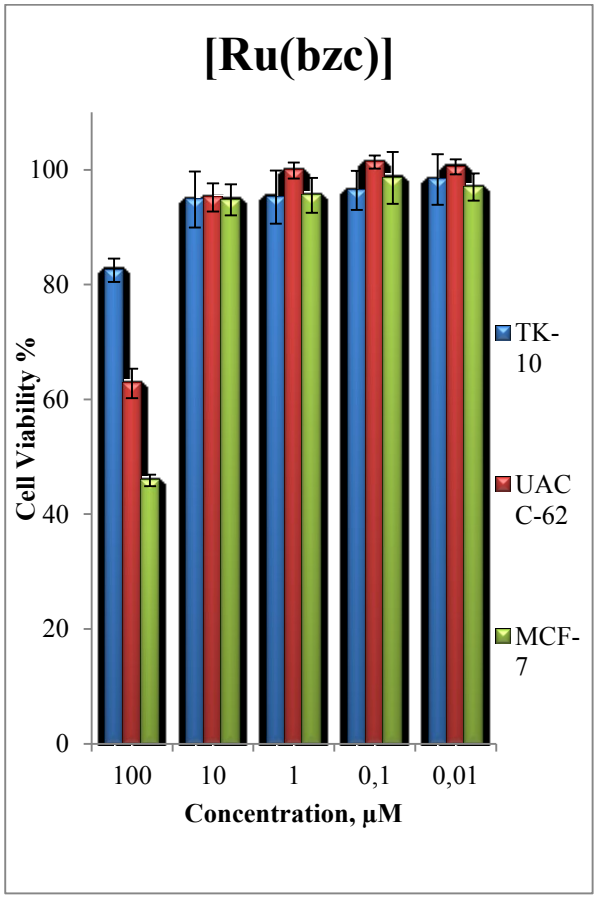
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Cell Viability %
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80
60
40
20
0
100
10
1
0,1
0,01
Concentration, µM
TK-10
UAC C-62
MCF-7