THE SYNTHESIS OF GROUP 10 METALS SCHIFF BASE COMPLEXES WITH ONN AND ONS GROUPS: CHARACTERIZATION AND THEIR CYTOTOXICITY AND ANTIBACTERIAL ACTIVITIES

MD AZHARUL ARAFATH

UNIVERSITI SAINS MALAYSIA
2018

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by

MD AZHARUL ARAFATH

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

February 2018

DEDICATION

To

My teacher and mentor,

oasis of the honesty and principle,

the source of heavenly love and affection

My Dear Father

LATE REAZ UDDIN

ACKNOWLEDGEMENT

All praise to the Almighty Allah for blessing me with sound health, mental strength and courage to conduct the research and write up of the PhD dissertation successfully. I would like to express my deepest acknowledgement to Universiti Sains Malaysia (USM) for giving me the opportunity to conduct the entire research smoothly with worthy facilities. I would like to acknowledge the authority of Shahjalal University of Science and Technology for granting study leave throughout my research period.

I am delighted here to extend sincere gratitude to my honourable supervisor Professor Dr. Farook Adam for his scholastic and informative guidance in research and write up of the thesis. I express profound gratitude to my dignified co-supervisors Dr. Mohd Rizal Razli for his worthy advice during my research work.

I express heartiest gratitude to The World Academy of Science (TWAS) and Universiti Sains Malaysia for (USM - TWAS) post graduate fellowship for satisfactory financial support to continue my PhD. I also admit gratitude for RU grant 1001/PKIMIA/811269 from Universiti Sains Malaysia that financially supported this research.

Sincere appreciation is forwarded to Mr. Kwong Huey Chong for X-ray diffraction data and Mr. Muhd Nizam bin Muhamad Isa for NMR data. I want to thank, Dr. Rabiatul Basria, Professor Dr. Amirul Islam, Dr. Khadeer and Dr. Fouad for biological application. I confess thankfulness to all staffs of USM for friendly cooperation and support.

I want to wish to all my family members as well friends and colleagues of USM and SUST for cordial affection and inspiring suggestion.

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LIST OF ABBREVIATIONS AND SYMBOLS

Å	Angstrom
Anal. Cal.	Analytical Calculation
Ar	Argon
aro	Aromatic
atm	Atmosphere
BPS	Phosphate Buffered Saline
cm^{-1}	Wavenumber
^{13}C NMR	Carbon-13 Nuclear magnetic resonance
Cy	Cyclohexyl
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
d	Doublet
d_6	Deuterated Solvent
DMF	Di-methylformamide
DCM	Di-chloromethane
Ea.hy926	endothelial normal cell lines
EtOH	Ethanol

E. coli	*Escherichia coli*
FTIR	Fourier-transform infrared spectroscopy
FBS	Fetal Bovine Serum
g	gram
h	Hour
Hz	Hertz
^{1}H NMR	Hydrogen -1 Nuclear magnetic resonance
Hela	Cervical carcinoma cell lines
HCT 116	Human colorectal carcinoma cell lines
IC_{50}	Inhibitory concentration (dose causing 50% inhibition of cell growth)
i.e.	That is
K_2PtCl_4	Potassium tetrachloroplatinate
L	Liter
LMS	Least Mean Square
MeOH	Methanol
MCF-7	Breast cancer cells
MIC	Minimum Inhibition Concentration
mL	Mililiter

mmol	millimole
mol	Mole
MTT	(3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide)
NaOH	Sodium Hydroxide
Ni(II)	Nickel ion
$Ni(OAc)_2 \cdot 4H_2O$	Nickel(II) acetate tetrahydrate
nm	nanometer
OAc	acetate
$Pd(OAc)_2$	Palladium acetate
Pd(II)	Palladium ion
Pt(II)	Platinum ion
Pyr	Pyridine
q	quartet
rmp	Rotation Per Minute
s	singlet
S. aureus	*Staphylococcus aureus*
SI	Selectivity Index
t	triplet

TSC	Thiosemicarbazone
THF	Tetrahydrofuran
°	degree
^{o}C	Degree Celsius (degree centigrade)
μL	microliter
λ	Wavelength
δ	chemical shift
%	percentage

SINTESIS KOMPLEKS SCHIFF BES LOGAM KUMPULAN 10 YANG MENGANDUNGI ONN DAN ONS: PENCIRIAN SERTA AKTIVITI SITOTOKSISITI DAN ANTIBAKTERIA

ABSTRAK

Dua puluh satu ligan bes Schiff daripada terbitan salisildihida yang mempunyai amina heterosiklik, anilina dan karbotioamida telah disintesis. Ligan telah dikoordinasikan dengan logam peralihan Kumpulan 10 seperti Ni(II), Pd(II) dan Pt(II). Sebatian yang telah disintesis telah dicirikan dengan parameter fizikal seperti takat lebur dan analisis unsur bahan. Sebatian ini juga telah dicirikan dengan teknik spektroskopi, i.e. FTIR, ^{1}H NMR, ^{13}C NMR, dan UV-Vis. Kebanyakan struktur ligan telah ditentukan dengan analisis pembelauan sinar-X kristal tunggal. Ligan poli dentat yang mengandungi kumpulan ONS dan ONN dikoordinatkan melalui kumpulan nitrogen imina, oksigen fenolik dan sulfur tiolat untuk membentuk kompleks planar persegi. Ligan tersebut membentuk kompleks satah segiempat sama dan oktahedron dengan ion Ni(II). Kompleks gugusan satah segiempat sama dan tetra-palladat diperoleh melalui koordinasi kumpulan ONS bersama Pd(II). Kompleks dan kompleks makro telah diperoleh melalui koordinasi Pt(II) dengan ligan yang mengandungi kumpulan ONS. Aktiviti biologi telah dinilai untuk potensi antikanser terhadap tiga jenis sel kanser iaitu kanser payudara manusia (MCF-7), kanser serviks (Hela), dan kanser kolon (HCT 116). Kompleks logam telah mempamerkan aktiviti yang lebih tinggi berbanding ligan sahaja terhadap semua jenis sel. Di dalam kebanyakan siri, kompleks Ni(II) telah menunjukkan aktiviti yang lebih tinggi berbanding kompleks Pd(II) dan Pt(II). Di antara sebatian yang telah disintesis, aktiviti tertinggi telah

ditunjukkan oleh **L7-Ni** terhadap karsinoma kolorektal dengan IC_{50} = 7.90 ± 0.40 μM, sedangkan piawai cisplatin menunjukkan IC_{50} = 10.1 ± 1.00 μM. Beberapa kompleks menunjukkan aktiviti yang sama seperti ubat yang sedia ada. Pada umumnya, kompleks logam mempamerkan aktiviti yang lebih tinggi daripada ligan. Secara umum semua ligan didapati tidak toksik terhadap sel-sel normal manusia (sel endotelial manusia Ea.hy926). Beberapa kompleks logam didapati toksik kepada sel-sel manusia biasa, contohnya IC_{50} **L2-Pt** dan **L3-Ni** masing-masing adalah 16.30 ± 0.90 μM dan 5.80 ± 0.10 μM. Potensi antibakteria beberapa ligan terpilih dan kompleksnya diuji untuk sifat antibakteria terhadap dua jenis bakteria, iaitu *Escherichia coli* (Gram-negative, ATCC 25922), dan *Staphylococcus aureus* (Gram-positif, ATCC 12600) melalui kaedah penyebaran cakera menggunakan antibiotik piawai. Di antara ligan dan kompleks yang diuji, beberapa daripadanya didapati aktif terhadap bakteria. Walau bagaimanapun, kompleks logam menunjukkan aktiviti yang lebih tinggi daripada ligan. Secara umumnya, kompleks Ni(II) dan Pd(II) mempamerkan aktiviti yang lebih tinggi daripada kompleks Pt(II).

THE SYNTHESIS OF GROUP 10 METALS SCHIFF BASE COMPLEXES WITH ONN AND ONS GROUPS: CHARACTERIZATION AND THEIR CYTOTOXICITY AND ANTIBACTERIAL ACTIVITIES

ABSTRACT

Twenty-one Schiff base ligands of salicyldehyde derivatives with heterocyclic amine, aniline and carbothioamide were synthesized. The ligands were coordinated to Group 10 transition metals Ni(II), Pd(II) and Pt(II). The synthesized compounds were characterized with physical parameters such as melting point and elemental analysis. These compounds were further characterized with spectroscopic techniques, i.e. FTIR, ^{1}H NMR, ^{13}C NMR, and UV-visible spectroscopy. The structure of most of the ligands were elucidated with single crystal X-ray diffraction analysis. The poly dentate ONS and ONN group containing ligands coordinated via imine nitrogen, phenolic oxygen and thiolate sulphur to form square planar complexes. The ligands form square planar and octahedral complexes with Ni(II). Square planar and tetra-palladate cluster complexes were obtained with Pd(II) with the ligands containing the ONS group. Square planar and macro complexes were obtained with Pt(II) and ONS group containing ligands. The biological activity was evaluated for their anticancer properties against three human breast cancer (MCF-7), cervical cancer (Hela), and colon cancer (HCT 116) cell lines. The complexes exhibited higher activity than ligands on all the cell types. In most of the series the Ni(II) complexes showed higher activity than Pd(II) and Pt(II). The **L7-Ni** showed highest activity against colorectal carcinoma with IC_{50} = .90 ± 0.40 µM among the synthesized compounds, whereas standard cisplatin shows IC_{50} =10.1 ± 1.00 µM. A few of the complexes showed very

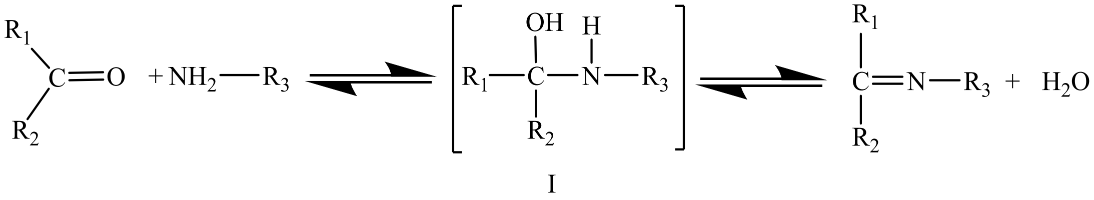
similar activity as standard existing drugs. Generally, the metal complexes exhibited higher activity than the ligands. In general all the ligands were found to be non-toxic to normal human cell line (human endothelial cell line Ea.hy926). Some metal complexes were found to be toxic to the normal human cell line, e.g. the IC_{50} of **L2-Pt** and **L3-Ni** is 16.30 ± 0.90 µM and 5.80 ± 0.10 µM respectively. The antibacterial activities of a few selective ligands and their complexes were tested for their antibacterial properties against two bacteria, i.e. *Escherichia coli* (Gram-negative, ATCC 25922), and *Staphylococcus aureus* (Gram-positive, ATCC 12600) via disc diffusion method using the standard antibiotic. Among the tested ligands and complexes a few of them were found active against bacteria. However, the metal complexes showed higher activity than ligands. Generally, the Ni(II) and Pd(II) complexes exhibited a higher activity than Pt(II) complexes.

CHAPTER ONE

INTRODUCTION

1.1 Schiff Base Compounds

Schiff base compounds are an important class of chemicals in this modern age of chemistry. Hugo Schiff was known as the first chemist who reported these compounds in 1864 [1]. Schiff bases are known as imines or azomethine compounds [1-5], generally having the formula $R_1R_2C=NR_3$ (Scheme 1.1), whereas R_1, R_2 and R_3 are alkyl, aryl, hydrogen or heteroaryl groups [1, 5, 6]. The Schiff base compounds and their coordination complexes with transitional metals has played a significant role in coordination chemistry, polymer chemistry, material science, medicinal and industrial chemistry [7-11]. Azomethine compounds having important C=N linkage show potential antibacterial, anticancer, antifungal and antidiuretic activities [3, 12-15].

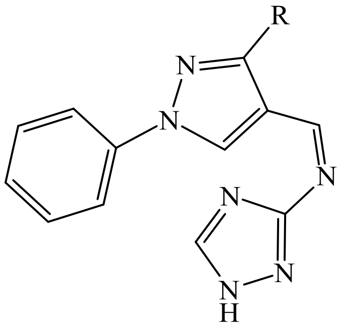


$R_1 = R_2 = R_3 =$ H, alkyl, aryl, heteroaryl,

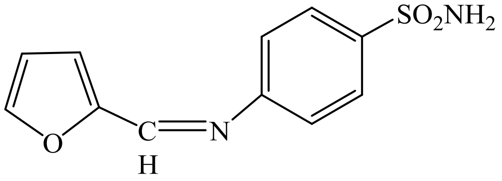
Scheme 1.1. The chemical reaction to show the synthesis of a Schiff base.

1.2 *N*-heterocyclic Schiff base compounds

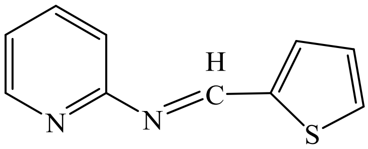
Heterocyclic Schiff base compounds having heteroatoms S, N and O (Scheme 1.2) show a wide variety of biological activity as well as show distinct chemical characteristics due to their diversity in bonding to transition metals [16-19]. Nitrogen and sulphur containing heterocyclic pyridine and thiazole moieties show potential biological activities against hyperthyroidism, human immunodeficiency virus (HIV), fungal, prion disease and gram-positive and gram-negative bacteria [20-22]. Imidazoles are potential biomolecules which show interesting biological activities such as antimicrobial, anti-candida, anti-tumor, anti-cancer, anti-inflammatory, cytotoxicity and anti-cryptococcal, like histidine, histamine and purine amino acids [23-26].



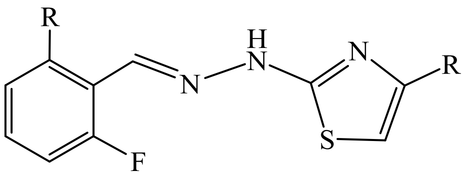
N-heterocyclic Schiff base
R = aryl



O-heterocyclic Schiff base



S-heterocyclic Schiff base



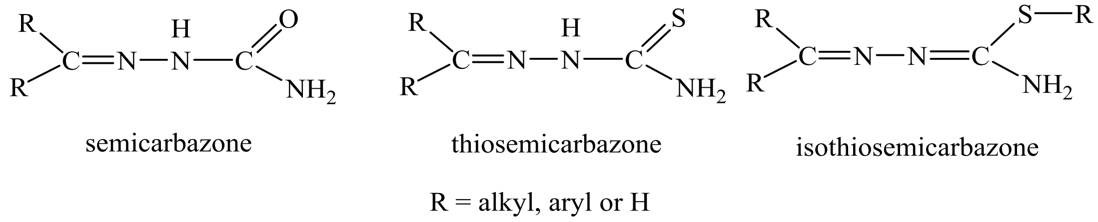
N,S-heterocyclic Schiff base
R= aryl, Cl, H

Scheme 1.2. The structure of heterocyclic Schiff base compounds.

1.3 Type of carbazone Schiff base compounds

Schiff bases of isothiosemi-, thiosemi-, and semicarbazone (Scheme 1.3), have been well studied due to their wide biological application, particularly their anti-cancer and anti-bacteria activities [27-33]. The interest in Schiff bases gradually increased due to the presence of hard or soft Lewis bases atoms together in the same molecule. The thiosemicarbazone Schiff base compounds contain soft Lewis base such as sulphur and nitrogen coordinating atoms as well as the hard Lewis base, oxygen atom

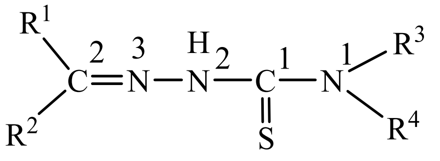
[34]. These Schiff base compounds are of special interest because of their diversity in hard and soft Lewis base metal complexation with hard and soft coordinating sites NSO [34-39].



Scheme 1.3. The structure of different type of carbazones.

1.3.1 Mono-thiosemicarbazone

Mono-thiosemicarbazone ($R^1R^2C^2$=N^3-N^2(H)-C^1(=S)$N^1R^3R^4$) **1** (Scheme 1.4) compounds have different subclasses, determined by the substituents R^1, R^2, R^3 and R^4. The compound which originated from aldehyde, i.e. one of two substituents of C^2 (R^1, R^2) is hydrogen and the substituents in N^1 (R^3, R^4) both can be hydrogen or one hydrogen and secondly the alkyl or alkyl.



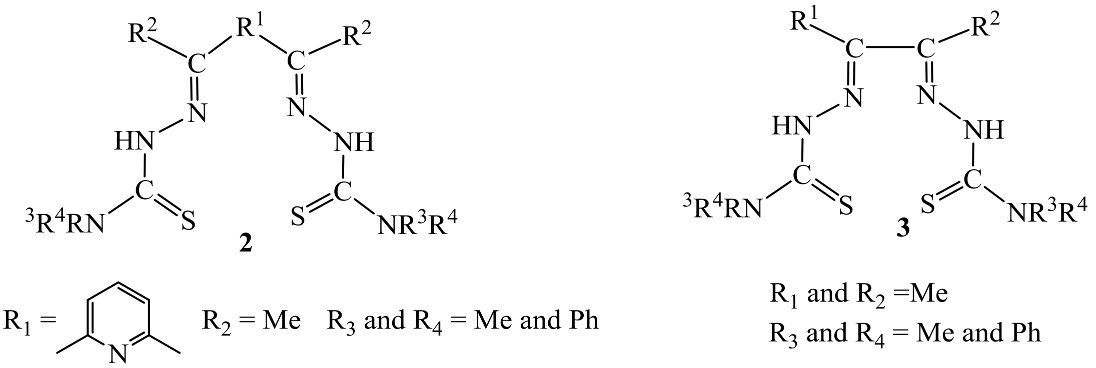
1

R_1 and R_2 = H
R_3 and R_4 = H
R_3 = H, whereas R_4 =aryl or alkyl

Scheme 1.4. The structure of thiosemicarbazone Schiff base compounds.

1.3.2 Bis-thiosemicarbazones

Bis-thiosemicarbazones are formed by connecting to separated thiosemicarbazones moieties connected via a ring or C-C bond, as shown in structures **2** and **3**, respectively (Scheme 1.5). These tetra-dentate ligands entrap metal inside to form square planar complexes [40-42]. The length of the C-C bond in the backbone affects the stability of the complexes. The higher number of C-C bonds via alkylation or arylation allow to better fit metal ions inside of the ligand cavity [41]. These tetra dentate ligands and transition metal complexes exhibit promising anticancer and antibacterial activity [43].



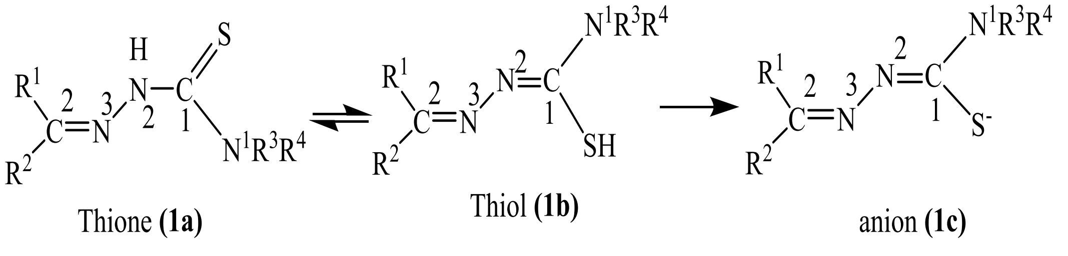
R_1 = (pyridine-2,6-diyl) R_2 = Me R_3 and R_4 = Me and Ph

R_1 and R_2 =Me
R_3 and R_4 = Me and Ph

Scheme 1.5. Bis-thiosemicarbazone Schiff base compounds.

1.4 Different molecular forms of thiosemicarbazones Schiff base compounds

Thiosemicarbazone can exists as thione-thiol tautomers **1a**-**1b** (Scheme 1.6) and both tautomers can coordinate to a metal centre. The anionic **1c** forms (Scheme 1.6) due to deprotonation of N^2H (**1a**) or SH(**1b**) [43]. There are different bonding modes of neutral and anionic forms according to substituent and central metal ion [44, 45].

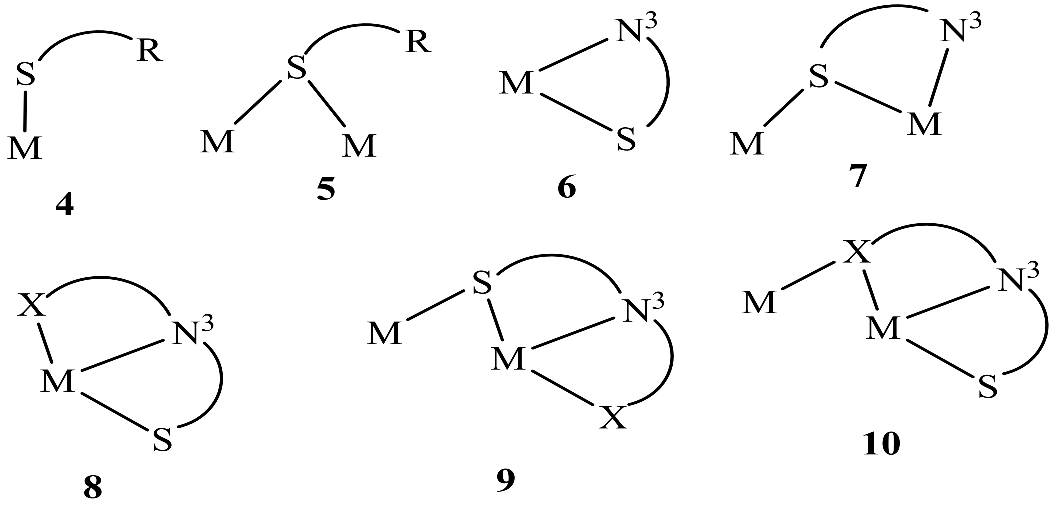


R_1 and R_2 = H
R_3 and R_4 = H
R_3 = H, whereas R_4 =aryl or alkyl

Scheme 1.6. The possible molecular forms of thiosemicarbazones in a solution neutral thione (**1a**), neutral thiol (**1b**) and anionic (**1c**).

1.4.1 Bonding approach in neutral form

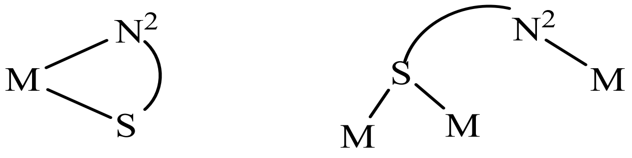
The thiosemicarbazone ligand series ($R^1R^2C^2{=}N^3\text{-}N^2(H)\text{-}C^1({=}S)N^1R^3R^4$) are coordinated to metal in neutral form, **1a** through the lone pair electrons of nitrogen atom (N^3) and S-chelation with X-bridging (X = N or O). The coordination occurs through only the S atom are also known as shown in **4-5** (Scheme 1.7) [46, 47] and the lone pair electrons of N^3 and S-chelation in **6** [48] of N^3 and S-chelation in **7** [47, 49]. The other bonding modes are via X, N^3 and S chelation in **8** [50, 51] and X, N^3 and S chelation with S-bridging in **9** [52]. X bridging mode with S, N^3 and X chelation in **10** (Scheme 1.7) are also observed [53].



Scheme 1.7. The coordination and bridging modes of neutral thiosemicarbazones ligands to metals.

1.4.2 Bonding approach in anionic form

The thiosemicarbazone series ($R^1R^2C^2{=}N^3\text{-}N^2(H)\text{-}C^1({=}S)N^1R^3R^4$) in the anionic form **1c** (Scheme 1.6) coordinate in different modes with metals as shown in **8-10** (Scheme 1.7) via N^3, S-chelation and X-bridging as well as N^3, S-chelation and S-bridging (where X = N or O). In the anionic form, the ligand will coordinate with metals via N^2 and S chelation as shown in **11**, whereas N^2 and S chelation and S bridging in **12** (Scheme 1.8) [54-60].

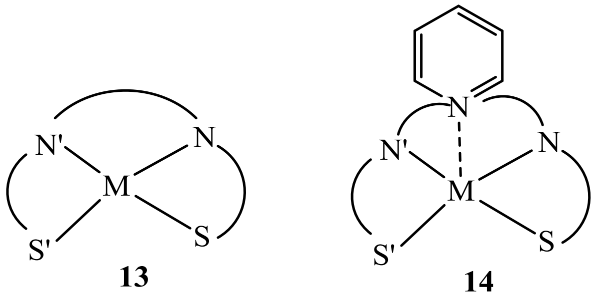


11 **12**

Scheme 1.8. The coordination mode of anionic thiosemicarbazone ligands to metals.

1.4.3 Bonding approach in bis-thiosemicarbazones

The bis-thiosemicarbazones can coordinate either in the anionic or in the neutral form via both wings. The specific approach depends on the central ring; the connections are shown in **13** and **14** (Scheme 1.9) [61-63]. The bis-thiosemicarbazone undergoes deprotonation at the NH group and the tetra-dentate N_2S_2 subsequently coordinates to metal ions forming square planar complexes [64, 65]. The bis-thiosemicarbazone chelating ligand form polynuclear complexes with different binding modes via N and S coordinating sites [66].



13 **14**

Scheme 1.9. The coordination mode of bis-thiosemicarbazone ligands to metals.

1.5 Transition metal complexes with thiosemicarbazone Schiff base compounds

Thiosemicarbazones having ONS coordinating sites are important in coordination chemistry due to their strong bonding ability with transition metals. These Schiff base compounds are of special interest because of their diversity in coordinating to hard and soft metals using the hard and soft coordinating sites. These compounds and their complexes with transition metals have wide biological and pharmaceutical applications.

1.5.1 Nickel complexes with thiosemicarbazone Schiff base compounds

Schiff base containing thiosemicarbazone moieties and their metal complexes have significant interest because of their potential in biological activity [67]. The Ni(II) complexes with Schiff base ligands having dithiocarbazate moiety have been noted to exhibit strong activities against lymphocytic leukaemia [68]. The NS donor Schiff base ligand having thiodipropanoic acid and antipyrine moieties coordinated to Ni(II) have shown potential antifungal and antibacterial activities [69]. The Ni(II) complexes with tetradentate N_2O_2 and tridentate N_2O Schiff base ligands were significantly studied for catalytic and biological activity [70-72]. The Ni(II) complexes with (Z)-2-hydroxy-N′-(2-oxoindolin-3-ylidene) benzohydrazide and similar ligands have shown potential antimicrobial, antitumor and anti-cancer activities compared to the free ligand [13, 72, 73]. The NSO coordinating site containing thiosemicarbazone ligand forming complexes with Ni(II) exhibit more antibacterial, anticancer and anticancer activity than the free ligand [74, 75].

1.5.2 Palladium Complexes with thiosemicarbazone Schiff base compounds

The thiosemicarbazones and dithiocarbazide with N and S donor site are known as soft Lewis base, thus theoretically strongly coordinated with soft Lewis acid such as palladium Pd(II) [76, 77]. The Pd(II) is d^8 metal ion, can form stable complexes with S containing thiosemicarbazone ligand due to strong σ-bond and d_{Π}- d_{Π} bonds by the donation of an electron pair to the ligand [30]. These Pd(II) complexes are more potent against virus such as malaria, tumour, cancer cells, bacteria, and fungi by inhibiting their ribonucleotide and transforming it to the deoxy-ribonucleotide [77-79]. Square planar complexes of palladium with Schiff base containing nitrogen and oxygen/sulphur coordinating sites are very important systems on account of their anticancer, catalytic and antitumor activities [80]. The Pd(II) complexes with nitrogen containing pyridine, quinoline, derivatives ligands shown promising anticancer and antitumor activity [81]. The trans-palladium(II) quinolylmethyl phosphonate complexes which exhibit potential *in vitro* anti-tumour activity [82]. The Pd(II) complexes of S-alkylthiosemicarbazone and thiosemi carbazone exhibited promising activity against bacteria such as *Candida albicans, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Escherichia coli* [83]. The NSO tridentate and NS bidentate thiosemicarbazone complexes with Pd(II) exhibited significant anticancer antibacterial, antifungal and antibacterial activity [76, 84, 85].

1.5.3 Platinum complexes with thiosemicarbazone Schiff base compounds

Cisplatin was synthesized by Pyrone in 1844 and was permitted for the treatment against human cancer in 1978. However, the biological effect of this compound on cancer was accidentally discovered by Rosenberg and co-workers in 1965. The emergence of modern medicinal chemistry, which was inspired by the discovery of cisplatin, has been contributed by the extensive knowledge and efforts of medicinal chemists on the coordination and biological properties of metal ions. The thiosemicarbazone moiety containing Schiff base ligands chelate with Pt(II) shows higher activity against antitumor and anticancer than their ligands; while metal-based drugs are more promising and convenient as therapeutic agent [86-89]. The complexes of Pt(II) with 4(N)- substituted derivatives of 2-acetylpyridine thiosemicarbazone exhibited potential antitumor, anticancer, antibacterial, antineoplastic and cytogenetic activities [88, 90, 91]. The NS coordinating site containing carbazate chelated to Pt(II) exhibits potential antibacterial and anticancer activity [92].

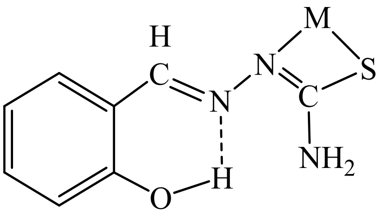
1.6 Antiproliferative activities of transition metal complexes of Schiff base compounds

Cancer is a deadly disease occurring both in male and female across the world. Almost half of all male and one-third of all female are diagnosed with cancer [93]. It is identified that one-quarter of adults' mortality is because of cancer [94]. However, cancer treatment is a massive task for oncologists to get feasible remedy with reasonable cost. Nowadays, many drugs are generally available to treat breast cancer, cervical and colon cancer such as Tamoxifen, 5-fluorouracil (5-FU) and Bevacizumab. However, these chemotherapeutic drugs are often less effective and sometimes cancer cells may exhibit or develop resistance to these drugs [95]. It was observed that DNA is one of the primary intracellular targets for anti-cancer drugs. It is thus important to understand the different binding modes of these small molecules towards DNA. The recognised noncovalent binding modes of small molecules to DNA are intercalative binding, groove binding and external electrostatic binding [96, 97]. Thiosemicarbazones Schiff base complexes with Cu(II) and hydrazine with Mn(II) exhibit potential antitumor and anticancer activity [98-101]. The tetradentate N^2O^2 Schiff bases complexes with Pt(II) and Pt(IV) exhibit promising anticancer activity [102] while the Cu(II) complexes of bidentate N^2 Schiff base exhibit affordable anticancer and antitumor activity [103].

1.7 Anti-bacterial activities of Nickel, Palladium and Platinum complexes with thiosemicarbazone Schiff base

Most of the Schiff bases have been shown to have a wide range of biological activities attributed to the –HC=N- imine functional group [104-107]. Biological activities have been shown to take place through hydrogen bonding (Fig. 1.8) of the imine of Schiff bases to the active site of the cell [108].

Metal coordinated Schiff base compounds are more effective in biological activity than uncoordinated ligands [109-111]. Thiosemicarbazones Schiff bases show uncommon coordination due to two factors. The factors responsible for uncommon coordination are: (a) intramolecular hydrogen bonding which reduces the ability of bonding of the lone pair electrons of the imine nitrogen; and (b) the bulky group on the chelating ligand (Scheme 1.10) [112].



Scheme 1.10. The possible bonding to metal and hydrogen bonding.

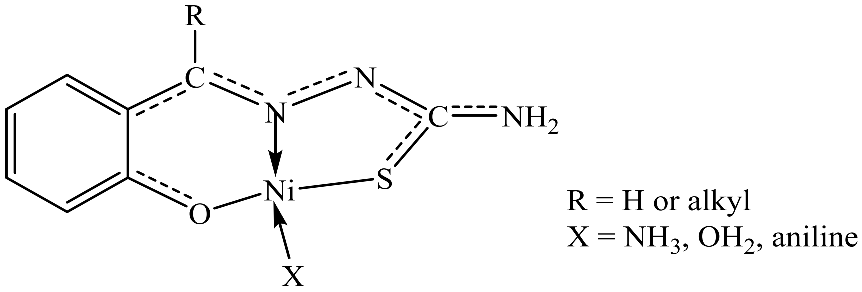
Transition metal complexes containing nitrogen, oxygen and sulphur donor sites possess unique configuration, structural lability and show biocidal activities [113]. The environment around the metal centre such as its coordination geometry, coordinated site and donor group are the key point for biological activities [114]. Thiosemicarbazone derivatives coordinated to metal ion forming chelates arise biological activities. The lipophilicity of the compounds regulates the access of constituents into the cell [115-117]. The peptidoglycan cell membrane of gram-positive bacteria is thicker than gram-negative bacteria, therefore Minimum Inhibition

Concentration (MIC) can be more for gram-positive bacteria than gram- negative bacteria due to different rate of the inoculation of compound diffusion [118].

The Pd(II) complexes with Schiff base derived from dithiocarbazic acids show potential biological activities due to their kinetic lability and thermodynamic stability [68, 119]. Nowadays, dangerous life-threatening contagious diseases initiated by multi - drug resistant Gram-positive and Gram-negative bacteria have been detected in alarming levels throughout the world [120]. Thiosemicarbazones, and nitrogen and sulphur containing chelating agents are becoming highly important in coordination chemistry and biology due to their physico-chemical properties and pharmacological properties [92, 121, 122].

1.8 Heterocyclic and thiosemicarbazone Schiff base compounds and their complexes

Thiosemicarbazones, particularly ($R^1R^2C^2=N^3-N^2(H)-C^1(=S)N^1R^3R^4$) are very important compounds with S and N coordinating centres; their coordination chemistry was firstly noted in the early sixties [123]. Ali and Livingstone [30] initially studied the chemistry of thiosemicarbazone which was successfully reviewed in 1975 by Campbell [28]. Patil and Shah in 1980 and subsequently Dave and Thampy in 1981 had reported the square planar Ni(II) complexes (Scheme 1.11) with 2-hydroxyacetophenone and its substituted derivatives thiosemicarbazone [124, 125].

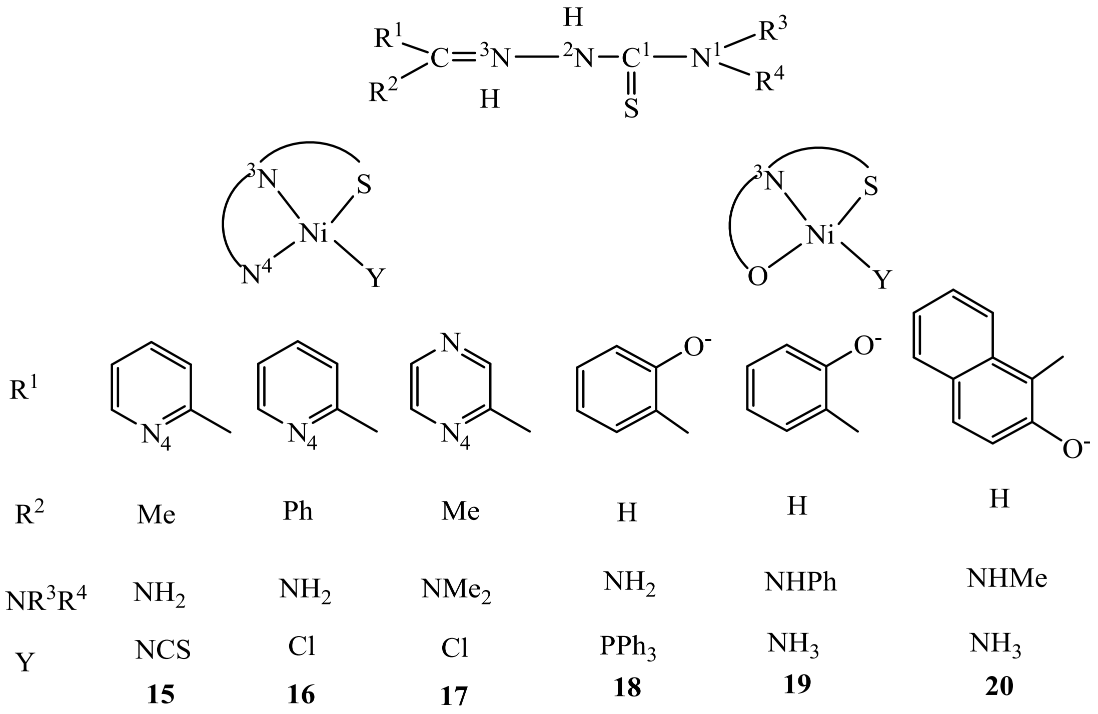


Scheme 1.11. The square planar Ni(II) complexes of thiosemicarbazone Schiff base ligands.

Metal-thiosemicarbazone coordination chemistry was studied in 1985 by Padhye and Kauffman [45], later by West et al in 1991 and 1993 [121, 126]. The Pd(II) and Pt(II) tetranuclear complexes with *p*-isopropylbenzaldehyde thiosemicarbazone was reported by Quiroga and Pérez in 1998 [79]. Further studies in this area was carried out by Casas et al in 1999 and 2000 [127, 128]. The reaction mixture of thiosemicarbazide and aldehyde or ketone was refluxed in methanol and in the presence of molecular sieves to synthesize thiosemicarbazone Schiff base compounds [43].

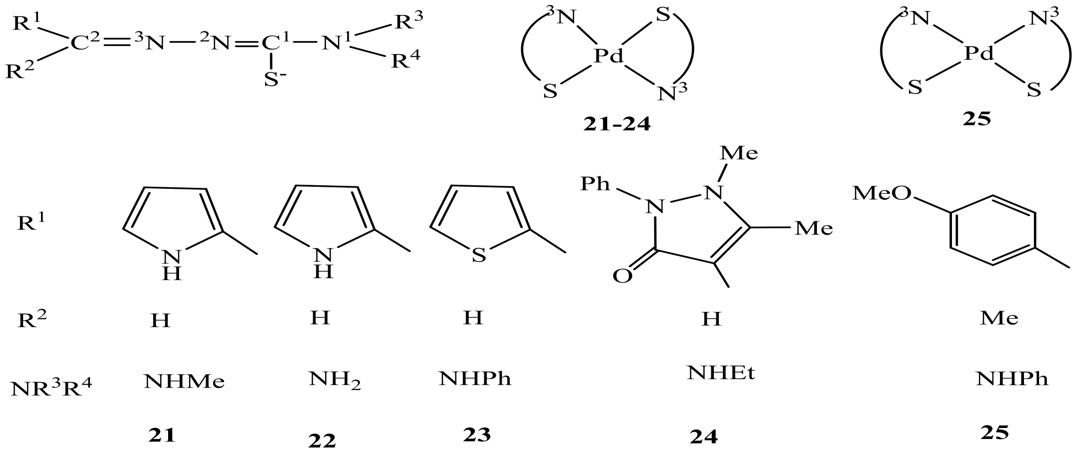
The synthesis of first row transition metal complexes with thiosemicarbazone were basically prepared from metal acetates, sulphates, perchlorates, halides or nitrates in organic solvent at room temperature or at refluxing temperatures [54, 129-137]. The complexes with 4*d* and 5*d* transition metals with thiosemicarbazone were prepared by different routes depending on the type of metals. The Pd(II) and Pt(II) complexes were synthesized using M_2PdCl_4 and M_2PtCl_4 (M = Li, K) [54, 87, 138-142].

Ni(II) form different types of complexes which are mononuclear and polynuclear with Schiff base chelating ligand was reported by Leovac et al in 2007 and Suni et al in 2007 [32, 143]. The Ni(II) is usually observed to coordinate with anionic bidentate or tridentate ligand via S or O donor site. The Ni(II) forms square planar complexes where the fourth position was occupied with NCS (N-bonding), Br, Cl, $MeCO_2$, PPh_3, and NH_3, while the thiosemicarbazone ligand was tridentate in the complexes **15-20** (Scheme 1.12) [144-150].



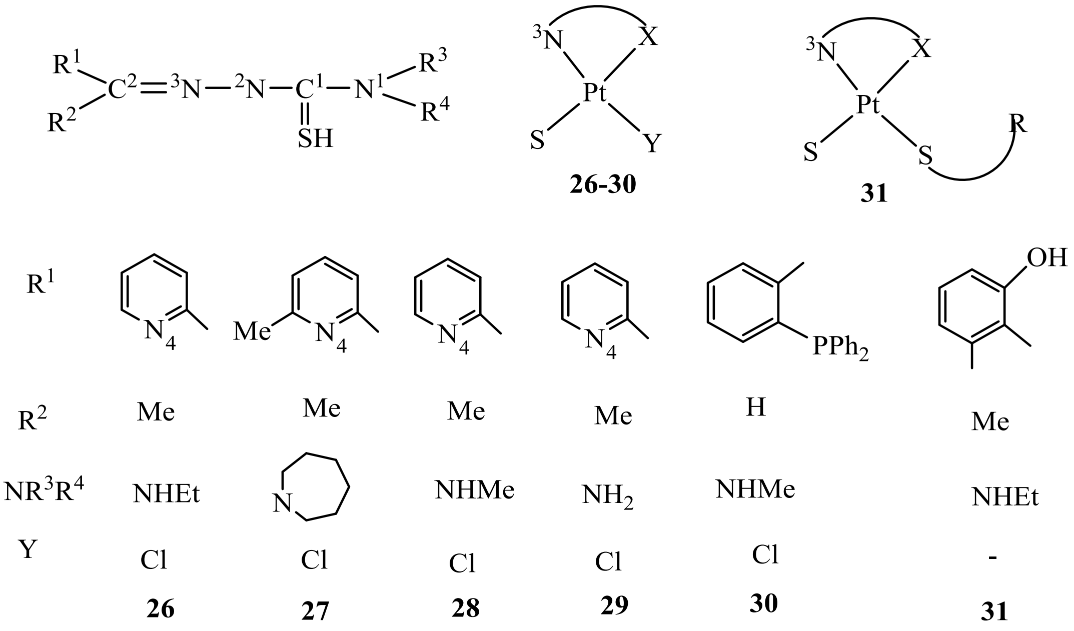
Scheme 1.12. The synthesized complexes of thiosemicarbazone Schiff base ligand to nickel.

The N^3 and S coordinating site containing thiosemicarbazone form trans square planer [PdL_2] complexes, **21-24** of Pd(II) (Scheme 1.13) [151-154]. At the same time Vila and Pereira reported the square planar [PdL_2]·$CHCl_3$ **25** (Scheme 1.13) complex with cis-position of S-donor site [155].



Scheme 1.13. The synthesized complexes of thiosemicarbazone Schiff base ligand to palladium.

Thiosemicarbazone uninegative tridentate NNS coordinating sites ligand form Pt(II) complexes with general formulae of [PtLY], shown as **26-30** (Scheme 1.14), whereas X = N or P [88, 90, 139, 142, 156]. The dinegative tridentate ONS coordinating site and the same ligand as neutral monodentate via S coordinating site form square planar Pt(II) complex, [PtL(H_2L)] **31,** whereas X = O [157].



Scheme 1.14. The synthesized complexes of thiosemicarbazones Schiff base ligand to platinum.

1.9 Anticancer and antibacterial activities of thiosemicarbazone and hydrazone Schiff base compounds and their complexes

The interest of Schiff base and its complexes with transition metals is increasing due to potential biological application [158]. Brockman and Thomson in 1956 had investigated the anti-leukemic activity of pyridine-2-carbaldehyde thiosemicarbazone but the compound was found to be toxic [159]. Antholine and Knight in 1976 reported the potential antitumor activity of iron and copper complexes of 5-substituted-2-formylpyridine thiosemicarbazones against tumor cell lines [160]. Dobek and Klayman in 1986 reported the remarkable antibacterial activity of 2-acetylpyridine-1-oxide thiosemicarbazones against *N. gonorrhoea, S. faecalis, N.*

meningitis and *S. aureus* [161]. Liu and Lin in 1992 observed the antitumor activity of 3- and 5-aminopyridine-2-carboxaldehyde thiosemicarbazones on L1210 leukaemia, whereas the 3-amino substituted complex was found to be more active [162]. Oxovanadium(IV) complexes of thiosemicarbazone of 2-formylpyridine showed potential *in vitro* activity against *E. coli* was reported by Maiti and Guha in 1988 [163]. The titanium and zirconium complexes of thiosemicarbazone derived from 2-acytylnaphtalene, 2-acytylthiopene, 2-acytylpyridine, and 2-acytylfuran exhibited potential activity against *E. coli, S. aureus*, *S. viridans* and *P. mirabilis* [164]. 3-aminopyridine-2-carboxaldehyde thiosemicarbazone has been shown to have potential activity against mouse M109 lung carcinoma and A2780 human ovarian carcinoma [165]. Bindu et al. in 1998 investigated antibacterial activity against pathogenic bacteria of human *Salmonella typhi, S. aureus* and, *Shigella dysentariae* using Cu(II) complexes of N-phenylsalicylaldehyde thiosemicarbazone, where they found that the complexes exhibited higher activity than the ligand [166]. Küçükgüzel et al in 1999 [167] studied the antibacterial activity against *Staphylococcus aureus* using ethyl-2-arylhydrazone-3-oxobytyrates derivatives. Kovala and Yadav in 2000 synthesized Pt(II) square planar complexes with 2-acetylpyridine thiosemicarbazone-N-Me [PtLCl] and tested against sophisticated ovarian cancer cell lines A2780/Cp8 and A2780. The IC_{50} values of 0.14 and 0.08 µM obtained respectively was higher than cisplatin [88]. Kovala and Demertzi in 2001 and 2003 studied the square planar and distorted square planar complexes of Pt(II) with thiosemicarbazone that exhibit lethal effect against Gram (+) bacteria [87, 90]. Singh and Narang [168] carried out the study on antibacterial activity against *Escherichia coli as well Aspergillus niger* using Co(II), Ni(II), Zn(II), Cu(II) and Cd(II) complexes with acetophenone-4-aminobenzoylhydrazone and 4-hydroxyacetophenone-4-aminobenzoylhydrazone.

Savini and Chiasserini in 2004 [169] had reported the anticancer activity of 3- and 5-methylthiophene-2-carboxaldehyde α-(N)-heterocyclic hydrazone derivatives. 6-Methyl-2-formyl pyridine Schiff bases of S-methyldithiocarbazates ligand (Hmpasme) form complexes [Pd(mpasme)Cl], [Pt(mpasme)Cl] and [Pt(Hmpasme)Cl] with (Pd(II) and Pt(II) respectively and were investigated against human ovarian cancer (Caov-3) cell lines. The ligand and platinum complex showed remarkable activity but Pd(II) complex showed only weak activity was reported by Ali et al in 2006 [170]. Al-Sha'alan in 2007 [171] evaluated the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* using Cu(II), Ni(II), Co(II), Mn(II) and Fe(II) complexes with 7-chloro-4-(benzylidenehydrazo) quinolone. Pyridyl thiosemicarbazone and its transition metal complexes were tested for anti-bacterial activities. The higher inhibitory effect was found for complexes because of more lipophilicity of complexes than ligand [43]. The Pd(II) and Pt(II) complexes of 2-acetyl-4-methylthiazo lethiosemi carbazone and 2-acetylpyrazinethiosemicarbazone exhibited potential activity against two human tumor cell lines HT-29 and HuTu-80 were reported in 2016 by Nimir et al [172]. Pt(II) complexes of methylhydrazine carbodithioate derivatives of indolin-2-one exhibited strong activity against HCT 116, MCF-7 was reported by Li et al in 2017 [173].

1.10 The Problem statement

The thiosemicarbazone and heterocyclic Schiff base compounds are important segment for chemistry, biology and medicinal chemistry. These compounds were synthesized by different techniques which gave low yield [174-176]. These compounds contain nitrogen, sulphur and oxygen together in the same molecule. The ONS and ONN group could be chelated strongly with transition metal ions. Very few of these compounds have been reported complexed to group 10 transition metal ions in the literature. The percentage yield was not satisfactory and the synthetic procedures were not described explicitly.

These compounds and their transition metal complexes might have good biological and medicinal properties. *Cis*-platin was the first group 10 metal chelated complex to be used as an anti-cancer agent. It has been identified that the nitrogen moiety in *cis*-platin plays an important role in its anti-cancer activity. Similar complexes as cis-platin could be a potential chemotherapeutic agent for cancer treatment besides surgery and radiation therapy. Therefore, the comparative investigation against cancer cell lines using thiosemicarbazone and heterocyclic Schiff base ligands and their complexes with Group 10 metal ions, seems to be an interesting option to be investigated.

Dangerous life-threatening contagious diseases initiated by multi-drug resistant gram-positive and gram-negative bacteria have been increasing at an alarming rate across the world. Thiosemicarbazones, particularly nitrogen and sulphur containing chelating agents have shown promising activities against such bacteria. In addition, the phenolic OH containing groups have shown potential antibacterial and antifungal activities [177, 178]. From the literature review the Schiff base ligands and their complexes with Group 10 metal ions have not been investigated comparatively against gram-positive and gram-negative bacteria. These compounds could be the potential agent against diseases originating from microorganism.

1.11 Objective of the research work

In this work, some new Schiff base compounds will be synthesized and complexed with group 10 transition metal ions. These complexes and their ligands will be subjected to the characterization and cytotoxicity and bacteriological studies. The specific objectives to fulfil the aim of this study are as follows:

1. To synthesize and fully characterize thiosemicarbazone, heterocyclic and oxygen bis Schiff base compounds having ONS and ONN coordination sites.
2. To synthesize and fully characterize the Ni(II), Pd(II) and Pt(II) complexes with the above Schiff base ligands.
3. To evaluate of their anti-proliferative activities against breast cancer (MCF-7), colon cancer (HCT 116), cervical cancer (Hela) and normal (endothelial cell Ea.hy926) cell lines.
4. To test of their antibacterial activities against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*.

CHAPTER TWO

EXPERIMENTAL

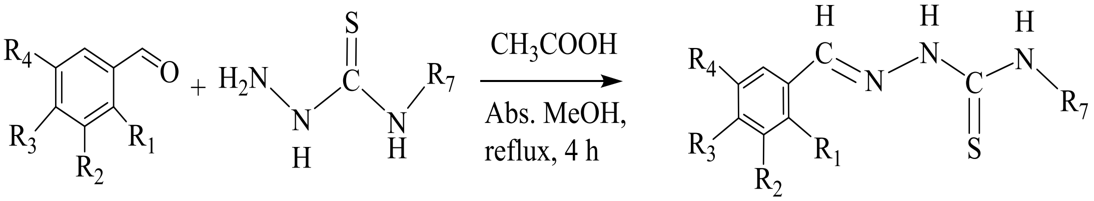
2.1 Material and measurement

All the reagents and chemicals were purchased from Sigma-Aldrich and Fluka. The solvents were purified with prescribed standard procedure [179]. FTIR data were obtained using the Perkin Elmer System 2000 FTIR as KBr disc in the range 400-4000 cm^{-1}. The melting points were recorded using a Gallenkamp melting point apparatus (model 7936B, England). The ^1H and ^{13}C NMR were recorded with Bruker Avance III 500 MHz and 125 MHz spectrophotometer respectively using TMS as internal standard and DMSO-d_6, CD_3OD-d_4, $OC(CD_3)_2$-d_6, and $CDCl_3$ as solvent. X-ray crystallographic data were recorded on a Bruker SMART APEXII CCD area-detector diffractometer using graphite-monochromated Mo*Kα* radiation (λ = 0.71073Å) in the range 100 K to 294 K. The data were collected and reduced using APEX2 and SAINT. The structures were solved using the SHELXS-97 program package, and refined using the SHELXL-97 program package. All none hydrogen atoms were anisotropically refined. Molecular graphics were created using SHELXTL-97.

2.2 Experimental procedure for the ligands synthesis

2.2.1 General procedure for the synthesis of Schiff base ligands

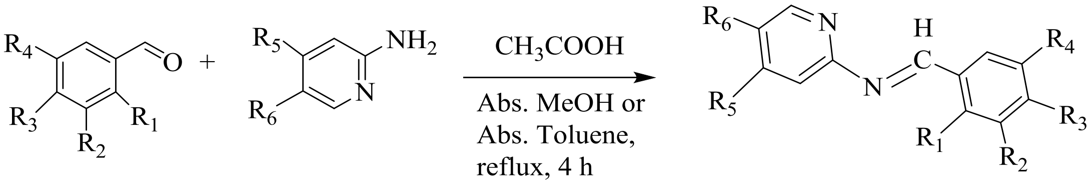
The aldehyde was dissolved in requisite amount of methanol or ethanol (20.0 mL) and 0.20 mL glacial acetic acid was added as catalyst [49, 90-91, 179]. The corresponding amine was dissolved in methanol or ethanol then added to the aldehyde solution. The resulting solution was refluxed for 4 h, in a round bottomed flask equipped with magnetic stirrer and water condenser. The product was dried under reduced pressure then washed with the appropriate solvent. The product was recrystallized with slow evaporation of solvent at room temperature and diffusion technique.



Scheme 2.1. Synthesis of Schiff base ligands. The scheme shows the reaction between benzaldehyde derivatives and carbothioamide derivatives.

Table 2.1. Ligand labels and their identity for compounds in Scheme 2.1.

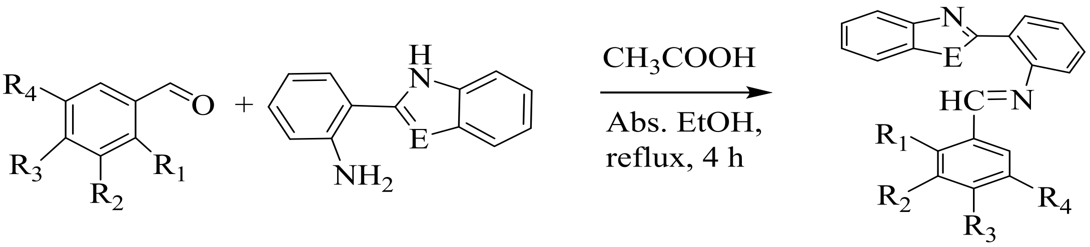
Sample Code	R_1	R_2	R_3	R_4	R_7
L1	OH	CH_3	H	H	CH_3
L2	OH	CH_3	H	H	C_6H_{11}
L3	OH	NO_2	H	OCH_3	C_6H_{11}
L4	OH	NO_2	H	OCH_3	CH_3
L5	OH	t-butyl	H	H	C_6H_{11}
L6	OH	H	H	t-butyl	C_6H_{11}
L7	H	OH	OCH_3	H	C_6H_{11}
L8	OH	H	OH	H	CH_3
L9	OH	H	H	H	C_6H_{11}
L10	H	OH	OCH_3	H	CH_3



Scheme 2.2. Synthesis of Schiff base ligands. The scheme shows the reaction between benzaldehyde derivatives and pyridine derivatives.

Table 2.2. Ligand labels and their identity for compounds in Scheme 2.2.

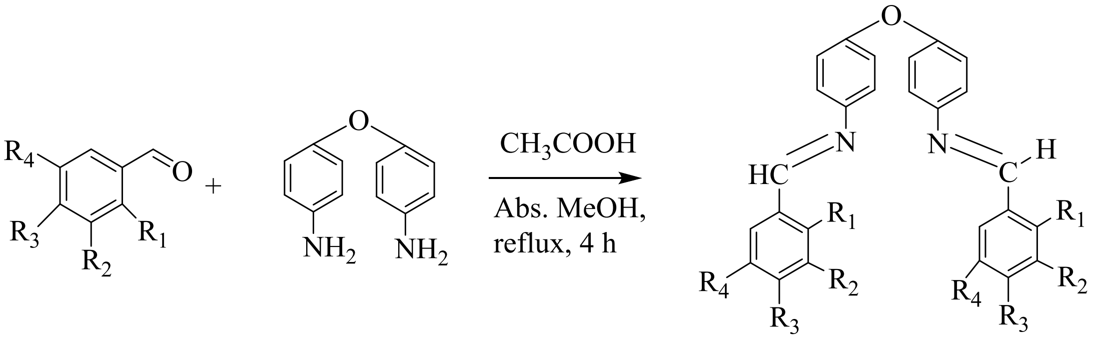
Sample Code	R_1	R_2	R_3	R_4	R_5	R_6
L11	OH	H	H	CH_3	H	CH_3
L12	OH	H	H	CH_3	CH_3	H
L13	OH	CH_3	H	H	CH_3	H
L14	OH	CH_3	H	H	H	CH_3
L15	OH	H	H	OCH_3	H	CH_3
L16	H	OH	OCH_3	H	CH_3	H



Scheme 2.3. Synthesis of Schiff base ligands. The scheme shows the reaction between benzaldehyde derivatives and imidazole derivatives.

Table 2.3. Ligand labels and their identity for compounds in Scheme 2.3.

Sample Code	R_1	R_2	R_3	R_4	E
L17	H	OH	OCH_3	H	N
L18	H	OH	OCH_3	H	S



Scheme 2.4. Synthesis of Schiff base ligands. The scheme shows the reaction between benzaldehyde derivatives and 4,4'-oxydianiline.

Table 2.4. Ligand labels and their identity for compounds in Scheme 2.4.

Sample Code	R_1	R_2	R_3	R_4
L19	OH	NO_2	H	OCH_3
L20	OH	CH_3	H	H
L21	H	OH	OCH_3	H

2.2.2 Synthesis of 2-(2-hydroxy-3-methylbenzylidene)-*N*-methyl hydrazine-1-carbothioamide (L1)

The 0.68 g (5.00 mmol) 2-hydroxy-3-methylbenzaldehyde was dissolved in 20.0 mL methanol, 0.20 mL glacial acetic acid was added and the mixture was refluxed for 30 minutes. A solution of 0.52 g (5.00 mmol) *N*-methyl hydrazinecarbothioamide in 20.0 mL methanol was added dropwise with stirring to the aldehyde solution. The resulting colourless solution was heated under reflux for 4 h with stirring. The crude product was washed with 5.0 mL *n*-hexane. The recovered product was dissolved in DMSO for purifying and recrystallization. Light yellow coloured single crystals suitable for X-ray diffraction study was obtained by slow evaporation of the solvent. Colour: Light yellow; M. P.: 181-182 °C; Yield (%): 94. anal. Cal. For $C_{10}H_{13}N_3OS$ (FW: 223.29 g mol^{-1}); C, 53.74; H, 5.83; N, 18.81; Found: C, 53.71; H, 5.79; N, 18.83

%. ^{1}H NMR (500 MHz, DMSO-d_6, Me_4Si ppm): δ 11.38 (s, N-NH), δ 9.39 (s, OH), δ 8.34 (s, HC=N), δ 8.44 (q, CS-NH), δ 7.42-6.81 (multiplet, aromatic), δ 3.00 (d, *J* = 4.5 Hz, N-CH_3), δ 2.20 (s, Ph-CH_3). The ^{13}C NMR (DMSO-d_6, Me_4Si ppm): δ 177.48 (C=S), δ 154.24 (C=N), δ 143.64-119.10 (C-aromatic), δ 31.05 (N-CH_3), δ 15.91(Ph-CH_3) ppm. IR (KBr pellets υ_{max}/cm^{-1}): 3418 υ(NH), 3133 υ(OH), 2983(NC-H_3, *sp*^{3}), 1618 υ(C=N), 1553 υ(C=C, aromatic), 1270 υ(C=S), 1251 υ(CH, bend., aromatic), 1085 υ(C-O). 1043 υ(C-N).

2.2.3 Synthesis of *N*-cyclohexyl-2-(2-hydroxy-3-methylbenzylidene) hydrazine -1 -carbothioamide (L2)

The 0.68 g (5.00 mmol) 2-hydroxy-3-methylbenzaldehyde was dissolved in 20.0 mL methanol. 0.20 mL glacial acetic acid was added to the mixture and was refluxed for 30 minutes. A solution of 0.87 g (5.00 mmol) *N*-cyclohexylhydrazine carbothioamide in 20.0 mL methanol was added drop wise with stirring to the aldehyde solution. The resulting colourless solution was refluxed for 4 h with stirring. The colourless precipitate was obtained with evaporation of solvent. The crude product was washed with 5.0 ml *n*-hexane. The recovered product was dissolved in acetonitrile and purified by recrystallization. A colourless single crystals suitable for X-ray diffraction was recovered with slow evaporation of acetonitrile. M. P.: 240-241 °C; Yield (%): 93. anal. Cal. For $C_{15}H_{21}N_3OS$ (FW: 291.41 g mol^{-1}); C, 61.77; H, 7.21; N, 14.42; Found: C, 61.81; H, 7.19; N, 14.42 %. ^{1}H NMR (500 MHz, DMSO-d_6, Me_4Si ppm): δ 11.27 (s, N-NH), δ 9.51 (s, OH), δ 8.34 (s, HC=N), δ 8.05 (d, *J* = 8.35 Hz, CS=NH), δ 7.39-6.81 (multiplet, aromatic-H), δ 2.20 (s, Ph-CH_3), δ 1.87-1.14 (multiplet, cyclohexyl-H) ppm. The ^{13}C NMR (DMSO-d_6, Me_4Si ppm): δ 175.79 (C=S), δ 154.29 (C=N), δ 143.76-119.17 (C-aromatic), δ 15.93(CH_3), δ 52.87-

24.90(C-cyclohexyl) ppm. IR (KBr pellets υ_{max}/cm^{-1}): 3364 υ(NH), 3148 υ(OH), 2989(CH_3), 2931 and 2854 υ(CH, cyclohexyl), 1620 υ(C=N), 1540 υ(C=C, aromatic), 1268 υ(C=S), 1218 υ(CH, bend., aromatic), 1122 υ(C-O). 1075 υ(C-N).

2.2.4 (*E*)-*N*-cyclohexyl-2-(2-hydroxy-5-methoxy-3-nitrobenzylidene)hydrazine-1-carbothioamide (L3)

The 2-hydroxy-5-methoxy-3-nitrobenzaldehyde 0.76 g (5.00 mmol) was dissolved in 20.0 mL methanol, 0.20 mL glacial acetic acid was added and the mixture was refluxed for 30 minutes. *N*-cyclohexylhydrazinecarbothioamide 0.54 g (5.00 mmol) solution in 20.0 mL methanol was added dropwise with stirring to the aldehyde solution. The resulting deep orange solution was refluxed for 4 h with stirring. A reddish orange precipitate was formed. The precipitate was filtered and washed with 5.0 mL hot methanol and 5.0 mL *n*-hexane. The recovered product was dissolved in a mixture of THF and toluene (4:1) to attempt purification by recrystallization. Only a reddish orange solid product was isolated. The product was washed with 5.0 mL methanol and 5.0 mL of *n*-hexane. Colour: Reddish Orange; M. P.: 245-246 ^oC; Yield (%): 98. Anal. Cal. For $C_{15}H_{20}N_4O_4S$ (FW: 352.41 g mol^{-1}); C, 51.10; H, 5.60; N, 15.80; Found: C, 51.15; H, 5.58; N, 15.70 %. ^1H NMR (500 MHz, DMSO-d_6, Me_4Si ppm): δ 11.57 (s, N-NH), δ 10.40 (s, OH), δ 8.41 (s, HC=N), δ 8.21 (d, *J* = 8.55 Hz, CS-NH), δ 8.03 (s, aromatic-H), δ 7.54 (s, aromatic-H), δ 3.83 (s, Ph-OCH_3), δ 1.86-1.14 (multiplet, cyclohexyl-H). The ^{13}C NMR (DMSO-d_6, Me_4Si ppm): δ 175.87 (C=S), δ 151.51 (C=N), δ 145.55-108.91 (C-aromatic), δ 56.02 (Ph-OCH_3), δ 52.94 - 24.97 (C-cyclohexyl). IR (KBr pellets υ_{max}/cm^{-1}): 3363 υ(NH), 3123 υ(OH), 2934 υ(CH_2, cyclohexyl), 1584 υ(C=N), 1532 υ(C=C, aromatic), 1431 υ(C-H, *sp*^{3}, bend), 1249 υ(NO_2), 1213 υ(C=S), 1052 υ(C-N), 937 υ(N-N).