

# Synthesis and Biological Activities of Metal Complexes of Schiff Bases Synthesized from Isoniazid



*Humaira Afzal*

**NUST201463655MSNS78214F**

**This work is submitted as an MS thesis in partial fulfillment of the  
requirement for the degree of**

**(MS in Chemistry)**

**Supervised By:**

*Dr. Muhammad Arfan*

**Department of Chemistry**


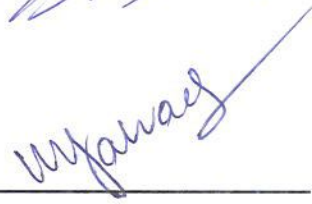
**School of Natural Sciences (SNS)**

**National University of Sciences and Technology (NUST), H-12**

**Islamabad, Pakistan**



**National University of Sciences & Technology****MS THESIS WORK**

We hereby recommend that the dissertation prepared under our supervision by: HUMAIRA AFZAL, Regn No. NUST201463655MSNS78214F Titled: Synthesis and Biological Activity of Metal Complexes of Schiff Bases Synthesized From Isoniazid be accepted in partial fulfillment of the requirements for the award of **MS** degree.

**Examination Committee Members**1. Name: DR. AZHAR MAHMOODSignature: 2. Name: DR. FAHAD EHSANSignature: 

3. Name: \_\_\_\_\_

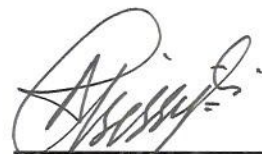
Signature: \_\_\_\_\_

4. Name: DR. TARIQ MAHMOODSignature: Supervisor's Name: DR. MUHAMMAD ARFANSignature: 

Head of Department

30-08-17

Date

**COUNTERSIGNED**Date: 30/8/17

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Signature:  \_\_\_\_\_

Name of Supervisor: Dr. Muhammad Arfan

Date: 30-08-17

Signature (HoD):  \_\_\_\_\_

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***IN THE NAME OF ALLAH, THE MOST  
GRACIOUS, THE MOST MERCIFUL.***

*Dedicated to*  
*My Parents, Grand Parents and Siblings*

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# List of Abbreviations and Symbols

7-ACA	7-Aminocephalosporanic acid
AIDS	Acquired Immune Deficiency Syndrome
6-APA	6-Aminopenicillanic acid
ApEMT	4-Acetylpyridenylideneamino-3-ethyl-5-mercapto-s-triazole
ApMMT	4-Acetylpyridenylideneamino-5-mercapto-3-methyl-s-triazole
Ar	Argon
Boc	Di- <i>tert</i> -butyl carbonate
Bz	Benzoyl
cm <sup>-1</sup>	Per centimeter
DNA	Deoxyribonucleic Acid
FT-IR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography Mass Spectrometry
ISE	Ion Selective Electrode
mL	Mililiter
NaBH <sub>4</sub>	Sodium Borohydride
NaOH	Sodium Hydroxide
PMBP	1-Phenyl-3-methyl-4-benzoyl—5-pyrazolone
RNA	Ribonucleic Acid
SRB	Sulphorhodamine B
TLC	Thin Layer Chromatography

## **Abstract**

The present research work deals with the synthesis of Schiff bases derived from Isoniazid and different aldehydes. Nine different ligands were synthesized by using variously substituted aldehydes. Benzoyl and Boc protection of the aldehyde were also carried out where necessary. The ligands were characterized by the melting point, FT-IR and GC-MS. After that synthesized ligands were used for complexation reaction by using metal halide salt i.e.  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ . All the synthesized complexes were characterized by melting point, FT-IR and UV-vis spectroscopy. The biological activity of the ligands as well as complexes was checked against Colorectal Carcinoma cell line HCT-116 and all the compounds show activity against cell line. The activity against cell line was carried out using four different dilutions and all the compounds were even active up to the fourth dilution.

# Chapter 1

## 1.1 Introduction

The rapid improvement in the area of bio-inorganic chemistry has created the concern towards metal complexes. The compounds formed between metal ions and neutral and negatively charged species are studied under the branch of Chemistry known as coordination chemistry. Much interest has been created in the metal complexes of organic chelating ligands due to their applications in different fields. Many chelating ligands have been used in the field of Coordination Chemistry, but Schiff bases become remarkable ligands due to their easy preparation and complexation [1]. Metal complexes of the Schiff bases have been reported since the mid of the nineteenth century [2]. The geometry of metal complexes has been proposed by the Alfred Werner in 1893 and much work has been done by him in the field of metal complexes and got Nobel Prize in 1913. Much interest in the field of metal complexes has been created due to their applications in various fields. Metal complexes have found many exciting properties that are why they show many applications in various aspects of human life. Metal complexes were not only found as biological active compound but also used in other fields such as catalysis, solvent extraction, leather tanning *etc.* [3-4].

### 1.1.1 Chemistry of Schiff Bases

Hugo Schiff was the German chemist and the first person who synthesized the imines by the condensation reaction of aldehydes with primary amines. Due to the condensation reaction  $\text{H}_2\text{O}$  molecule is also eliminated. Although these are not the bases but the designation for these compounds is bases and still used now. The imines were named Schiff bases after Hugo Schiff [5].

The general formula of the Schiff bases are represented as  $\text{R}_3\text{R}_2\text{C}=\text{NR}_1$  and are also known as imines and azomethine. The substituent  $\text{R}_2$  and  $\text{R}_3$  may be alkyl, aryl, heteroaryl or hydrogen. Schiff base becomes a stable imine when substituent may be alkyl or aryl. Schiff

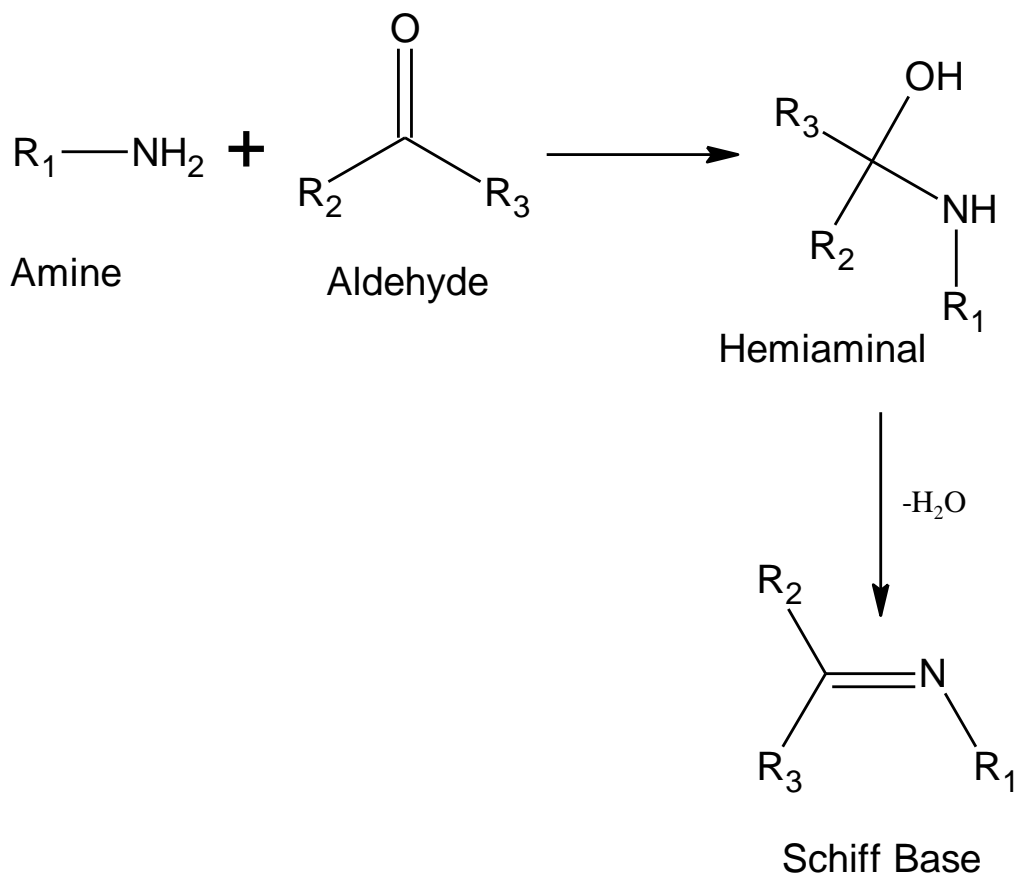
## Chapter 1

base can be synthesized by the reaction of primary amine and a carbonyl compound. The reaction is nucleophilic addition reaction forming a hemiaminal, which is followed by the dehydration to generate imine [6]. For more than hundred years the physical properties and reactivity of Schiff bases have been studied [7].

The Schiff base ligands are considered as privileged ligands [8] and are capable to stabilize metal in different oxidation states [9]. The lone pair of electron in  $sp^2$  hybridized orbital of nitrogen is responsible for the chemical and biological importance of the Schiff bases and has been shown through several studies [10-13].

Ligands other than Schiff bases, having  $sp^2$  hybridized nitrogen atom show much consideration in the coordination chemistry [14-16]. Schiff bases are conceived as excellent chelating ligands due to their synthetic flexibility, easy synthesis and special property of C=N group [17-18] and their coordinating property increases if functional groups like -OH or -SH are present close to the imine group. The general scheme for the synthesis of Schiff base ligand is shown in the Figure 1.1.

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**Figure 1.1: Synthesis of Schiff base Ligand through Condensation Reaction**

### 1.1.2 Formation of Schiff Bases

The Schiff base ligands can be synthesized by the condensation of aldehyde/ketone and amines in different reaction conditions and also various solvents are used. Methanol and ethanol are the most frequently used solvents for the synthesis of Schiff bases and these can be carried out either by stirring at room temperature or under the reflux. Following methods can be used to synthesize Schiff bases and their metal complexes.

#### 1.1.2.1 Direct Ligand Synthesis Followed by Complexation

Using this procedure, before complexation with metal the separation and purification of Schiff bases are performed. The advantage of this method is the characterization of ligands and complexes can be compared.

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### **1.1.2.2 Template Synthesis**

This is the one step reaction in which metal complexation is carried out without isolation of Schiff bases [19-23]. In this method, the metal ion catalyzes the reaction by acting as reaction template.

## **1.2 Application of Schiff Bases and Their Metal Complexes**

A wide range of application has been shown by Schiff bases and their metal complexes. Much enthusiasm for the development medicines based on metals started in the mid-1960s. A large portion the model investigation of Schiff base metal complexes have concentrated on the binding mode of the ligands. Metal complexes containing Schiff base ligands are when incorporated to the drug molecule, the drug lipophilicity, toxicity and bioavailability will be improved [24]. Schiff bases mostly act as tridentate ligands. These coordinate to the transition metals through the phenolato oxygen, imine nitrogen and the carboxylate oxygen [25]. This had been revealed through the X-ray crystal structure studies of the complexes [26].

Schiff bases are widely studied because of their interesting applications in industrial and biological areas [27]. It has also been studied that the activities are enhanced when the drug or the ligand is coordinated to the metal and form metal complexes [28-29]. The biological activities shown by almost all Schiff base metal complexes are anti-bacterial, anti-viral, anti-fungal and anti-tubercular activity [30-33]. The biological activities are shown by these compounds are due to the unpaired electron of nitrogen atom. These participate in the hydrogen bonding with NH or OH groups of amino acids, proteins, DNA or RNA of the species.

The ion selective electrode (ISE) is used for clinical, chemical and environmental analysis and these ISEs are based on polymeric membranes comprises of the ionophores. With transition metal ions, Schiff bases form very stable metal complexes also act as ion carrier in the polymeric membrane [34].



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Schiff base compounds also act as fluorescent and electronic material especially Zn(II) complexes of Schiff bases, e.g. 2-(hydroxymethyl)-4-methyl-6-((quinolinyl-8-imino)methyl) phenol and its Zn (II) complex [35].

Another important use of the Schiff bases is their role as an efficient corrosion inhibitor. The Schiff bases immediately form monolayer on the surface which must be protected and this ability is due to the C=N linkage of Schiff bases [36]. Even in the acidic media, Schiff base compounds protected the various metals like steel, copper and aluminum from corrosion [37-42].

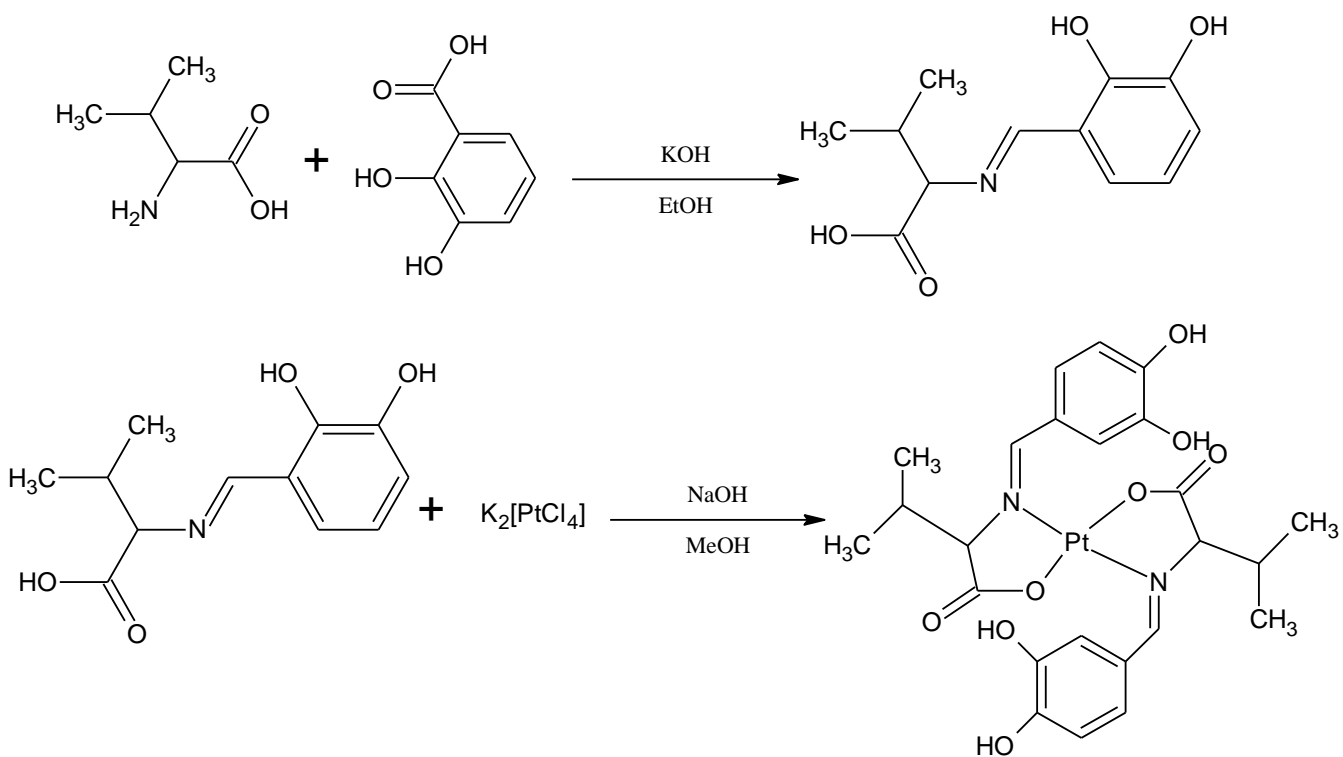
Metal complexes of Schiff bases have been employed as dyes which provide fast colour to leather, food packages and wool especially chromium and cobalt complexes. These complexes have excellent light resistance and stability even in the presence of acidic media [43].

### 1.3 Synthesis of Schiff Bases and Their Metal Complexes

#### 1.3.1 From Amino Acids

Alsalmeh *et al.* (2016) synthesized Schiff base using amino acid and aldehyde by stirring the mixture at room temperature. The yield obtained was 82 %. The synthesized ligands were then used to synthesize the metal complex in refluxing condition with occasional stirring [44].

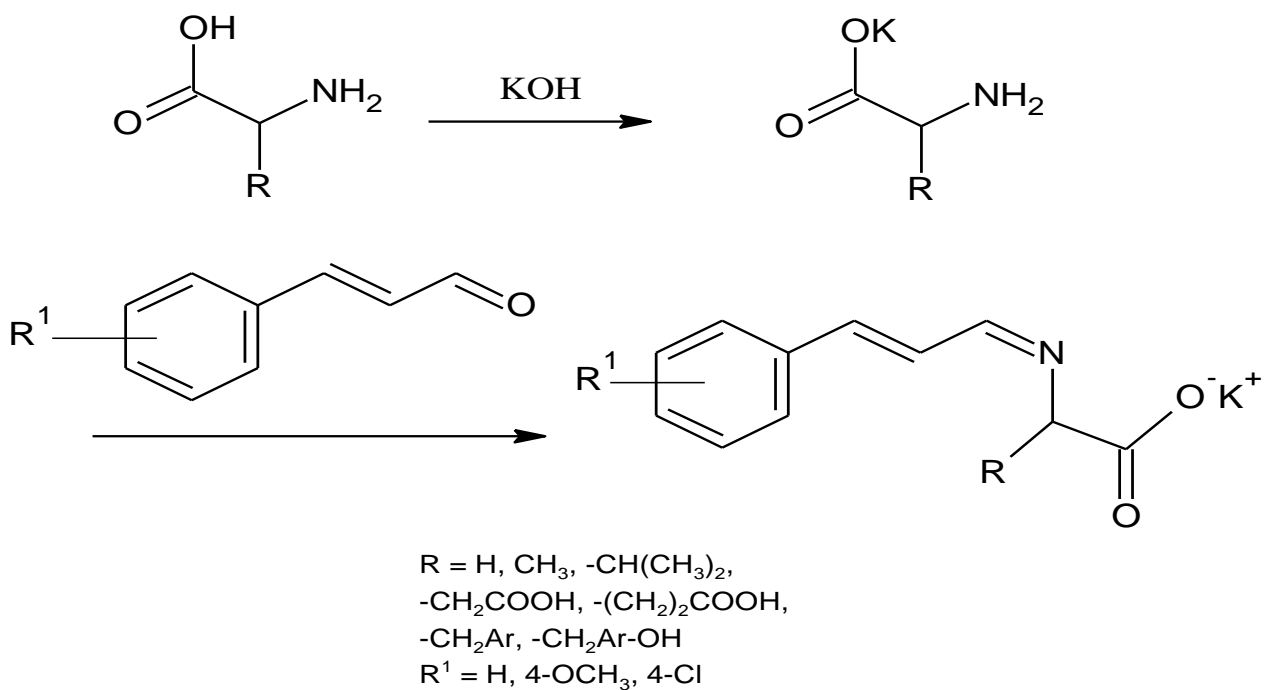
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**Scheme 1.1: Synthesis of the Schiff Base Ligand and its Metal Complex**

In **2016**, the hydrophilic Schiff base ligands were synthesized by Hui Wang and coworkers and cinnamaldehyde derivatives along with different amino acids were used for this synthesis by using simple approach [45].

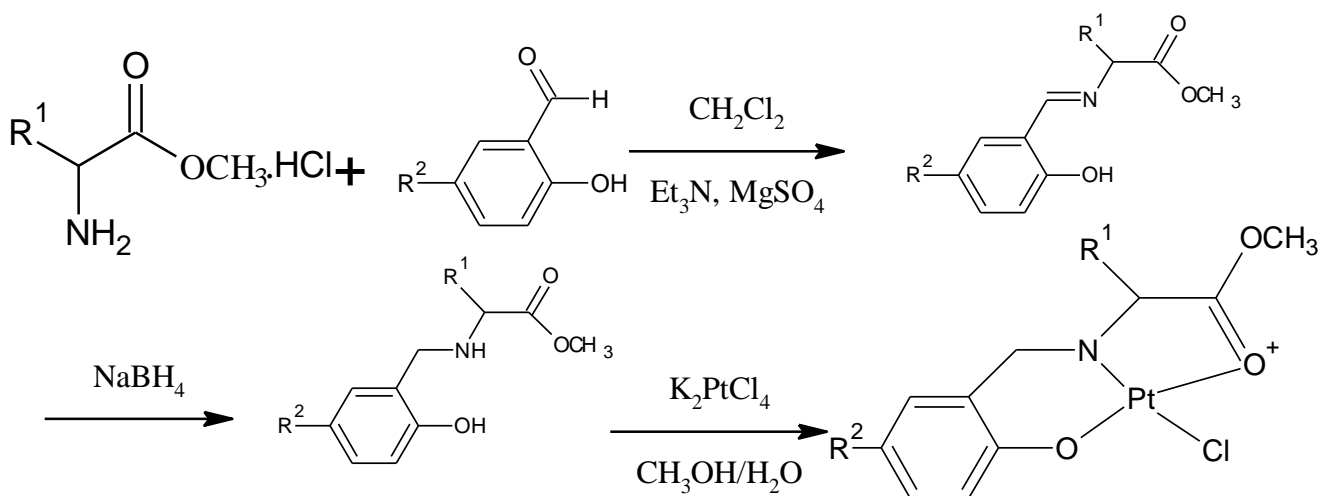
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### Scheme 1.2: Synthesis of the Schiff Base of Cinnamaldehyde with Amino Acids

Li and co-workers (2014) had synthesized the Schiff bases from amino acid ester and aldehyde in the presence of anhydrous magnesium sulphate and triethylamine at room temperature. The product was treated with sodium borohydride. The yield of the products varies [46].

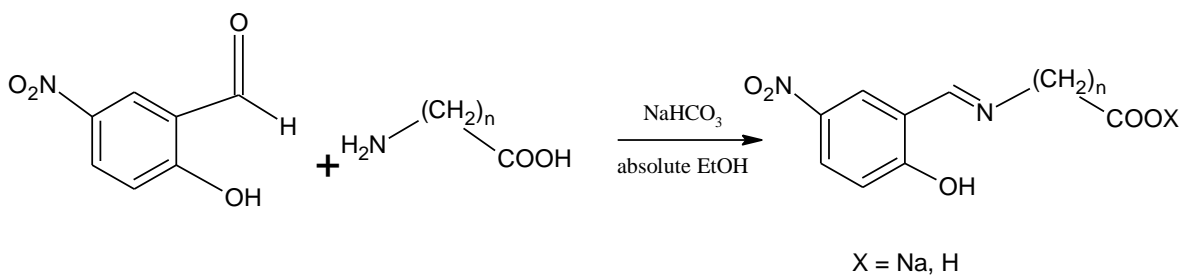
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- a:  $R^1 = i\text{Bu}$   $R^2 = \text{H}$
- b:  $R^1 = \text{Me}$   $R^2 = \text{H}$
- c:  $R^1 = \text{CH}_2\text{OH}$   $R^2 = \text{H}$
- d:  $R^1 = (1H\text{-indole-3-yl})\text{methanide}$   $R^2 = \text{H}$
- e:  $R^1 = \text{Bn}$   $R^2 = \text{Br}$
- f:  $R^1 = i\text{Bu}$   $R^2 = \text{Cl}$

**Scheme 1.3: Synthetic Route for the Synthesis of the Compounds**

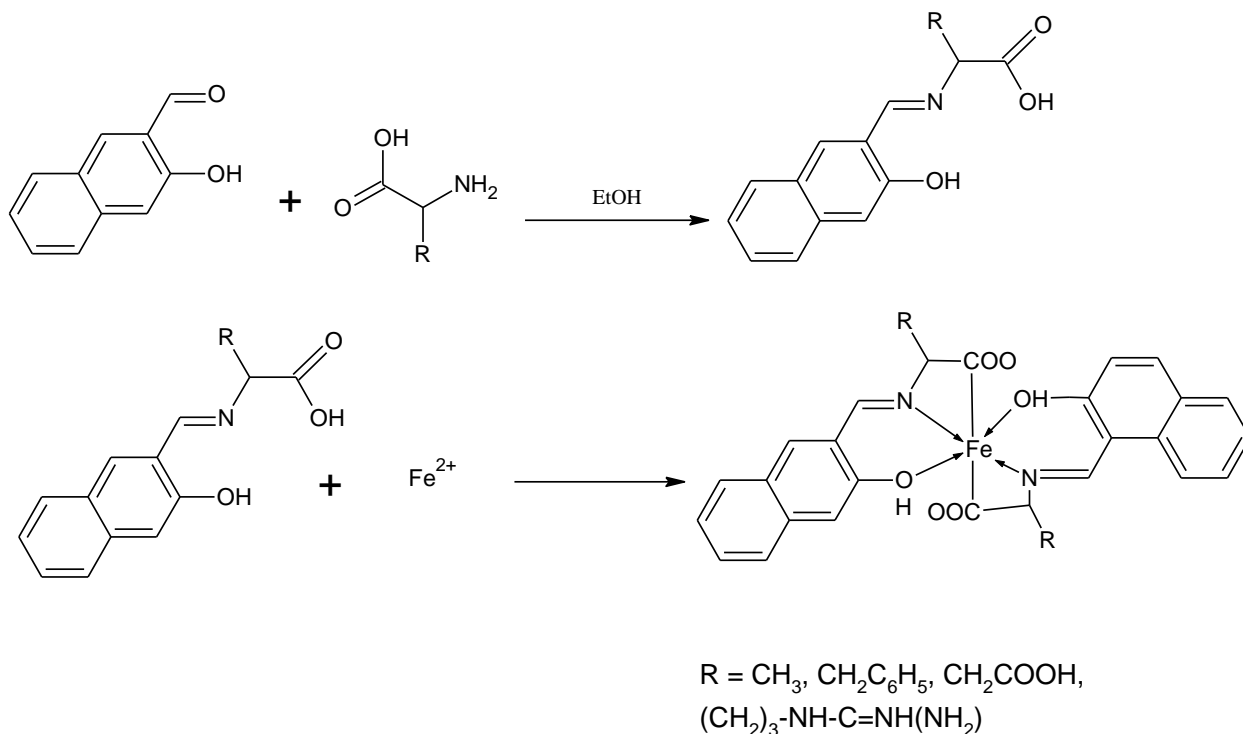
Nitro substituted compounds has been considered as effective compounds for anti-bacterial activity. In **2014**, Gungor and co-workers synthesized Schiff bases using 5-nitrosalicylaldehyde and higher amino acids both as monosodium salt and neutral form. The pH of solution was adjusted at 5.0-6.0 [47].



**Scheme 1.4: Synthesis of Schiff Base Ligands from Higher Amino Acids**

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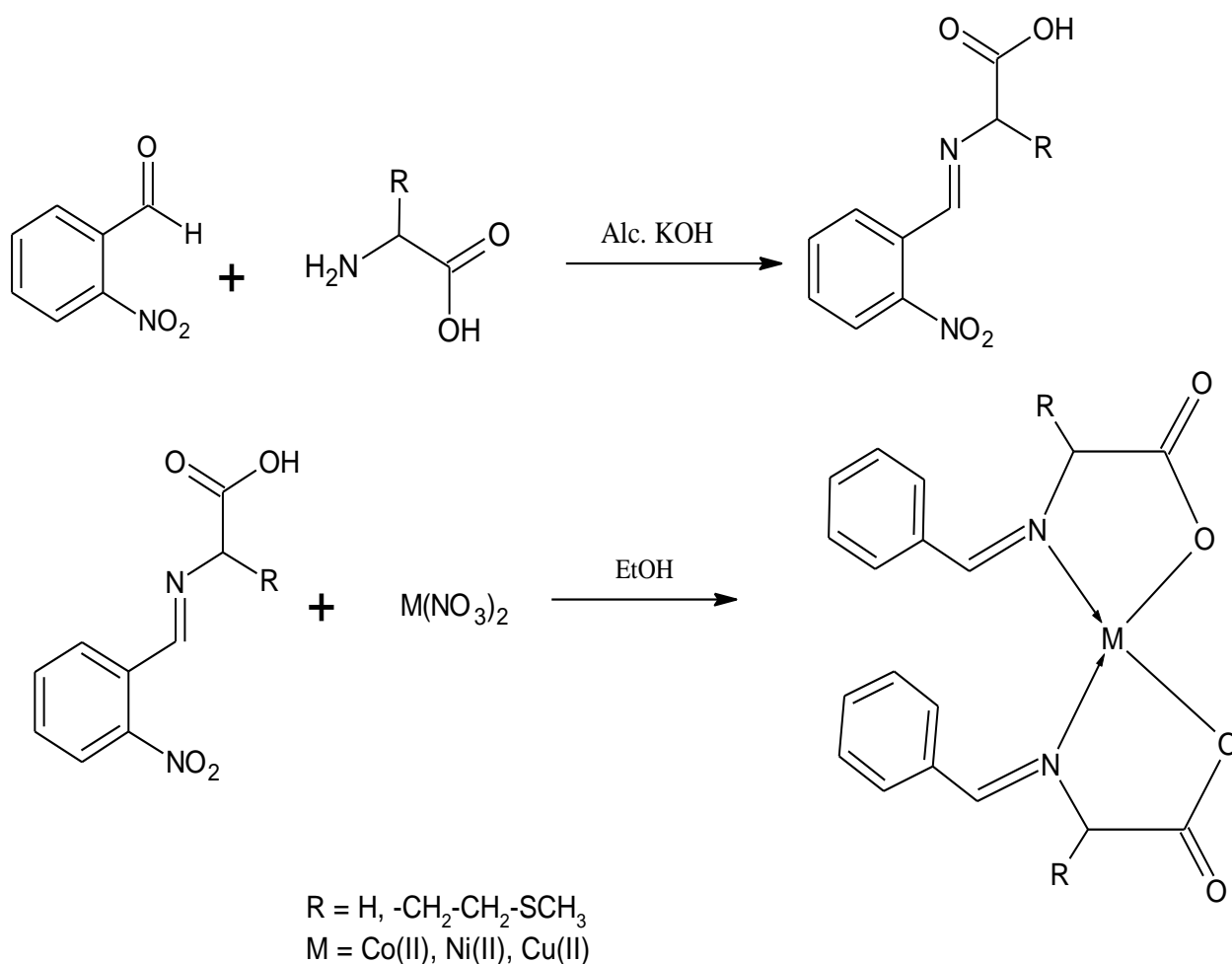
Laila H. and coworkers (2013) had synthesized Schiff bases by reacting derivative of hydroxyl-naphthaldehyde with different amino acids by simple reflux. The products were obtained in good yield. The ligands were further used for the synthesis of iron complexes. This synthesis was carried out in the acidic media [48].



### Scheme 1.5: Formation of the Schiff Base Ligands and their Complexes.

Singh *et al.* (2012) synthesized Schiff bases using amino acids and aldehyde with nitro substituent by refluxing the mixture for 3 hours. The yield obtained through this method was 89-90 %. The Co(II), Cu(II) and Ni(II) complexes were then prepared from the ligands by stirring the mixtures and appropriate yield was obtained after drying [49].

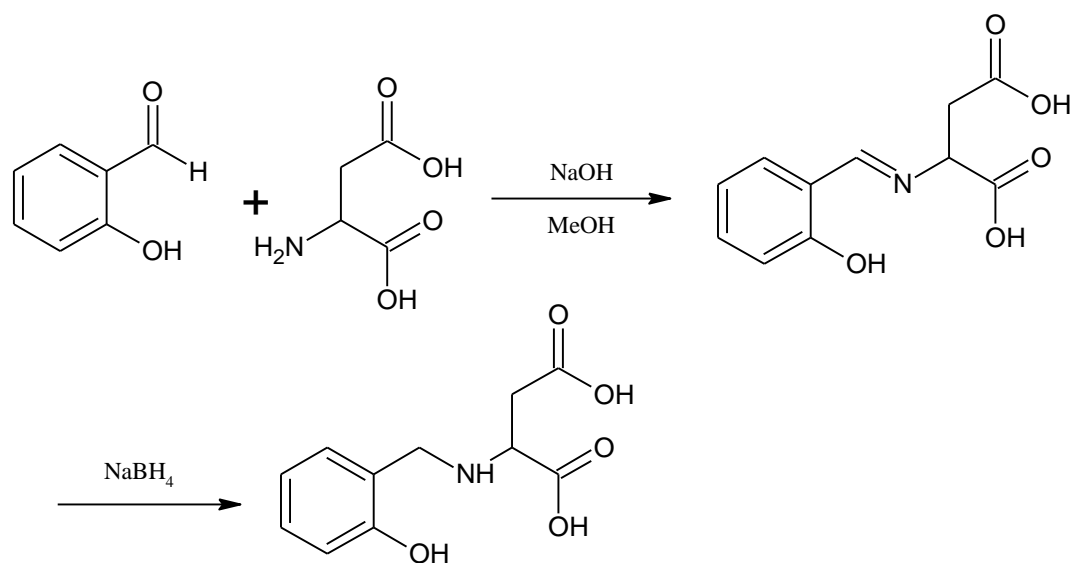
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**Scheme 1.6: Synthesis of Schiff Bases and their Metal Complexes**

Jia and co-workers (2009) synthesized the Schiff base ligands by reacting the L-aspartic acid with aldehyde. The solutions were prepared in methanol. The reaction was carried out in the basic media. In this reaction the ligands were reduced using  $\text{NaBH}_4$ . The product was obtained in good yield. The synthesized ligands were then used to prepare its Zn(II) and Cu(II) polymeric complexes [50].

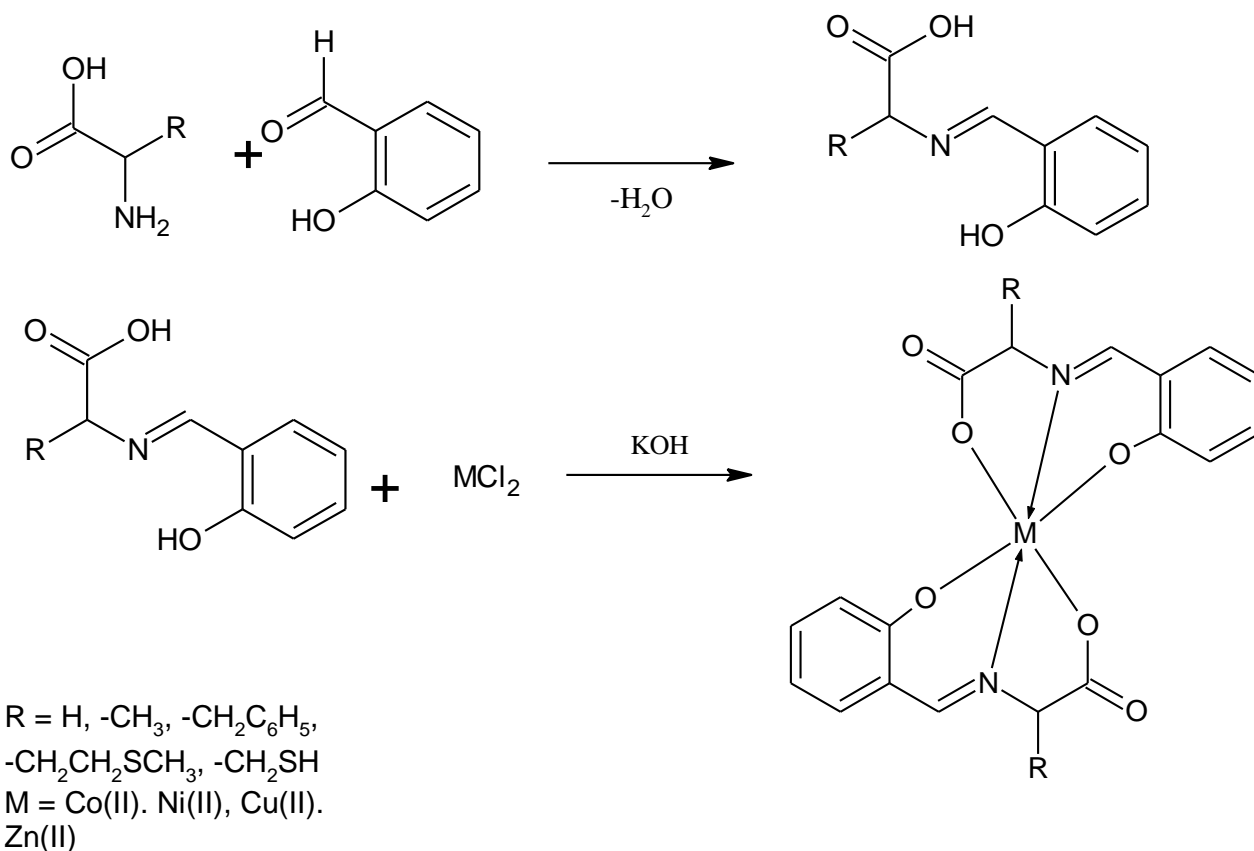
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**Scheme 1.7: Synthesis of Reduced Schiff Base Ligand from L-Aspartic acid.**

Z.H. Chohan and coworkers (2007) synthesized Schiff base by reacting salicylaldehyde with different amino acids. The synthesized Schiff bases were further used for complexation reaction where tridentate ligand coordinate to the divalent metal ions [51].

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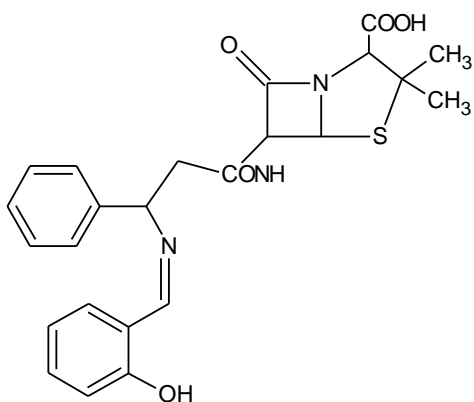
**Scheme 1.8: Preparation of Schiff base Ligands from Amino Acids**

### 1.3.2 From Drugs

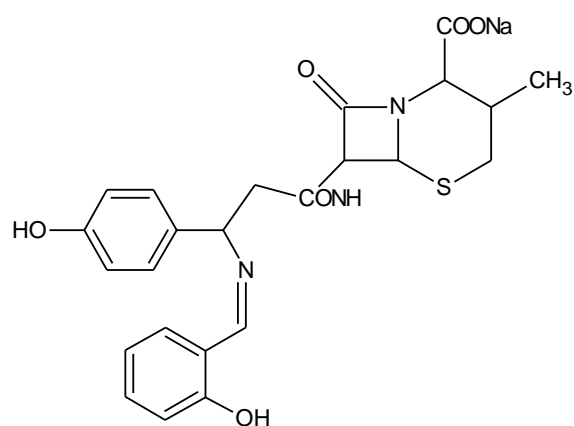
In **2014**, K.P. Srivastava *et al.* have synthesized the unsymmetrical Schiff bases by the reaction of salicylaldehyde with various drugs namely amoxicillin, cephalexin, sulphamethoxazole and trimethoprim. The reaction was performed under microwave irradiation in the presence of basic catalyst. The synthesized ligands were further used for the complexation reaction [52].



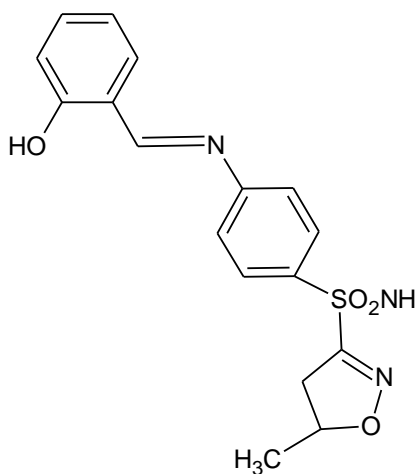
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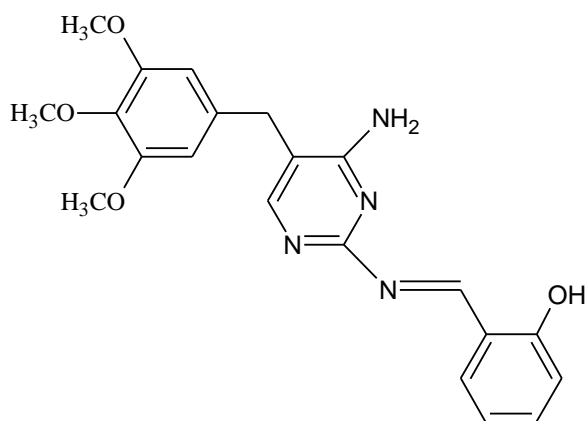
Salicylideneamoxicillin



Salicylidenecephalexin



Salicylidenesulphamethoxazole

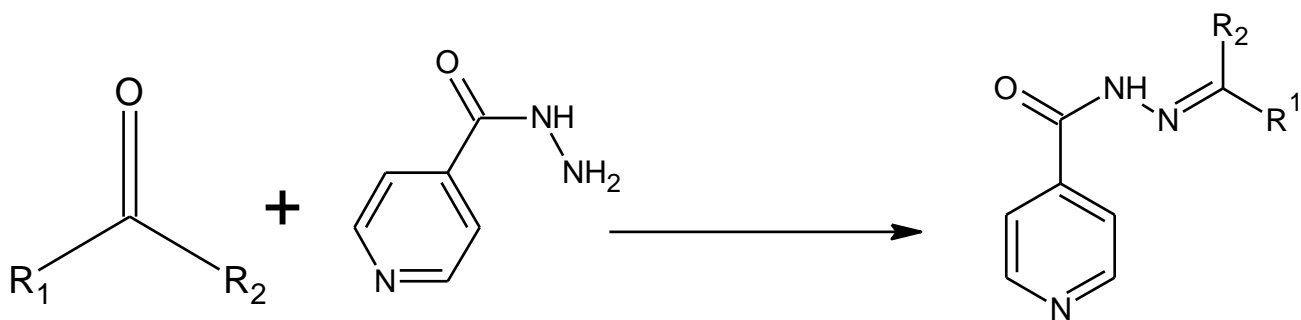


Salicylidenetrimethoprim

### Scheme 1.9: Structural Formulae of the Ligands

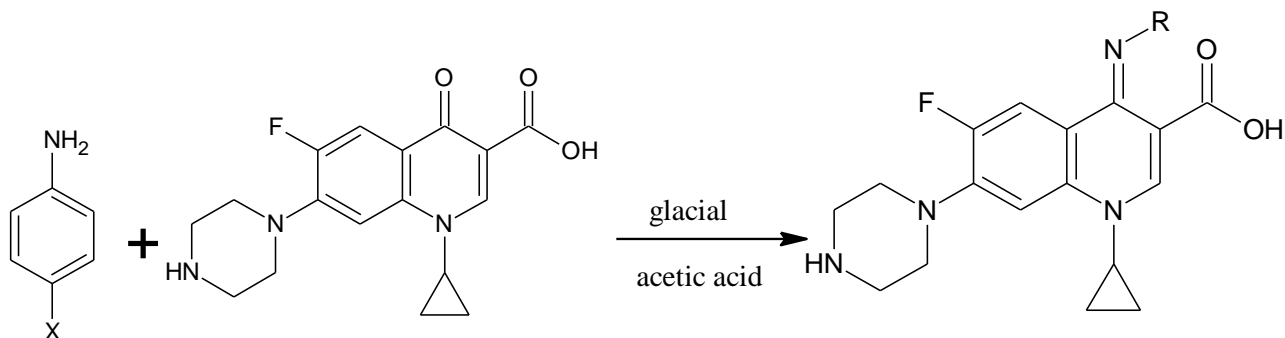
Hearn *et al.* (2009) has synthesized the Schiff bases by the reaction of the drug Isoniazid with different aldehyde and ketones. The method used was the simple reflux method. The yield obtained with aldehyde was more than the yield obtained with ketones [53].

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### Scheme 1.10: Preparation of Schiff Bases from Carbonyl Precursor

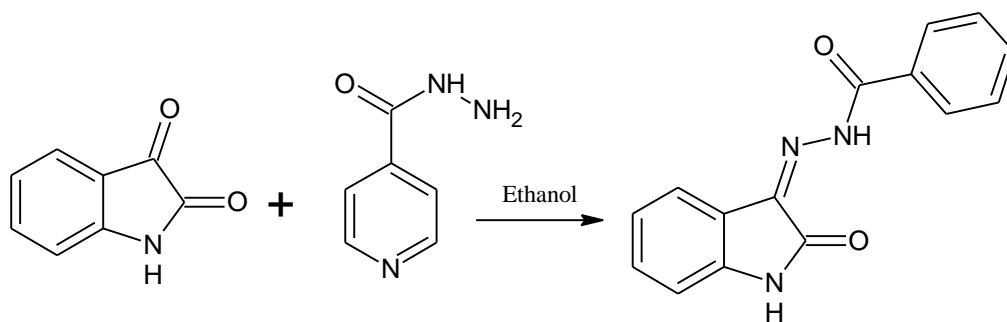
Schiff bases were synthesized from the drug ciprofloxacin and *p*-substituted aniline by Imran *et al.* in **2007**. The reaction was done in the presence of acidic media [54].



### Scheme 1.11: Synthesis of Schiff Bases from Ciprofloxacin

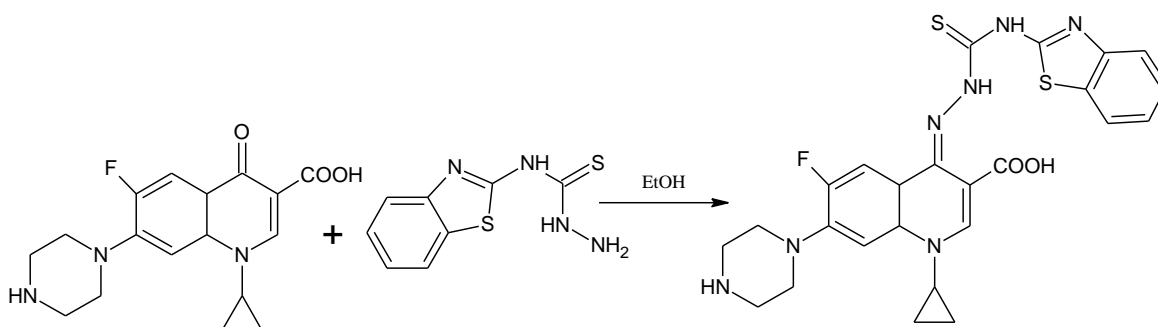
Aboul-Fadl *et al.* (**2003**) has prepared the Schiff bases by the reaction of the isoniazid drug with isatin. The catalyst used during this reaction was acid catalyst the product was obtained in good yield [55].

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**Scheme 1.12: Synthesis of Schiff Bases from Isatin**

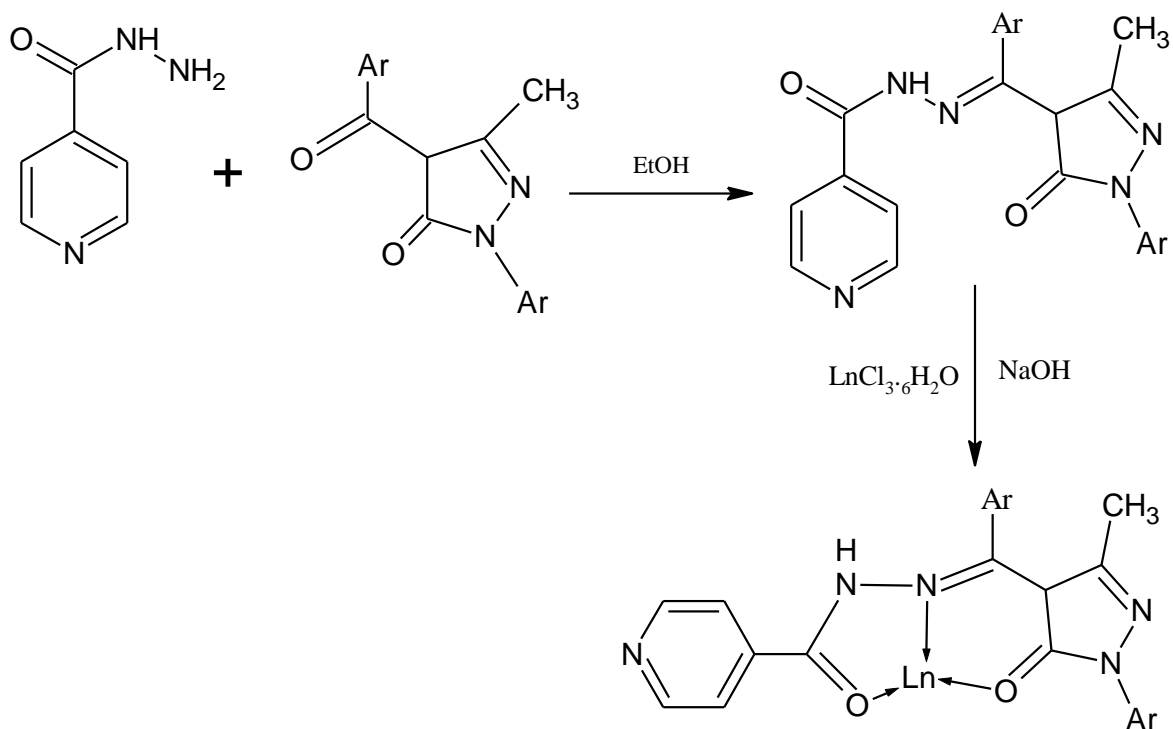
Rosy and co-workers (2015) have synthesized the Schiff base ligands by the chemical reaction of the ethanolic solution of the drug levofloxacin with hydrazine carbothioamide. The reaction was carried out in the acidic media [56].



**Scheme 1.13: Synthesis of Schiff Base from Levofloxacin**

In 2000, Yang and coworkers have synthesized the Schiff base by the chemical reaction of the drug Isoniazid and ketone (PMBP). The reaction was carried out by the reflux method. The yield obtained through this method was good. The rare earth metal complexes were synthesized from the ligands in the presence of NaOH to adjust the pH [57].

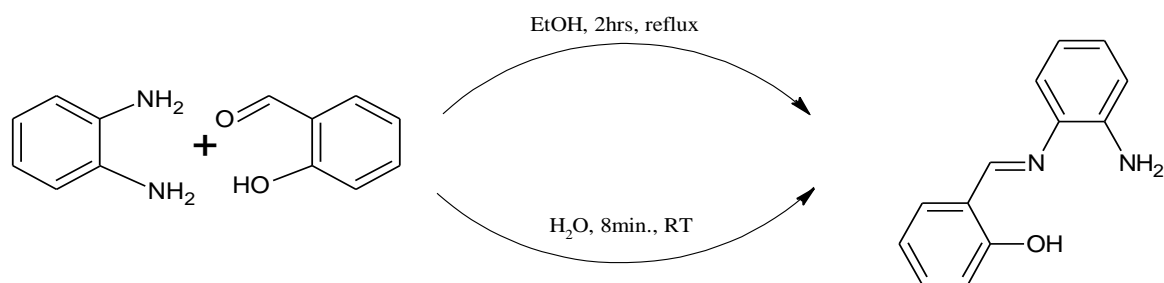
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**Scheme 1.14: Synthesis of Schiff Base Ligand from PMBP**

### 1.3.3 From Diamines

Rao and co-workers (2010) have synthesized the Schiff bases by treating the diaminobenzene with aldehydes using  $\text{NaOH}$ . The reaction has been done through green route method as well as standard method. The yields were compared [58].

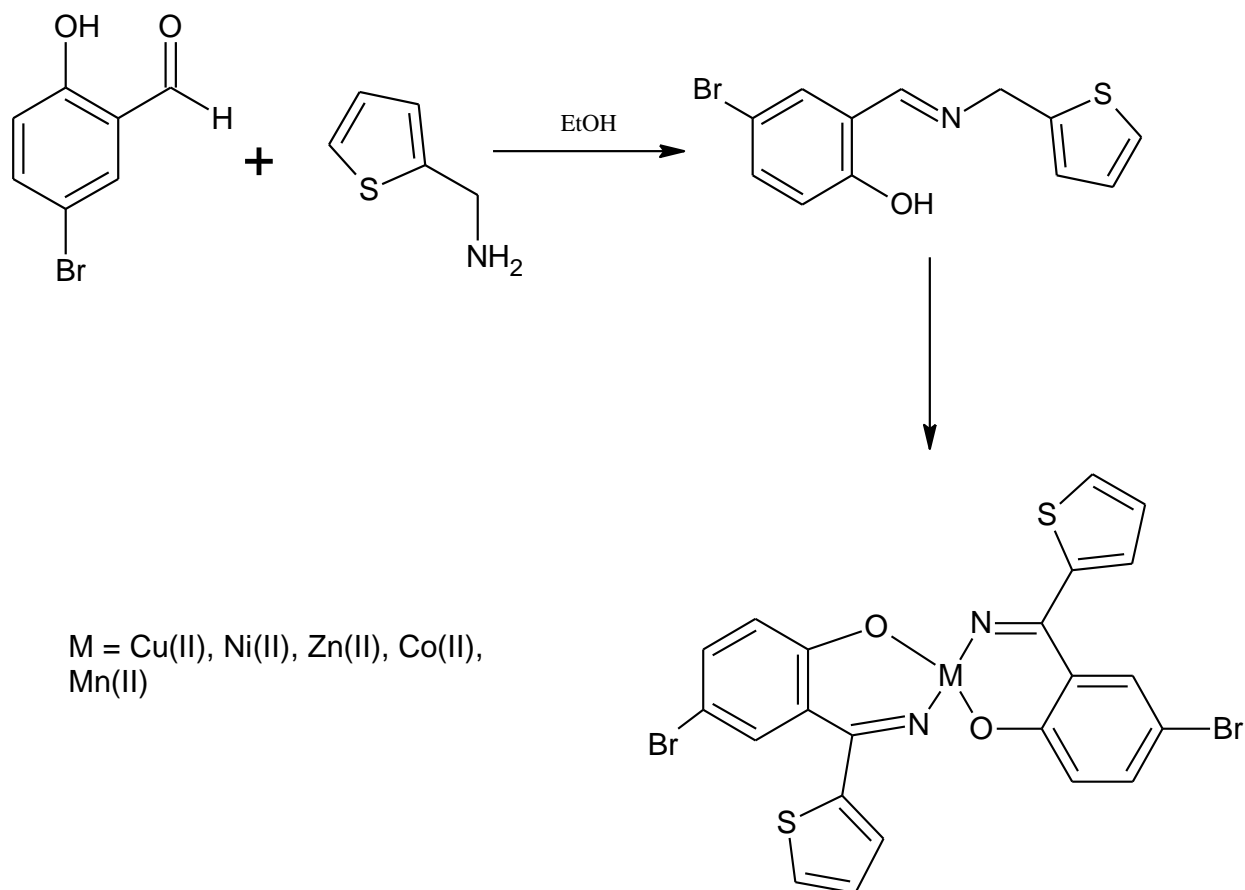


**Scheme 1.15: Preparation of 2-((Z)-(2-aminophenylimino)methyl) phenol**

### 1.3.4 Miscellaneous Reactions

## Chapter 1

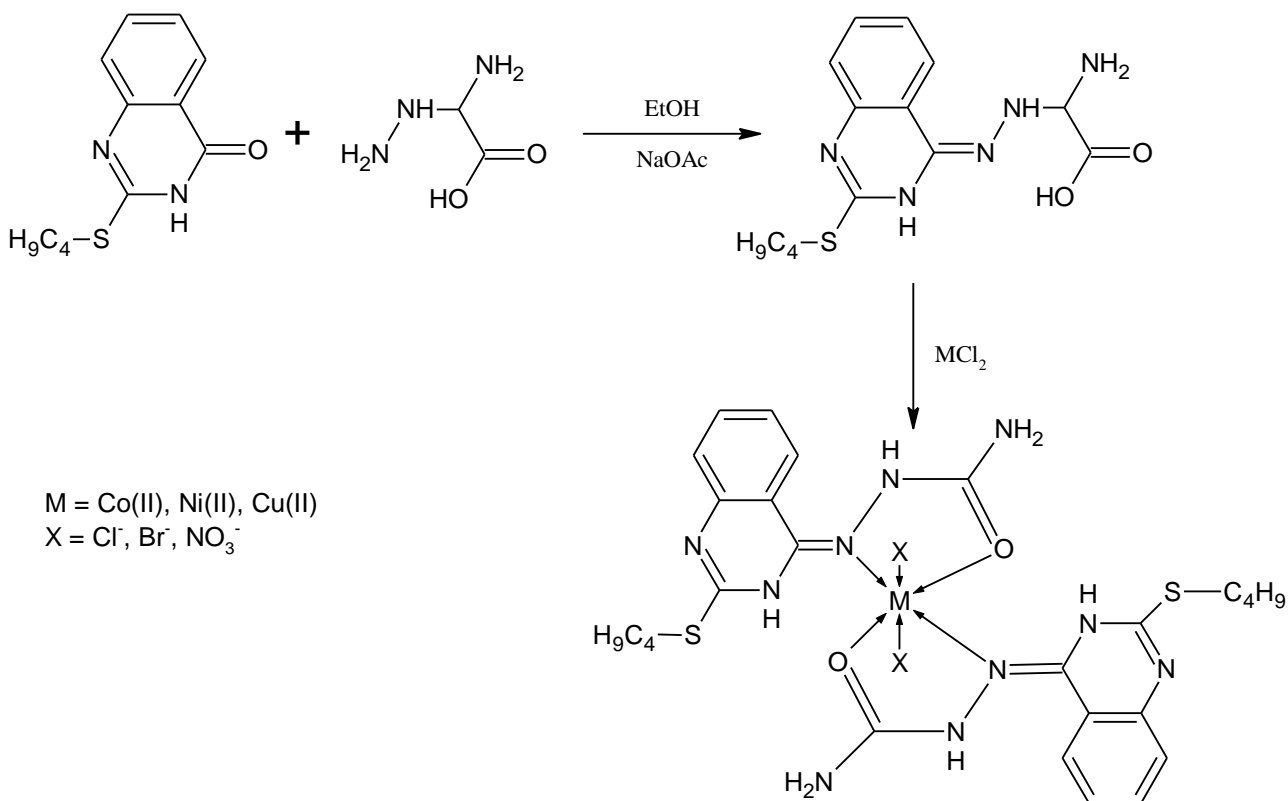
El-Sherif *et al.* (2011) have synthesized the Schiff base ligands by refluxing the bromo substituted aldehyde with amino thiophene. The product was obtained in good yield and dried at room temperature [59].



**Scheme 1.16: Synthesis of Schiff Base Ligand and its M (II) Complexes**

Rai and co-workers (2014) synthesized the Schiff bases by stirring the quinazoline with semicarbazides using sodium acetate as catalyst. The optimum yield was obtained through this reaction. Metal (II) halide salts were used in (1:2) to synthesize the metal complexes of ligands [60].

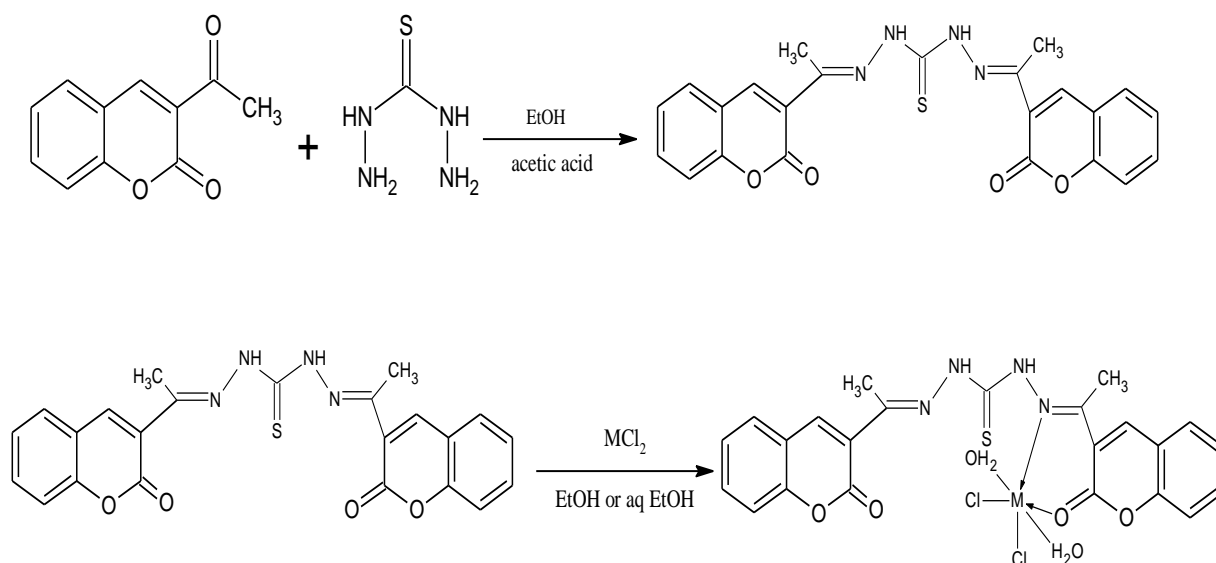
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### Scheme 1.17: Synthetic Route of Schiff Base from Semicarbazides and its M (II) Complexes

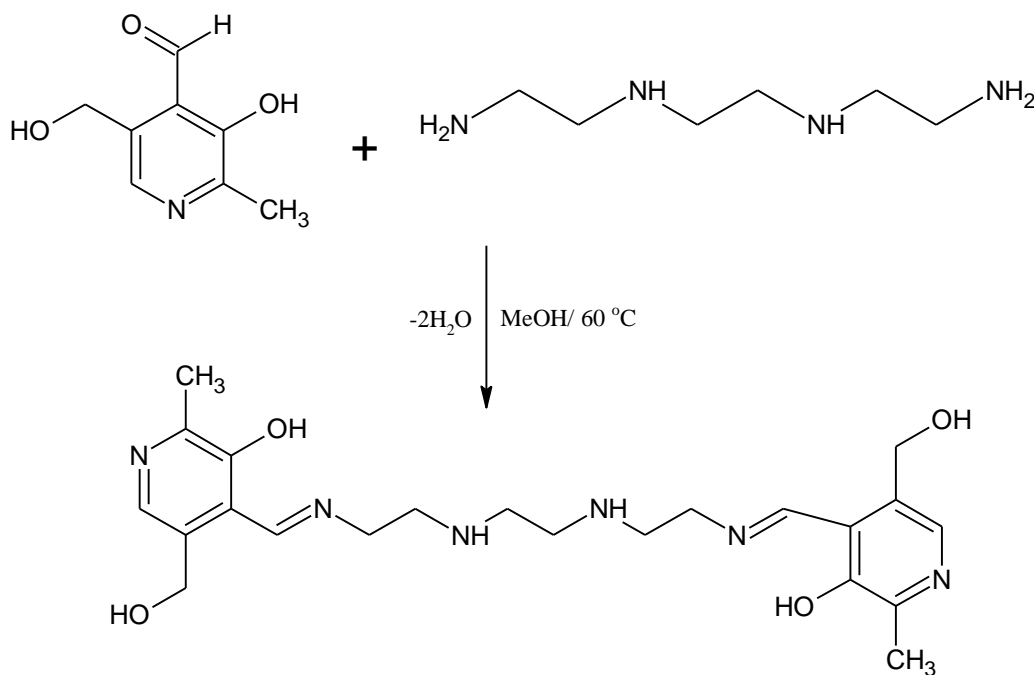
Sathisha and co-workers (2008) have synthesized the ligands by refluxing the carbohydrazide with coumarin by using the catalyst acetic acid. The good yield was obtained through this method and was purified by recrystallization [61].

## Chapter 1



**Scheme 1.18: Synthesis of the Schiff Bases and Complexes**

Back and co-workers (2015) have synthesized the Schiff base ligands by treating the pyridoxal with triethylenetetramine. An atmosphere was provided. The complexes were synthesized using template method [62].



**Scheme 1.19: Preparation of Schiff Base Ligand**

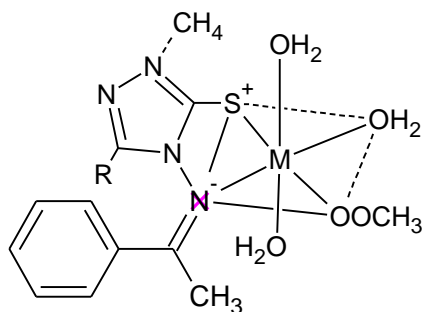
## Chapter 1

### 1.4 BIOLOGICAL ACTIVITIES

#### 1.4.1 Anti-bacterial Activities

The infectious diseases are caused due to bacteria and that are resistant to antibiotics. Due to these infectious diseases the mortality rate increases. The exploration and improvement of more effective antibacterial drugs are required and some of the Schiff bases are known to be new antibacterial drugs [63].

K. Singh *et al.* (2006) have synthesized 4-acetylpyridenylideneamino-5-mercapto-3-methyl-s-triazole (ApMMT) and 4-acetylpyridenylideneamino-3-ethyl-5-mercapto-s-triazole (ApEMT) and also synthesized their Ni, Zn, Co, Cu complexes and checked their activity against *B. subtilis*, *E. coli*, *S. dysenteriae* and *Pseudomonas* sp. The ApEMT-Co (1:1) and ApEMT-Co (1:2) showed highest activity against all types of bacteria. The compounds ApMMT, ApEMT, ApMMT-Ni (1:1) and ApMMT-Ni (1:2) showed inhibitory action against *B. subtilis* and *E. coli*. ApMMT-Zn (1:2) showed anti-bacterial activity against *Pseudomonas* sp. and *E. coli*. The copper complexes inhibited no activity against all types of bacteria [64].



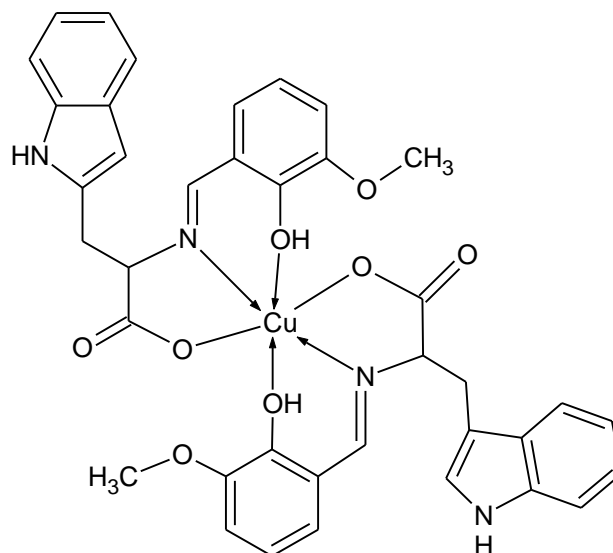
**Figure1.2: Structure of Metal Complexes of ApMMT and ApEMT**

L.H. Abdel-Rahman *et al.* (2016) have synthesized the Schiff base ligands MST (3-methoxysalicylaldehyde tryptophan), DSP (4-diethylaminosalicylaldehyde phenylalanine), DSH (4-diethylaminosalicylaldehyde histidine) and their Cu complexes. Gram positive and Gram negative bacteria were used for the anti-bacterial activity. It was observed that anti-bacterial activity of ligands had been enhanced when they coordinated to Cu(II) ion.



## Chapter 1

Having the structural difference between gram positive and gram negative bacteria, the compounds inhibited more strongly the gram positive bacteria [65].



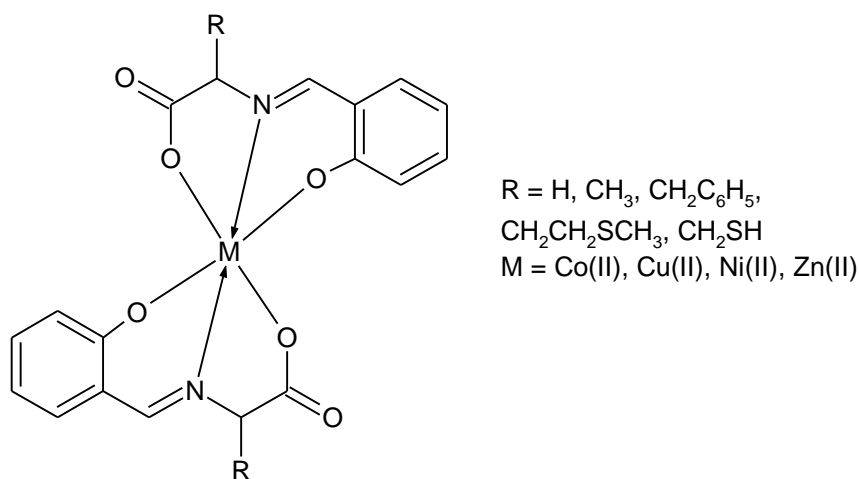
**Figure1.3: Structure of Cu Complex of MST**

### 1.4.2 Anti-fungal Activities

The infections caused due to fungi are not only related to tissues but also such fungal infections has been reported which are very dangerous for life [66-67]. The main reason for this is that the patients who are in the advanced age, major surgery, immunosuppressive therapy, AIDS, cancer treatment are increasing and are at high risk [68-69]. So, there is the need of more effective anti-fungal drugs [70] and some of the Schiff bases are used as anti-fungal agents.

Z.H. Chohan *et al.* (2007) using salicylaldehyde and various amino acids synthesized the ligands. Then their Co(II), Cu(II), Ni(II) and Zn(II) complexes were synthesized. The anti-fungal activities of the ligands as well as metal complexes were checked against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glaberata*. All the synthesized ligands and complexes were inactive against all the species. Only compound 14, 16 and 20 show anti-fungal activity against the species under determination [71].

## Chapter 1



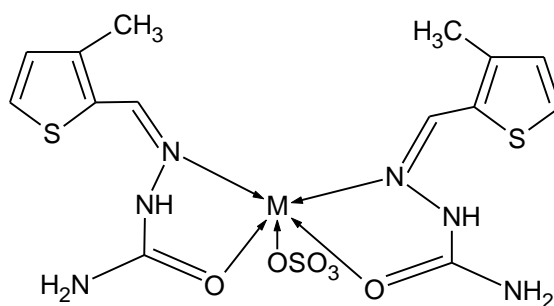
**Figure 1.4: Proposed Structure of Metal (II) Complex**

M.A. Ashraf *et al.* (2011) have synthesized the Schiff base of 2-amino-benzothiazole, 4-amino-salicylic acid and 4-aminophenol and checked their anti-fungal activity against *Aspergillus niger* and *Chalara corda*. It was observed that benzothiazole Schiff bases showed more inhibitory action towards fungi species as compared to other ligands. The Schiff base of salicylic acid showed good activity as compared to standard drug [72].

O. Ozdemir *et al.* (2016) have synthesized the amino acid Schiff base derivatives of 6-aminopenicillanic acid and 7-aminocephalosporanic acid and checked their antifungal activity against *C. albican* and *Candida kрузei*. All the synthesized compounds showed inhibitory action against fungal strains. The results indicated that monosodium Schiff bases expressed less activity as compared to the neutral Schiff bases and 6-APA and 7-ACA showed more activity than Schiff bases [73].

S. Chandra *et al.* (2015) have synthesized the hydrazine carboxamide, 2-[3-methyl-2-thienyl methylene] and its Ni and Cu complexes. All the complexes were screened against fungal strains *i.e.* *Aspergillus niger* and *Aspergillus flavus*. Among all the compounds the [Ni(L)<sub>2</sub>SO<sub>4</sub>] showed maximum inhibitory action [74].

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**Figure 1.5: Proposed Structure of Metal Complex of Hydrazine carboxamide, 2-[3-methyl-2-thienyl methylene]**

### 1.4.3 Anti-tumor Activities

Tremendous attempts have been applied for the development of chemical compounds to control the cancer [75]. It is the most thoroughly studied topic in the coordination chemistry that transition metal complexes of Schiff base ligands are biologically more active as compared to non-Schiff base complexes [76].

A.A. El-Sherif, T.M.A. Eldebss (2011) have synthesized 4-bromo-2-(thiophen-2-yl-imino)methylphenol (ATS) and its metal complexes. Cytotoxic studies of the compounds have been monitored against colon carcinoma (HCT116) and larynx carcinoma (HEP2). The results showed that ligands are not active against cell lines. The maximum activity has been shown by the complexes of Cu and Zn. The complexes show activity in the following order *i.e.* Ni < Zn < Cu [77].

H.-Q. Chang *et al.* (2016) had synthesized the  $[\text{Ni}_2(\text{L}^1)_2(\text{tren})_2(\text{H}_2\text{O})](\text{ClO}_4)_3$  (1),  $[\text{NiL}^2(\text{tren})_2](\text{ClO}_4)_2 \cdot 2.5\text{H}_2\text{O}$  (2) and  $[\text{NiL}^2(\text{tren})_2]\text{I} \cdot 1.5\text{H}_2\text{O} \cdot \text{CH}_3\text{OH}$  (3). The synthesized complexes have been checked against human hepatic cancer (HepG2), human cervical cancer (HeLa) and human prostate (PC3). All the synthesized compounds exhibited cytotoxic activity but the complex showed higher activity as compared to others because of its dinuclear structure [78].

### Work Plan

- To synthesize Schiff base ligands from Isoniazid and different aldehydes.

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- To protect the amino group using Boc and benzoyl protecting group.
- To synthesize Ni(II) Complexes from Schiff base ligands and NiCl<sub>2</sub>.6H<sub>2</sub>O.
- To characterize the synthesized compound by Melting Point, FT-IR, UV-vis and GC-MS.
- To check and compare the anti-tumor activity of the ligands as well as complexes.

**2.1 EXPERIMENTAL****2.1.1 Chemicals**

The reagents used were benzaldehyde, salicylaldehyde, *p*-methoxybenzaldehyde, thiophene carbaldehyde, furaldehyde, 2-chlorobenzaldehyde, 4-chlorobenzaldehyde, *p*-amino benzaldehyde (99.9 %), glacial acetic acid, and metal salt  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  and active compound Isoniazid drug (99.9 %).

**2.1.2 Solvents**

All the solvents ethanol, methanol and chloroform were of analytical grade and used without further purification.

**2.1.3 Instrumentation**

To weigh different chemicals electronic analytical balance ATY224 was used. The progress of the reaction was monitored through TLC which was analyzed under UV- lamp. The solvents were evaporated using Rotary evaporator R-210. The melting points were recorded in open capillary in melting point apparatus SMP10 which were incorrect. The functional groups of the compounds were identified through FT-IR equipped with ATR model ALPHA 200488. UV 2800 spectrophotometer was used to record the UV-vis spectra of complexes in the range of 800-200 nm. The GC-MS of the products were recorded with the help of PerkinElmer Clarus600 GC coupled with 600c Ms Quadpole EI (electron impact). Other details of the GC-MS are:

Column: Elite-5

Column Inner Diameter: 0.25 mm

Carrier gas: Helium

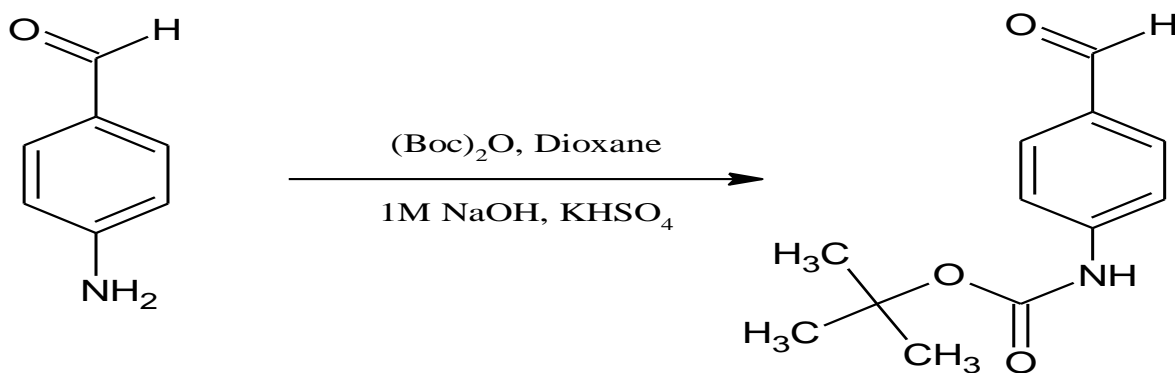
Column Flow: 1mL/min

## Chapter 2

To check the purity of the compounds GC was performed. After confirmation of purity, mass spectrometry of the compounds was carried out.

### 2.1.4 General Procedure for *N*-Boc Protection of Amino Group of Aldehyde

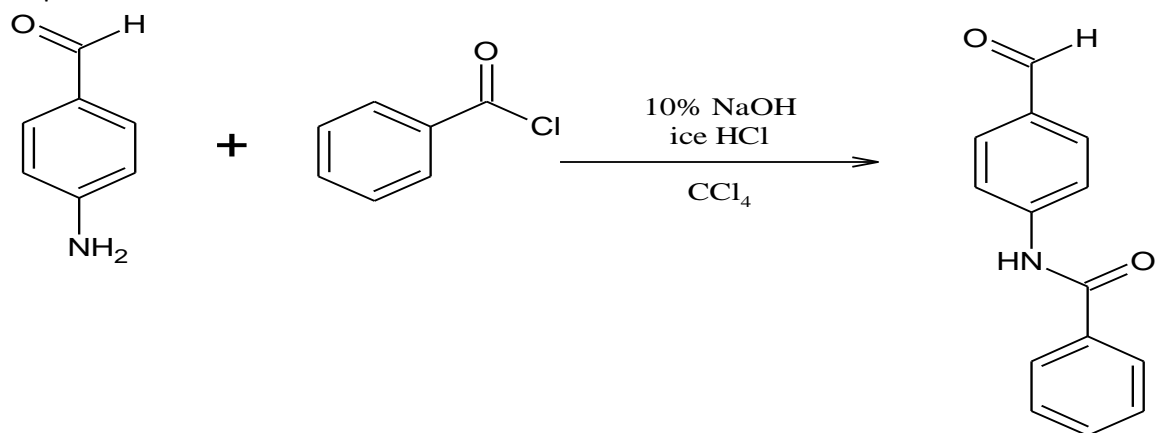
The aldehyde (13 mmol, 0.5 g) was dissolved in mixture of dioxane: water (8.28: 4.19 mL). Then 4.19 mL of 1M NaOH solution was added. The mixture was stirred for half an hour in ice bath. To this solution (Boc)<sub>2</sub>O (4.54 mmol, 0.9 g) was added and again stirred for one hour at room temperature. The mixture was concentrated through rotary evaporator. The pH of the solution was maintained 2-3 by adding dilute solution of KHSO<sub>4</sub>. The extraction was carried out with ethyl acetate, washed with water, brine and dried over MgSO<sub>4</sub>. FT-IR: ( $\nu_{\max}$ , cm<sup>-1</sup>) 1656 (C=O), 1587 (C=C), 1368 (C(CH<sub>3</sub>)<sub>3</sub>).



**Scheme 2.1: Boc Protection of *p*-Amino Benzaldehyde**

### 2.1.5 General Procedure for *N*-Benzoyl Protection of Amino Group of Aldehyde

Dissolve aldehyde (13 mmol, 0.5 g) in 10 % NaOH (2 mL) and stirred the mixture for half an hour then added benzoyl chloride (0.68 mL) in three portions. Again stirred for half an hour and added ice to reaction mixture. To maintain pH at 3, concentrated HCl was added slowly. The resulting crude product was washed with CCl<sub>4</sub> (4 mL), filtered and dried. FT-IR: ( $\nu_{\max}$ , cm<sup>-1</sup>) 1676 (C=O), 1452, 1417 (C=C).

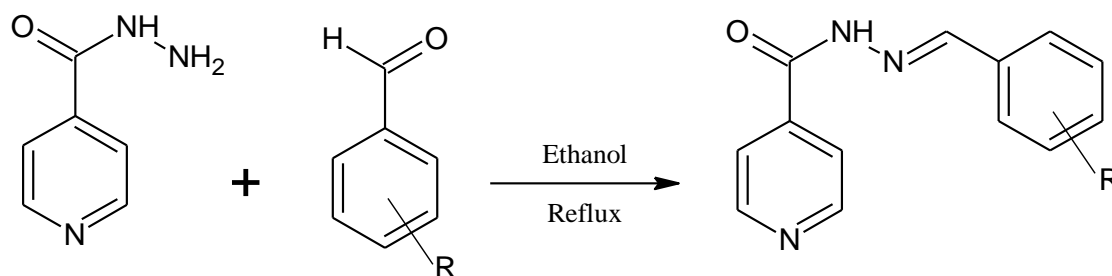


**Scheme 2.2: N-Benzoyl Protection of *p*-Amino Benzaldehyde**

### 2.1.6 General Procedure for the Synthesis of Schiff Base Ligands (GP1)

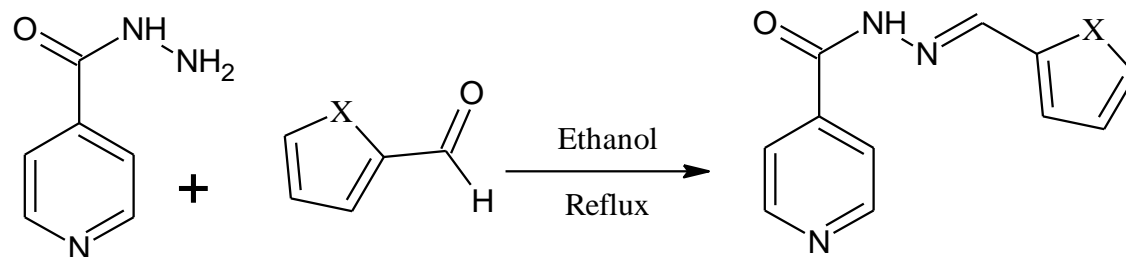
The reaction was carried out by reported procedure [53]. The isoniazid (INH) (3.6 mmol, 0.5 g) was dissolved in 10 mL of ethanol by heating the mixture upto boiling. The aldehyde (3.6 mmol, 0.4 mL) was added to the mixture drop wise. Then 3 mL of ethanol was again added. The mixture was refluxed for 3 hours. The excess solvent was removed by rotary evaporator. The mixture was left over night. The precipitates were filtered.

The general scheme for the synthesis of Schiff bases is as follows



R= H, 2-OH, 4-OCH<sub>3</sub>, 2-Cl, 4-Cl, 4-NHBz, 4-NHBoc

**Scheme 2.3: Synthesis of Schiff Base Ligand from Aromatic Aldehyde**

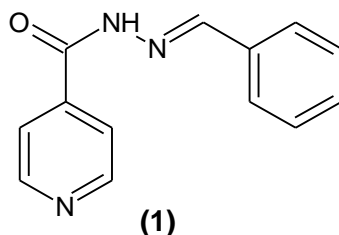


X= O, S

### Scheme 2.4: Synthesis of Schiff Base Ligand from 2-Substituted Aldehyde

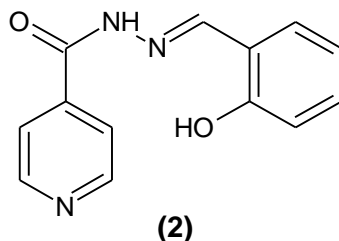
#### *N'*-[(*E*)-phenylmethylidene]pyridine-4-carbohydrazide (1)

Cream colour powder, Yield: 72 %, Reaction time: 1:30 hours, m.p. 196-198 °C, FT-IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 1683 (C=O amide), 1575 (C=N), 1563(C=C aromatic), 1549(NNH). MS m/z (%): 225 ( $\text{M}^+$ , 52%), 148 ( $\text{M}^+$ -C<sub>6</sub>H<sub>5</sub>, 60%), 135 ( $\text{M}^+$ -C<sub>7</sub>H<sub>6</sub>, 25%), 121 ( $\text{M}^+$ -C<sub>7</sub>H<sub>6</sub>N, 85%), 106 ( $\text{M}^+$ -C<sub>7</sub>H<sub>7</sub>N<sub>2</sub>, 47%), 78 ( $\text{M}^+$ -C<sub>8</sub>H<sub>7</sub>N<sub>2</sub>O, 63%).



#### *N'*-[(*E*)-(2-hydroxyphenyl)methylidene]pyridine-4-carbohydrazide (2)

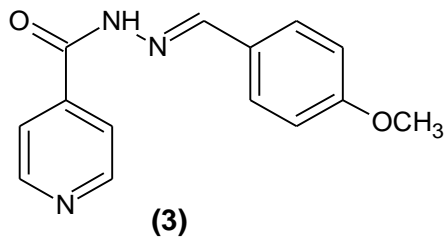
White powder, Yield: 84 %, Reaction time: 1:30 hours, m.p. 260-262 °C, FT-IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 1681 (C=O amide), 1612 (C=N) 1565 (NNH), 1489 (C=C, aromatic). MS m/z (%): 241( $\text{M}^+$ , 55%), 163 ( $\text{M}^+$ -C<sub>5</sub>H<sub>4</sub>N, 60%), 148 ( $\text{M}^+$ -C<sub>6</sub>H<sub>5</sub>O, 34%), 135 ( $\text{M}^+$ -C<sub>6</sub>H<sub>4</sub>NO, 46%), 106 ( $\text{M}^+$ -C<sub>7</sub>H<sub>7</sub>N<sub>2</sub>O, 92%), 93 ( $\text{M}^+$ -C<sub>7</sub>H<sub>7</sub>N<sub>2</sub>O, 21%), 78 ( $\text{M}^+$ -C<sub>8</sub>H<sub>7</sub>N<sub>2</sub>O<sub>2</sub>, 11%).



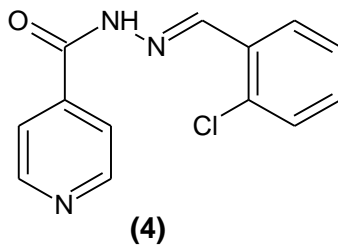


***N'*-[(*E*)-(4-methoxyphenyl)methylidene]pyridine-4-carbohydrazide (3)**

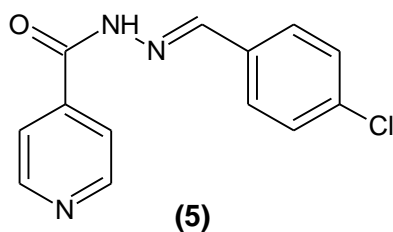
White solid, Yield: 92 %, Reaction time: 1:30 hours, m.p. 134-137 °C, FT-IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 1654 (C=O amide), 1596 (C=N), 1513 (C=C, aromatic), 1525 (NNH). MS  $m/z$  (%): 255 ( $\text{M}^+$ , 49%), 177 ( $\text{M}^+$ - $\text{C}_5\text{H}_4\text{N}$ , 28%), 150 ( $\text{M}^+$ - $\text{C}_6\text{H}_4\text{NO}$ , 37%), 148 ( $\text{M}^+$ - $\text{C}_7\text{H}_7\text{O}$ , 52%), 108 ( $\text{M}^+$ - $\text{C}_7\text{H}_7\text{N}_3\text{O}$ , 61%), 106 ( $\text{M}^+$ - $\text{C}_8\text{H}_9\text{N}_2\text{O}$ , 86%), 78 ( $\text{M}^+$ - $\text{C}_9\text{H}_{10}\text{N}_2\text{O}_2$ , 21%).

***N'*-[(*E*)-(2-chlorophenyl)methylidene]pyridine-4-carbohydrazide (4)**

White solid, Yield: 95 %, Reaction time: 1:30 hours, m.p. 221-223 °C, FT-IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 1669 (C=O amide), 1600 (C=N), 1565 (C=C, aromatic), 1548 (NNH). MS  $m/z$  (%): 259 ( $\text{M}^+$ , 32%), 181 ( $\text{M}^+$ - $\text{C}_5\text{H}_4\text{N}$ , 28%), 154 ( $\text{M}^+$ - $\text{C}_6\text{H}_4\text{NO}$ , 49%), 148 ( $\text{M}^+$ - $\text{C}_6\text{H}_4\text{Cl}$ , 78%), 112 ( $\text{M}^+$ - $\text{C}_7\text{H}_7\text{N}_3\text{O}$ , 13%), 106 ( $\text{M}^+$ - $\text{C}_7\text{H}_6\text{N}_2\text{Cl}$ , 25%), 78 ( $\text{M}^+$ - $\text{C}_8\text{H}_6\text{N}_2\text{OCl}$ , 28%).

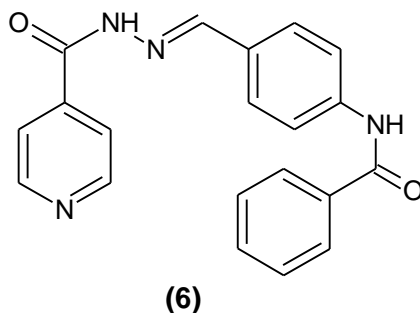
***N'*-[(*E*)-(4-chlorophenyl)methylidene]pyridine-4-carbohydrazide (5)**

White solid, Yield: 88 %, Reaction time: 1:30 hours, m.p. 224-226 °C, FT-IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 1661 (C=O, amide), 1602 (C=N), 1549 (NNH), 1489 (C=C, aromatic). MS  $m/z$  (%): 259 ( $\text{M}^+$ , 35%), 181 ( $\text{M}^+$ - $\text{C}_5\text{H}_4\text{N}$ , 15%), 154 ( $\text{M}^+$ - $\text{C}_6\text{H}_4\text{NO}$ , 56%), 148 ( $\text{M}^+$ - $\text{C}_6\text{H}_4\text{Cl}$ , 41%), 112 ( $\text{M}^+$ - $\text{C}_7\text{H}_7\text{N}_3\text{O}$ , 16%), 106 ( $\text{M}^+$ - $\text{C}_7\text{H}_6\text{N}_2\text{Cl}$ , 72%), 78 ( $\text{M}^+$ - $\text{C}_8\text{H}_6\text{N}_2\text{OCl}$ , 37%).



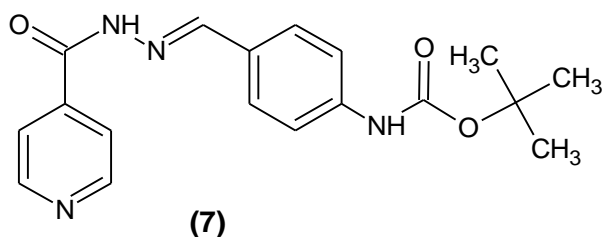
***N*-(4-{(E)-[2-(pyridin-4-ylcarbonyl)hydrazinylidene]methyl}phenyl)benzamide (6)**

Orange solid, Yield: 81 %, Reaction time: 1:30 hours, m.p. 205-207 °C, FT-IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 1661 (C=O, amide), 1602 (C=N), 1588 (C=C, aromatic), 1535 (NNH). MS m/z (%): 344 ( $\text{M}^+$ , 44%), 267 ( $\text{M}^+ - \text{C}_6\text{H}_5$ , 100%), 239 ( $\text{M}^+ - \text{C}_7\text{H}_5\text{O}$ , 32%), 148 ( $\text{M}^+ - \text{C}_{13}\text{H}_{10}\text{NO}$ , 80%), 106 ( $\text{M}^+ - \text{C}_{14}\text{H}_{12}\text{N}_3\text{O}$ , 20%), 78 ( $\text{M}^+ - \text{C}_{15}\text{H}_{12}\text{N}_3\text{O}_2$ , 41%).



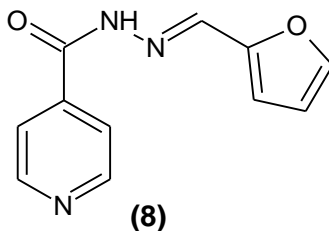
***Tert*-butyl(4-{(E)-[2-(pyridin-4-ylcarbonyl)hydrazinylidene]methyl}phenyl)carbamate (7)**

Yellow solid, Yield: 81 %, Reaction time: 1:30 hours, m.p. 192-194 °C, FT-IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 1712 (C=O, ester), 1662 (C=O, amide), 1602 (C=N), 1535 (NNH), 1547 (C=C, aromatic). MS m/z (%) 340 ( $\text{M}^+$ , 45%), 312 ( $\text{M}^+ - \text{CO}$ , 29%), 285 ( $\text{M}^+ - \text{C}_4\text{H}_7$ , 17%), 267 ( $\text{M}^+ - \text{C}_4\text{H}_9\text{O}$ , 95%), 257 ( $\text{M}^+ - \text{C}_5\text{H}_7\text{O}$ , 53%), 239 ( $\text{M}^+ - \text{C}_5\text{H}_9\text{O}_2$ , 38%), 211 ( $\text{M}^+ - \text{C}_6\text{H}_9\text{O}_3$ , 19%), 121 ( $\text{M}^+ - \text{C}_{12}\text{H}_{16}\text{NO}_3$ , 41%), 108 ( $\text{M}^+ - \text{C}_{13}\text{H}_{18}\text{NO}_3$ , 23%), 78 ( $\text{M}^+ - \text{C}_{13}\text{H}_{20}\text{N}_3\text{O}_3$ , 45%).

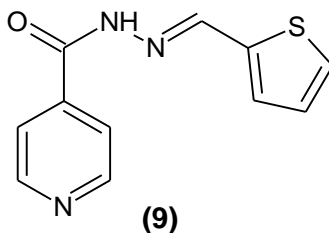


***N'*-[*E*]-furan-2-ylmethylidene]pyridine-4-carbohydrazide (8)**

Dusty white solid, Yield: 88 %, Reaction time: 1:30 hours, m.p.218-220 °C, FT-IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 1650 (C=O, amide), 1619 (C=N), 1535 (NNH), 1474 (C=C, aromatic). MS m/z (%): 215 ( $\text{M}^+$ , 46%), 148 ( $\text{M}^+$ -C<sub>4</sub>H<sub>3</sub>O, 61%), 137 ( $\text{M}^+$ -C<sub>5</sub>H<sub>4</sub>N, 44%), 109 ( $\text{M}^+$ -C<sub>6</sub>H<sub>4</sub>NO, 11%), 106 ( $\text{M}^+$ -C<sub>5</sub>H<sub>5</sub>N<sub>2</sub>O, 91%), 80 ( $\text{M}^+$ -C<sub>6</sub>H<sub>5</sub>N<sub>3</sub>O, 13%), 78 ( $\text{M}^+$ -C<sub>6</sub>H<sub>5</sub>N<sub>2</sub>O<sub>2</sub>, 39%).

***N'*-[*E*]-thiophen-2-ylmethylidene]pyridine-4-carbohydrazide (9)**

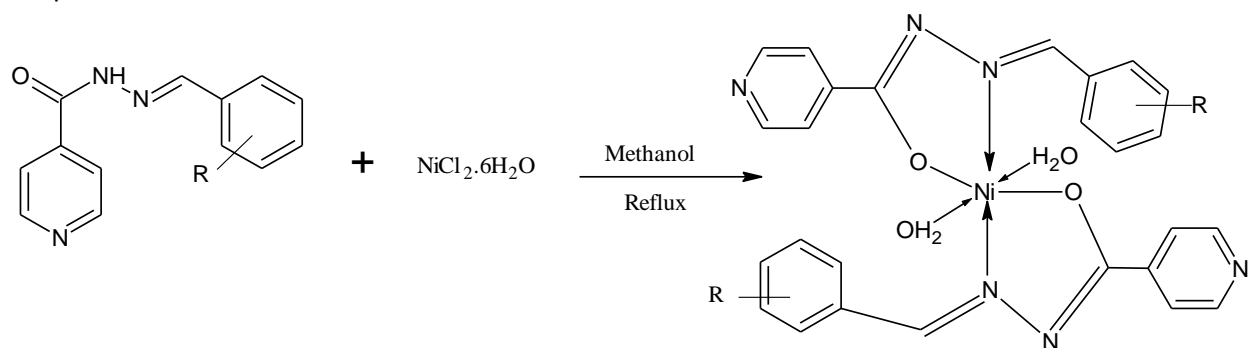
White solid, Yield: 87 %, Reaction time: 1:30 hours, m.p. 244-247 °C, FT-IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 1663 (C=O, amide), 1575 (C=N), 1535 (NNH), 1495 (C=C, aromatic). MS m/z (%):231 ( $\text{M}^+$ , 51%), 153 ( $\text{M}^+$ -C<sub>5</sub>H<sub>4</sub>N, 22%), 148 ( $\text{M}^+$ -C<sub>4</sub>H<sub>3</sub>S, 59%), 125 ( $\text{M}^+$ -C<sub>6</sub>H<sub>4</sub>NO, 27%), 106 ( $\text{M}^+$ -C<sub>5</sub>H<sub>5</sub>N<sub>2</sub>S, 82%), 96 ( $\text{M}^+$ -C<sub>6</sub>H<sub>5</sub>N<sub>3</sub>O, 19%), 78 ( $\text{M}^+$ -C<sub>6</sub>H<sub>5</sub>N<sub>2</sub>OS, 32%).



### 2.1.7 General Procedure for the Synthesis of Metal Complexes of Schiff Base Ligands (GP2)

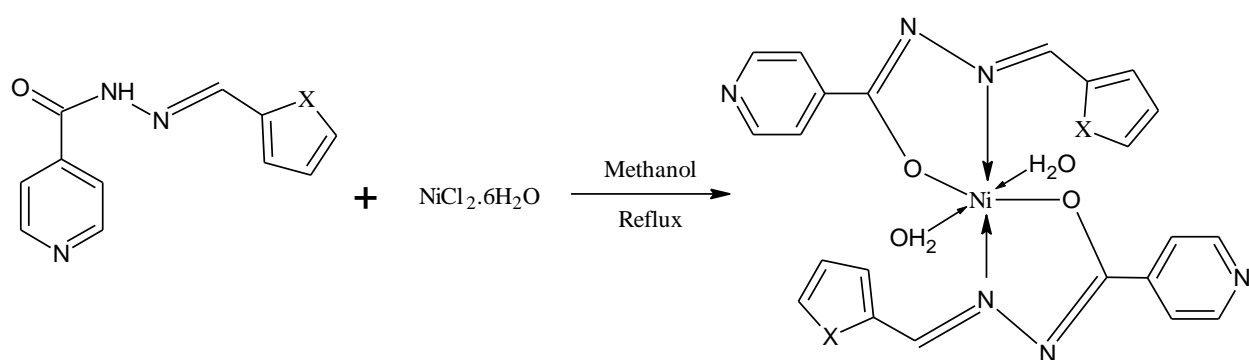
The reaction was carried out following the literature reported procedure [51]. The Schiff base ligand (1 mmol, 0.3g) was dissolved in 10 mL of methanol. The metal salt (0.5 mmol, 0.12g) was dissolved in 3 mL of methanol. Then metal salt solution was added to ligand solution and refluxed for 3 hours. The solution was kept overnight. The precipitates obtained were filtered and washed with ethanol.

Chapter 2



R= H, 2-OH, 4-OCH<sub>3</sub>, 2-Cl, 4-Cl, 4-NHBz, 4-NHBoc

**Scheme 2.5: Synthesis of Nickel Complex using Aromatic Schiff Base Ligand**

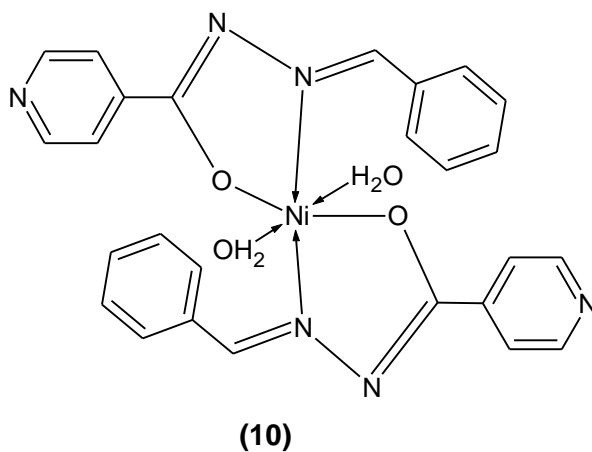


X=O, S

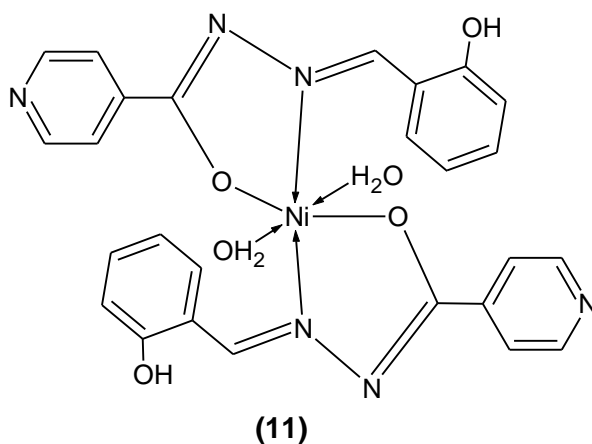
**Scheme 2.6: Synthesis of Nickel Complex using 2-Substituted Schiff Base Ligand**



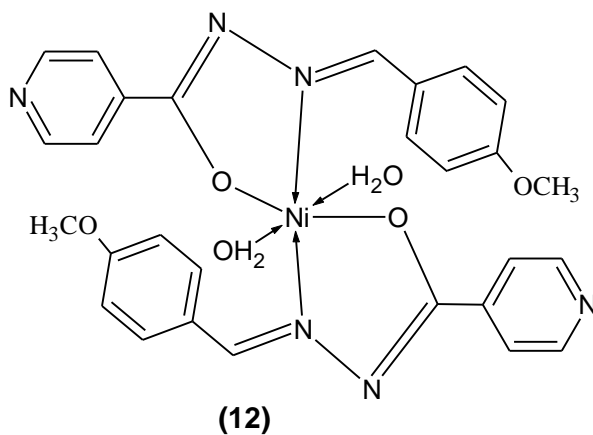
Grassy green powder, Yield: 73 %, m.p. 295 °C (decomp.), FT-IR: ( $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ) 3177 (OH), 1549 (C=N), 508 (M-O), 420 (M-N).



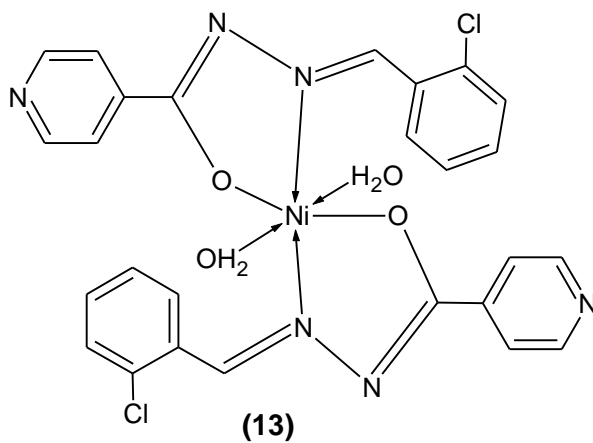
Mustard color powder, Yield: 67 %, m.p. 238 °C (decomp.), FT-IR: ( $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ) 3056 (OH), 1548 (C=N), 522 (Ni-O), 420 (Ni-N).



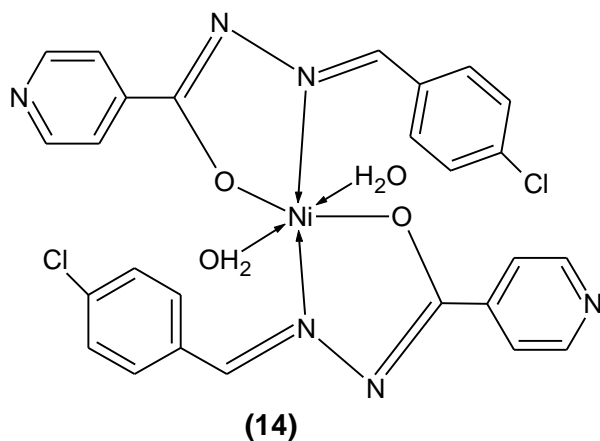
Mustard color powder, Yield: 65 %, m.p. 270 °C (decomp.), FT-IR: ( $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ) 3207 (OH), 1552 (C=N), 525 (Ni-O), 420 (Ni-N).



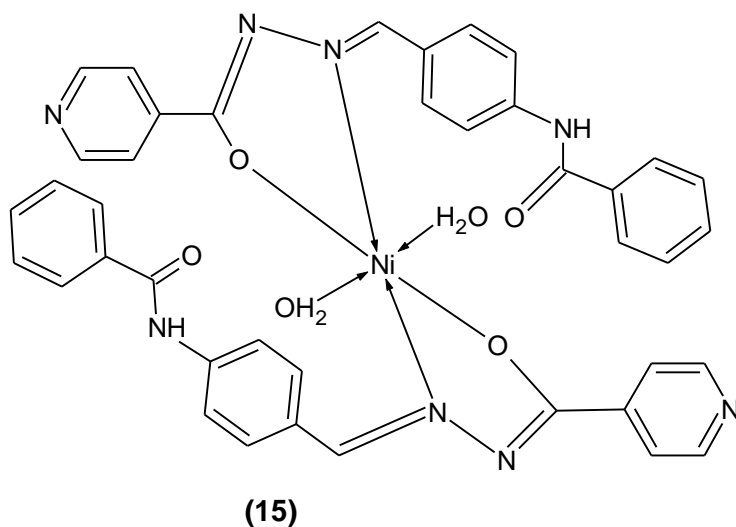
Pale green powder, Yield: 66 %, m.p. 285 °C (decomp.), FT-IR: ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ) 3230 (OH), 1548 (C=N), 527 (Ni-O), 420 (Ni-N).



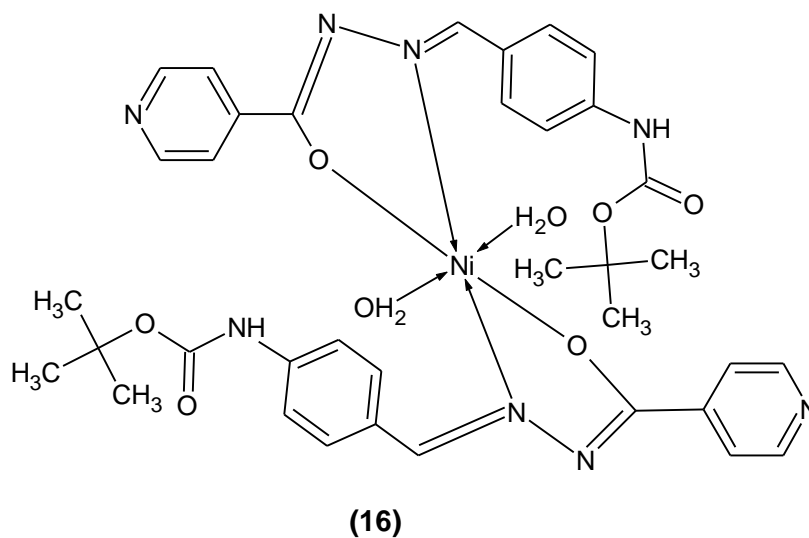
Yellowish green powder, Yield: 69 %, m.p. 280 °C (decomp.), FT-IR: ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ) 3186 (OH), 1594 (C=N), 542 (Ni-O), 420 (Ni-N).



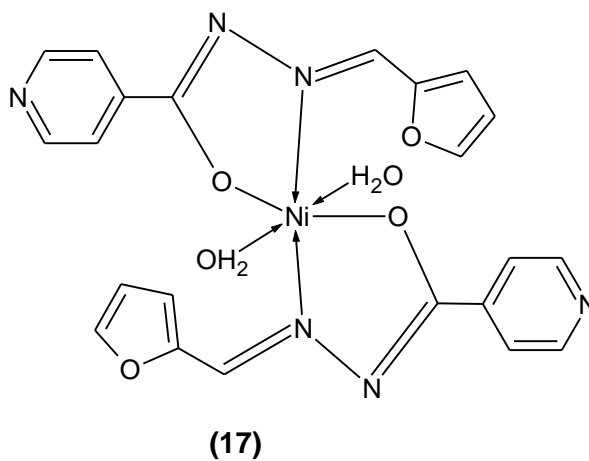
Brick red crystal, Yield: 64 %, m.p. 260 °C (decomp.), FT-IR: ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ) 3407 (OH), 1590 (C=N) 580 (Ni-O), 420 (Ni-N).



Brick red powder, Yield: 63 %, m.p. 245 °C (decomp.), FT-IR: ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ) 3410 (OH), 1591 (C=N), 1365 (C(CH<sub>3</sub>)<sub>3</sub>) 576 (Ni-O), 420 (Ni-N).

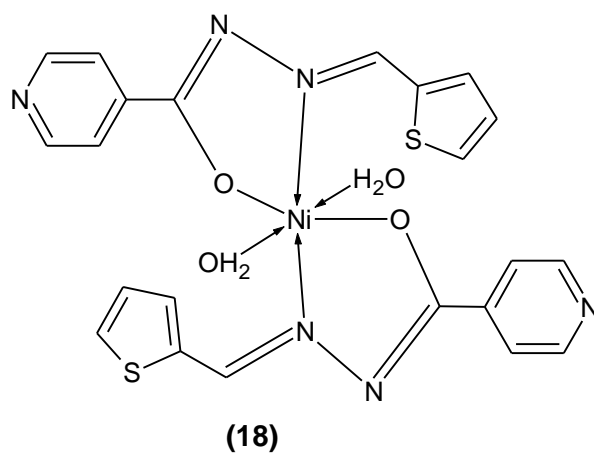


Mud green powder, Yield: 63 %, m.p. 228 °C (decomp.), FT-IR: ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ) 3118 (OH), 1557 (C=N), 566 (Ni-O), 420 (Ni-N).



Mustard colour powder, Yield: 71 %, m.p. 250 °C (decomp.), FT-IR: ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ) 3365 (OH), 1542 (C=N), 524 (Ni-O), 420 (Ni-N).





# Chapter 3

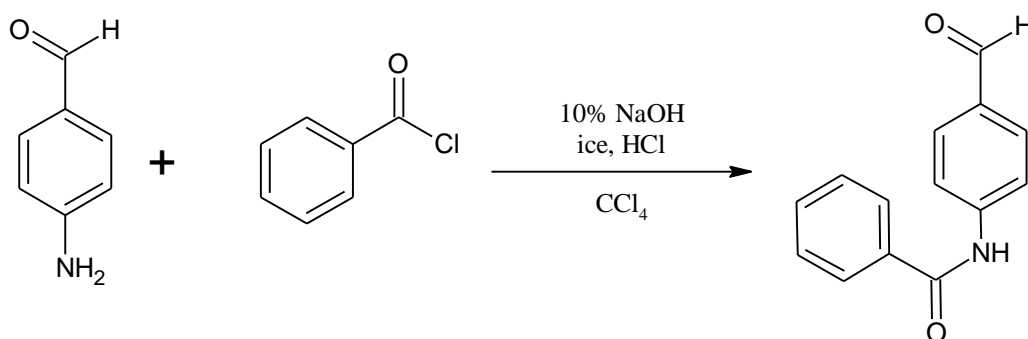
## 3.1 RESULTS AND DISCUSSION

This chapter deals with the results and discussion of the synthetic strategies used to accomplish the plan of work, described in schemes (3.1-3.3, 3.11, 3.14 and 3.15). According to the plan of work, attempts were made to synthesize target molecules.

The reaction was carried out by reported method [53]. The aldehydes used for the synthesis of ligands were benzaldehyde, salicylaldehyde, *p*-methoxy benzaldehyde, furaldehyde, thiophene carbaldehyde, 2-chloro benzaldehyde, 4-chloro benzaldehyde and *p*-amino benzaldehyde. All the aldehydes were used as such but the *p*-amino benzaldehyde containing amino group at the para position to aldehyde which can cause hindrance during the reaction. So, it is necessary to protect its amino group and then further use it for the ligand formation. Many protecting groups reported for the protection of amino group but in this work, two of them *i.e.* benzoyl chloride and di-*tert*-butyl carbonate (Boc) were used.

Benzoyl chloride was used for the protection of *p*-amino benzaldehyde by following the reported method. The reaction progress was monitored through TLC. After the completion of the reaction, the FT-IR of the product was recorded. The appearance of amidic carbonyl at  $1670-1690\text{ cm}^{-1}$  indicated that the protection of amino group had been done.

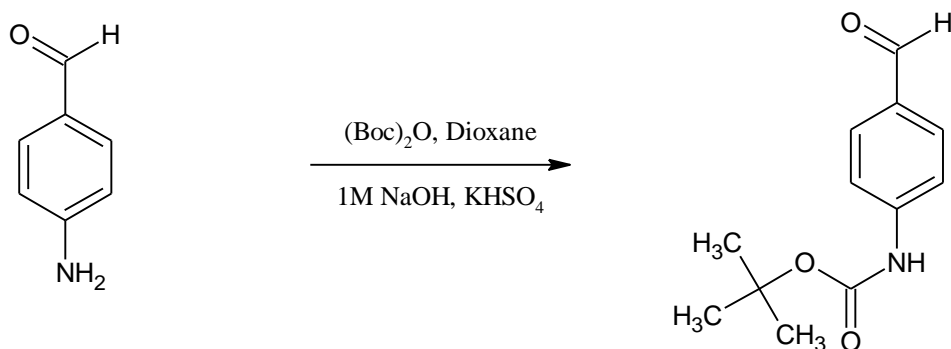
The scheme of the reaction is as follows:



**Scheme 3.1: Benzoyl Protection of *p*-Amino Benzaldehyde**

Boc was also used for the protection of amino group. The reaction was carried out and after the completion of the reaction; FT-IR of the product was noted. The completion of the reaction was indicated by the presence of characteristic ester carbonyl band at  $1676\text{ cm}^{-1}$ , band of *tert*-butyl group of the Boc was observed at  $1368\text{ cm}^{-1}$  and absence of  $\text{NH}_2$  duplet band at  $3400\text{-}3200\text{ cm}^{-1}$ .

The reaction equation is as follows:



**Scheme 3.2: Boc Protection of *p*-Amino Benzaldehyde**

### 3.1.1 Synthesis of Schiff Base Ligands from Aromatic Aldehydes

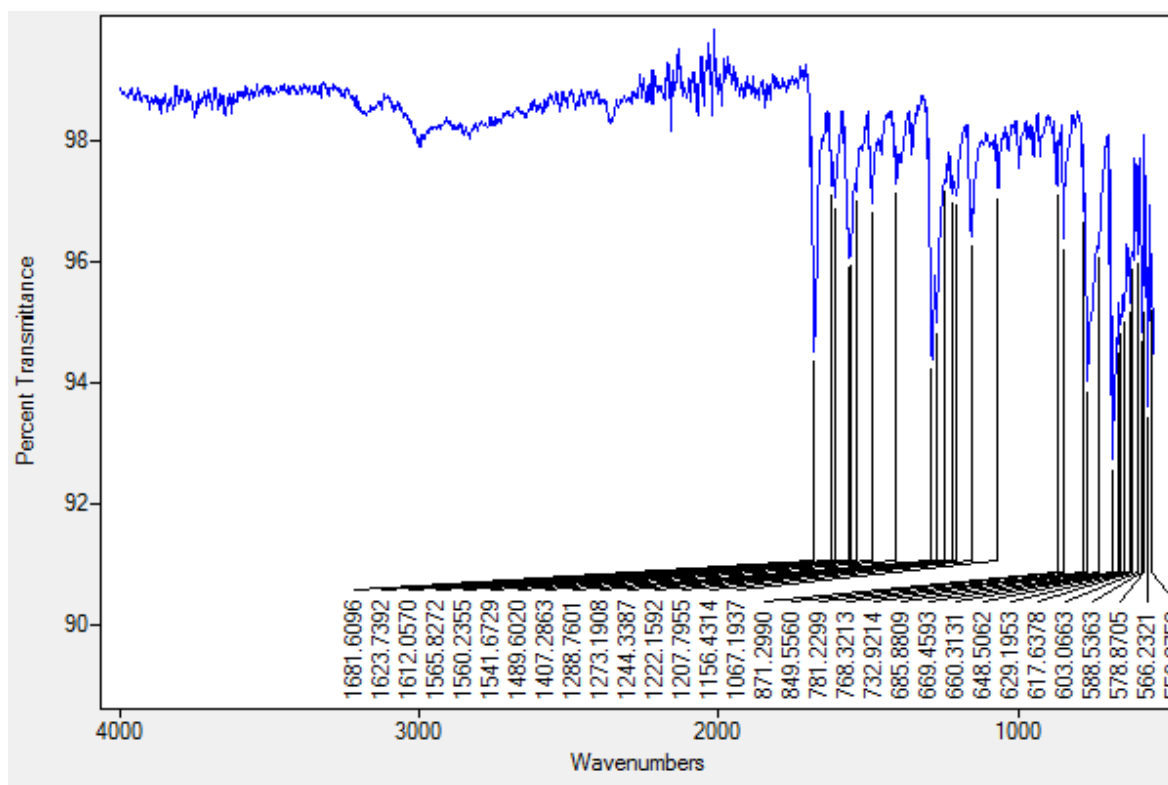
Schiff base ligands (**3a-3g**) were synthesized by the reaction of *o* and *p*-substituted aldehydes with isoniazid. The products (**3a-3g**) were characterized by spectroscopic studies. The physical and spectral data of the compounds are given in the Tables 3.1- 3.2.



appearance of the band for C=N in the spectra indicated the Schiff base's formation. The Table 3.2 represents the complete FT-IR data of the synthesized compounds.

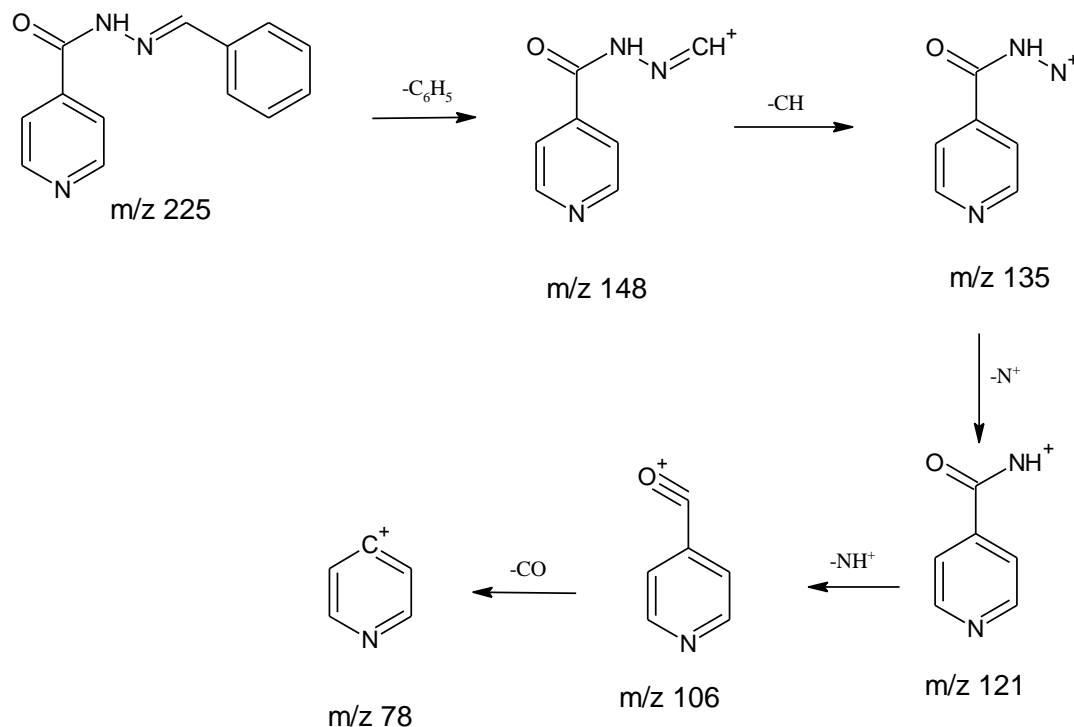
**Table 3.2: FT-IR Spectral Data of the Compounds (3a-3g)**

Compound No.	C=O (amide) ( $\nu$ $\text{cm}^{-1}$ )	C=N ( $\nu$ $\text{cm}^{-1}$ )	C=C (aromatic) ( $\nu$ $\text{cm}^{-1}$ )	NNH ( $\nu$ $\text{cm}^{-1}$ )
<b>3a</b> (R=H)	1683	1575	1563	1549
<b>3b</b> (R=OH)	1681	1612	1489	1565
<b>3c</b> (R=OCH <sub>3</sub> )	1654	1596	1513	1525
<b>3d</b> (R=2-Cl)	1669	1600	1565	1548
<b>3e</b> (R=4-Cl)	1661	1602	1489	1549
<b>3f</b> (R=4-NHBz)	1661	1602	1523	1535
<b>3g</b> (R=4-NHBoc)	1662	1602	1498	1535



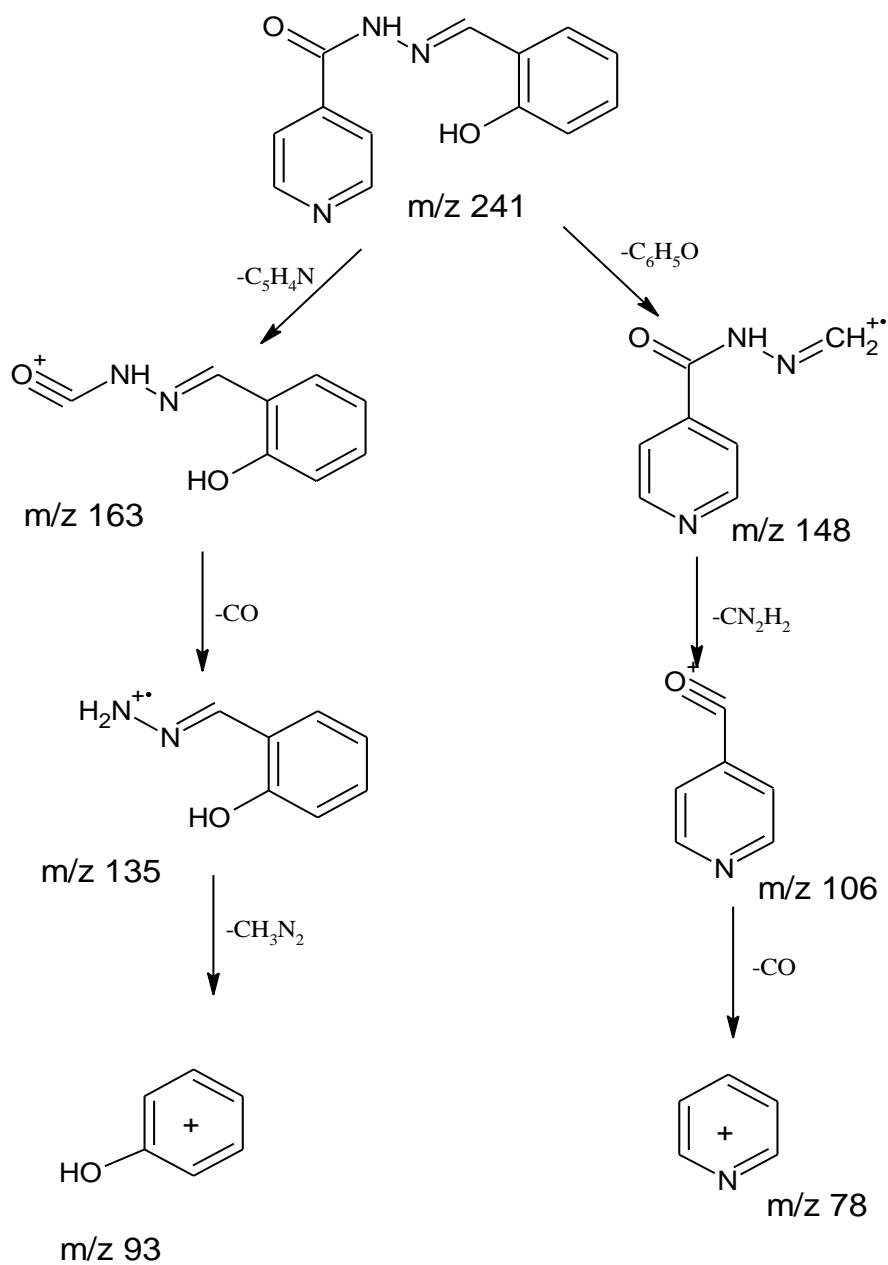
**Figure 3.1: FT-IR Spectrum of the Compound (3b)**

In GC-MS spectra of the synthesized compounds, the molecular ion peak appeared which confirmed the product formation. The molecular ion peak of **(3a)** was observed at  $m/z$  225 and the other peaks were shown at  $m/z$  148, 135, 121, 106 and 78 which confirmed the formation of *N'*-[(*E*)-phenylmethylidene]pyridine-4-carbohydrazide **(3a)**. The mass fragmentation of the compound 3a is given in the scheme 3.4.



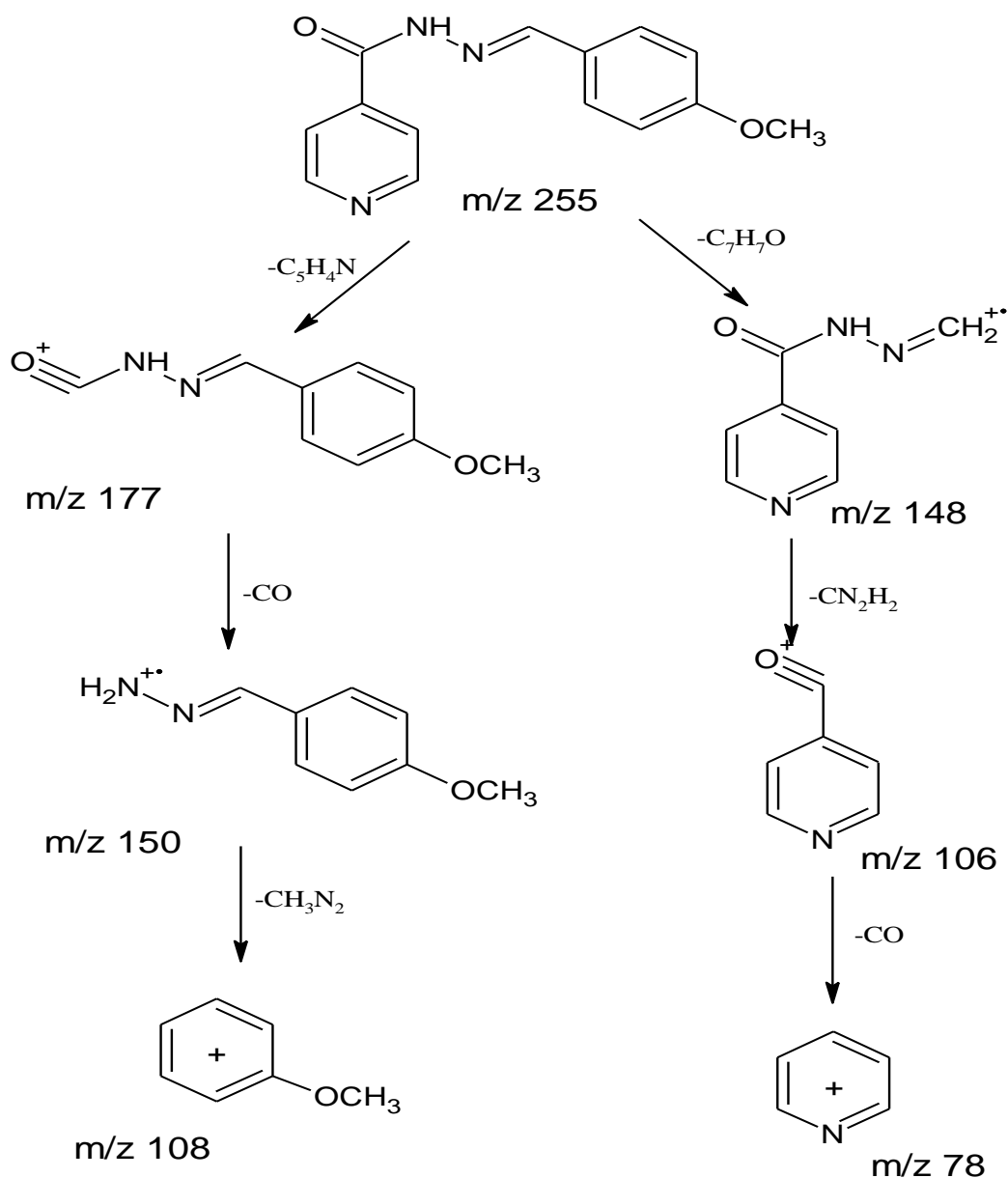
**Scheme 3.4: Mass Fragmentation Pattern of Compound (3a)**

*N'*-[(*E*)-(2-hydroxyphenyl)methylidene]pyridine-4-carbohydrazide **(3b)** was synthesized from salicylaldehyde. The molecular ion peak in the case of Schiff base ligand **(3b)** was observed at  $m/z$  241. The other fragments of the compounds were 163, 148, 135, 106, 93 and 78 which confirmed the product formation. The mass fragmentation pattern of **(3b)** was shown in scheme 3.5.



**Scheme 3.5: Mass Fragmentation Pattern of Compound (3b)**

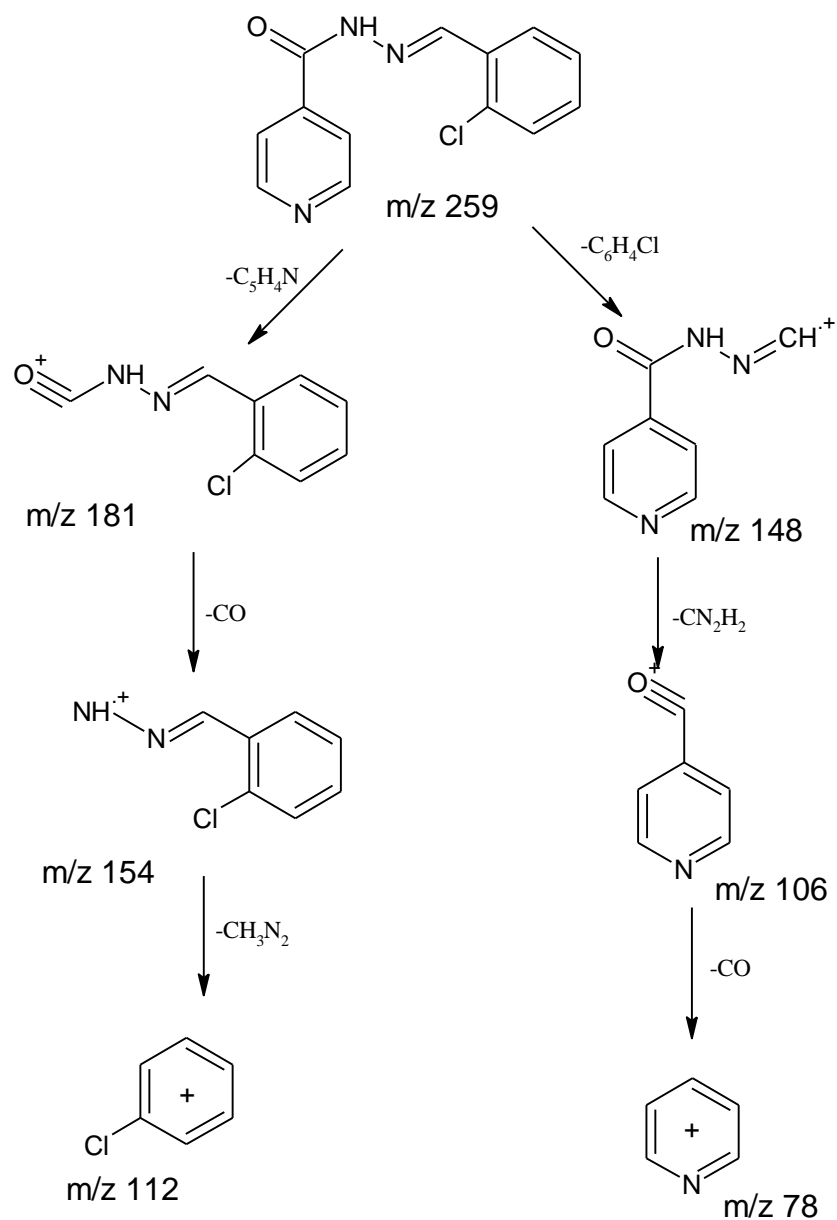
The compound (**3c**) was synthesized from *p*-methoxy benzaldehyde and isoniazid. The molecular ion peak of the synthesized compound (**3c**) was appeared at m/z 255. The other fragments were observed at m/z 177, 150, 148, 108, 106 and 78. The mass fragmentation confirmed the product formation. The fragmentation pattern of (**3c**) was given in the scheme 3.6.



**Scheme 3.6: Mass Fragmentation Pattern of Compound (3c)**

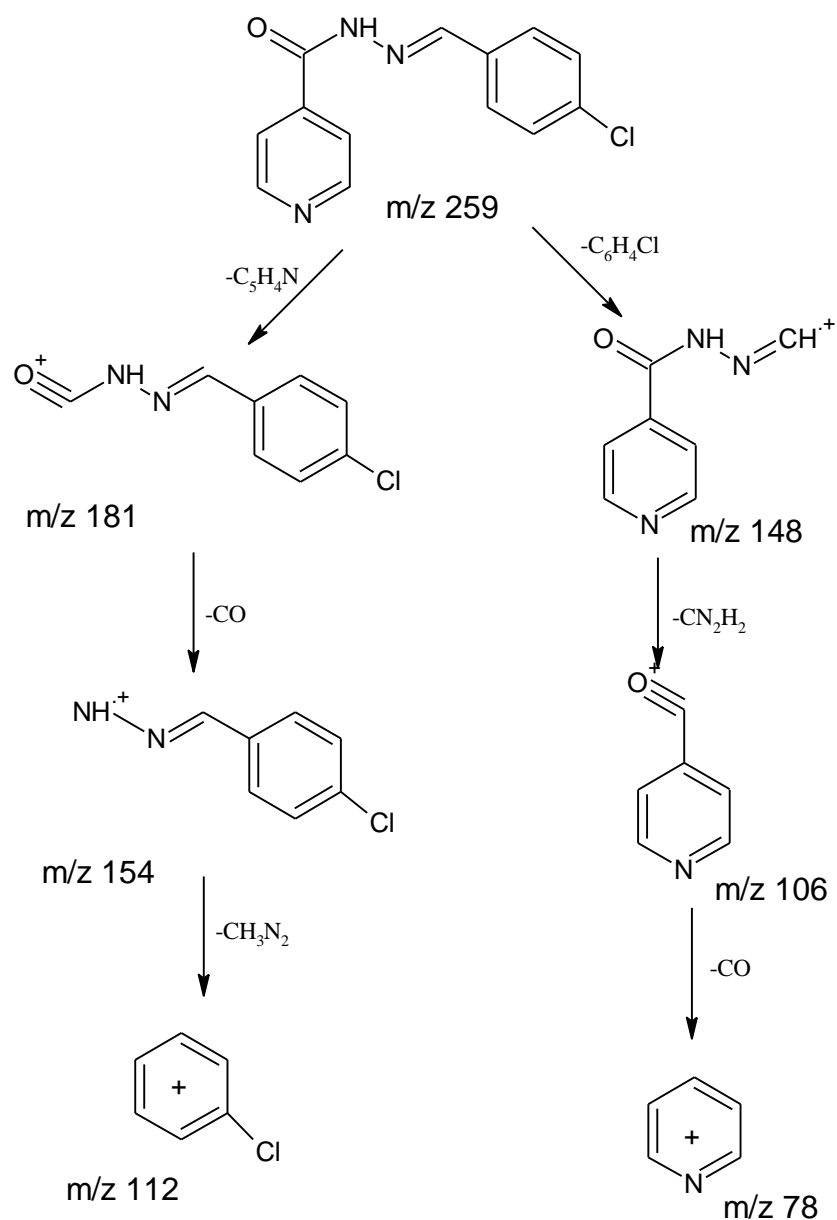
The Schiff base ligand (**3d**) was synthesized from 2-chloro benzaldehyde. The FT-IR analysis of the synthesized compound showed the C=N band at  $1600\text{ cm}^{-1}$  which indicated the product formation. The molecular ion peak of (**3d**) was observed at  $m/z$  259. The other mass fragments were appeared at  $m/z$  181, 154, 148, 112, 106 and 78. The mass fragmentation pattern of (**3d**) was shown in scheme 3.7.





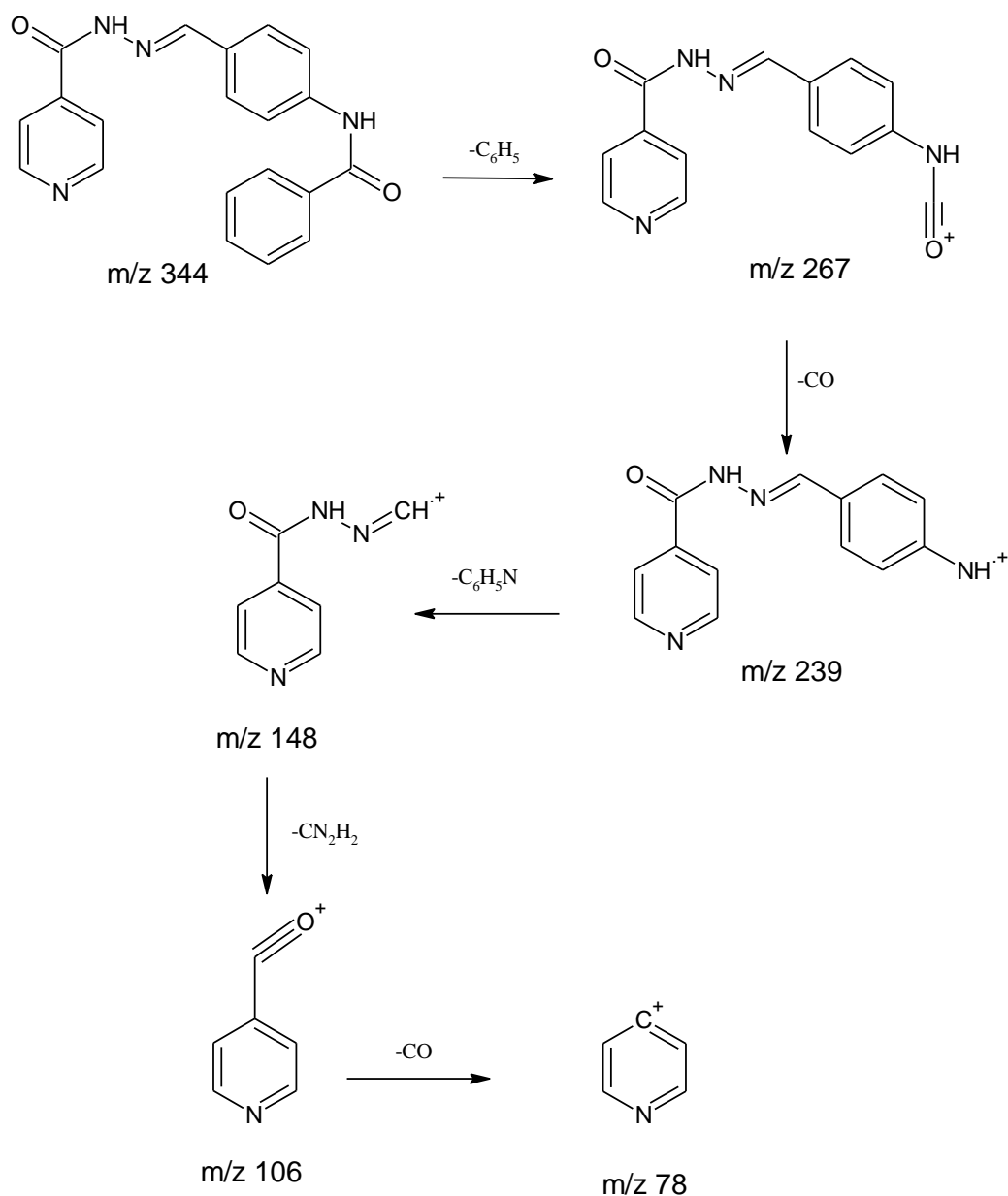
**Scheme 3.7: Mass Fragmentation Pattern of Compound (3d)**

To synthesize the Schiff base ligand (**3e**), 4-chloro benzaldehyde was used. The FT-IR of the compound indicated the product formation. The GC-MS of the product was recorded. The molecular ion peak was appeared at  $m/z$  259. The other fragments observed were  $m/z$  181, 154, 148, 112, 106 and 78. The mass fragmentation pattern of compound (**3e**) was shown in the scheme 3.8.



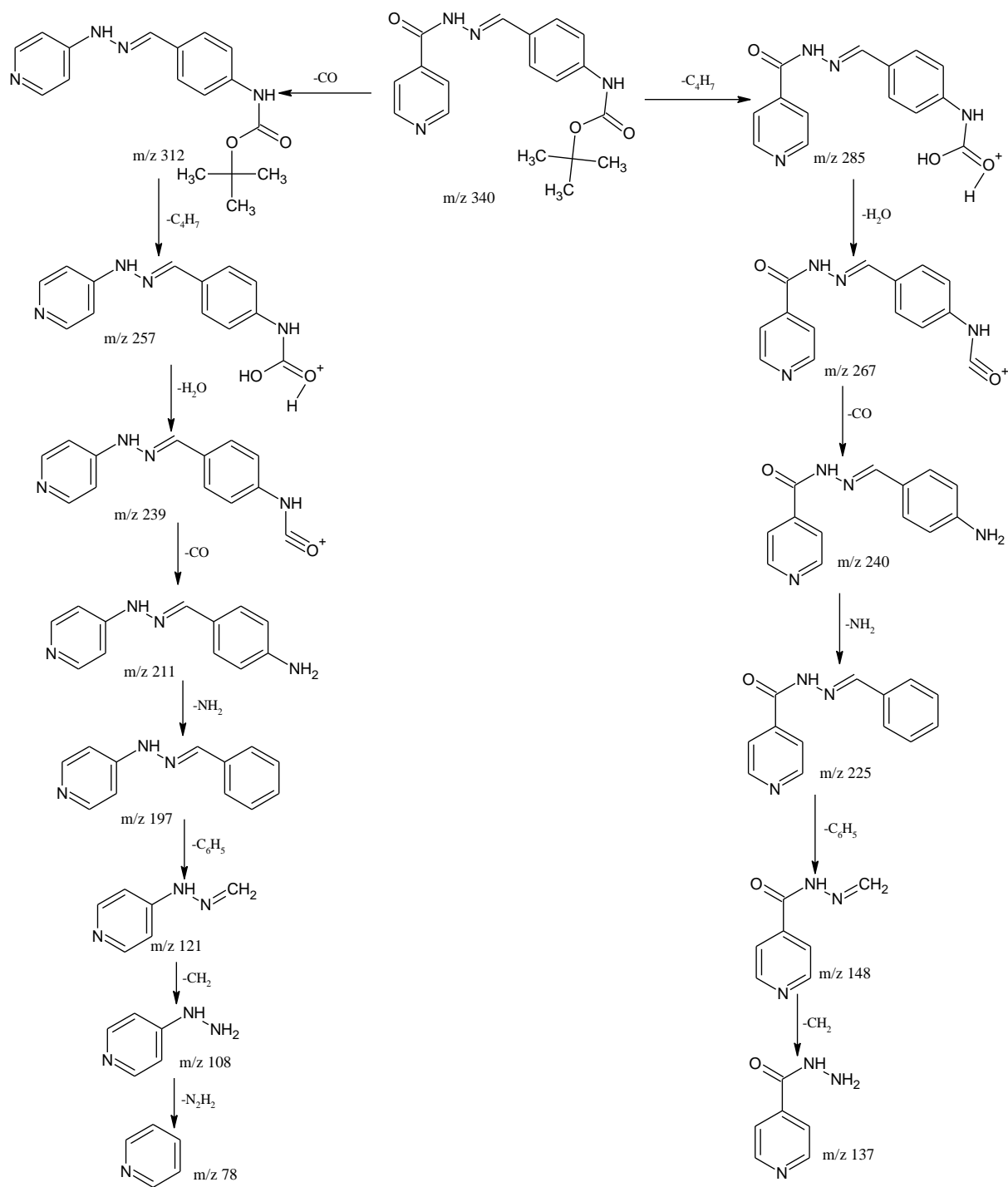
**Scheme 3.8: Mass Fragmentation Pattern of Compound (3e)**

*p*-Amino benzaldehyde was first protected using benzoyl chloride. Then protected aldehyde was used to synthesize the Schiff base ligand (**3f**). The product formation was indicated through FT-IR which showed characteristic band for C=N at  $1588\text{ cm}^{-1}$  and amidic carbonyl at  $1661\text{ cm}^{-1}$ . The GC-MS spectrum of the compound showed molecular ion peak at m/z 344. The other mass fragments were appeared at m/z 267, 239, 148, 106 and 78. The mass fragmentation pattern of the compound (**3f**) was shown in the scheme 3.9.



**Scheme 3.9: Mass Fragmentation Pattern of Compound (3f)**

Di-*tert*-butyl carbonate (Boc) was used to protect the amine group of the aldehyde at the para position. The Schiff base ligand (**3g**) was synthesized by using the N-Boc protected aldehyde and the drug isoniazid. The GC-MS of the compound was observed. The molecular ion peak was recorded at  $m/z\ 340$  the other fragments were appeared at  $m/z\ 312$ , 285, 267, 257, 239, 211, 121, 108 and 78. The mass fragmentation pattern of the compound (**3g**) was shown in scheme 3.10.



**Scheme 3.10: Mass Fragmentation Pattern of Compound (3g)**

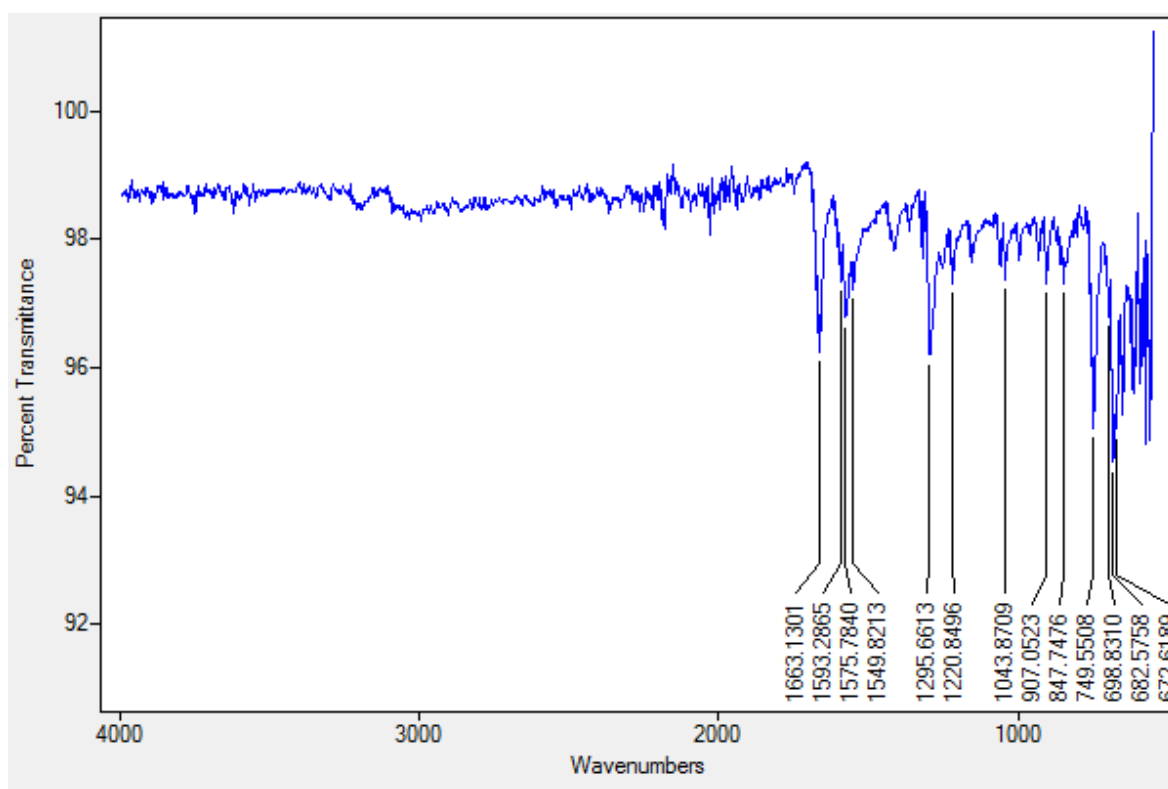
### 3.1.2 Synthesis of Schiff Base Ligands from 2-Substituted Aldehydes

$N'$ -[(*E*)-furan-2-ylmethylidene]pyridine-4-carbohydrazide (5a) and  $N'$ -[(*E*)-thiophen-2-ylmethylidene]pyridine-4-carbohydrazide (5b) were synthesized by reacting

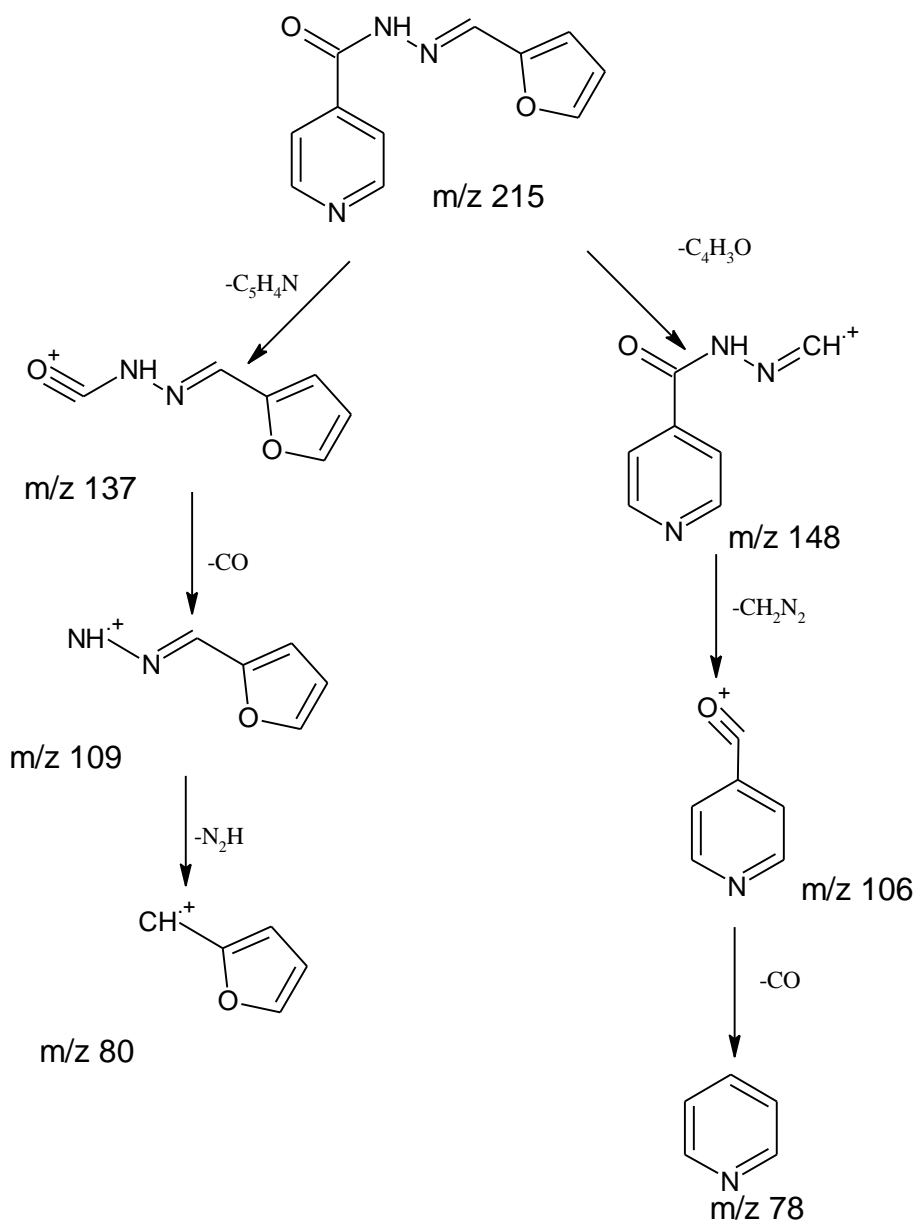


**Table 3.4: FT-IR Data of the Compounds (5a-5b)**

Compound No.	5a (X=O) ( $\nu$ $\text{cm}^{-1}$ )	5b (X=S) ( $\nu$ $\text{cm}^{-1}$ )
C=O (amide)	1650	1663
C=N	1619	1575
C=C (aromatic)	1474	1495
NNH	1535	1535

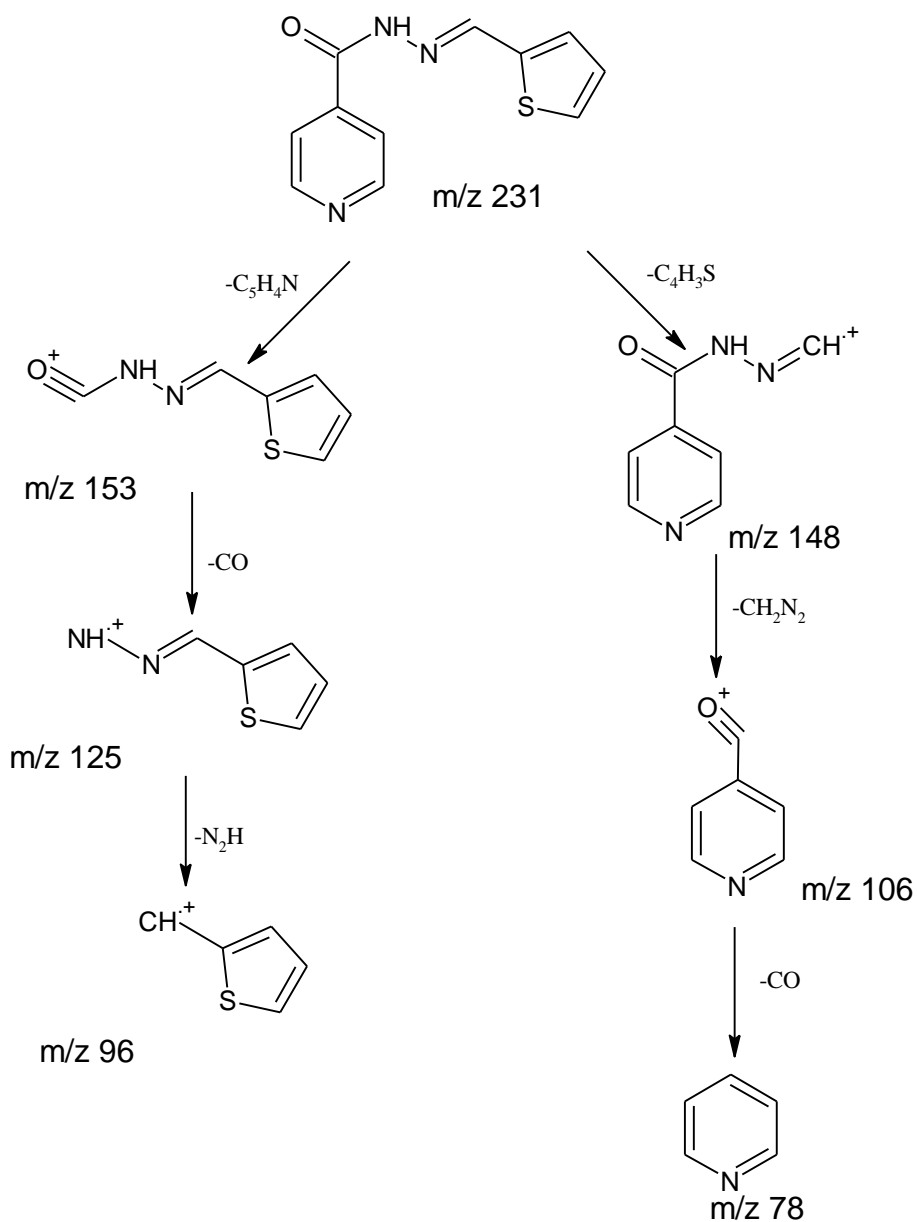
**Figure 3.2: FT-IR Spectrum of the Compound (5b)**

The GC-MS of the compounds provided the confirmation about product formation. The molecular ion peak of the *N*'-[(*E*)-furan-2-ylmethylidene]pyridine-4-carbohydrazide (**5a**) was observed at  $m/z$  215. The other fragments of the compound were appeared at  $m/z$  148, 137, 109, 106, 80 and 78. These were due to the loss of different fragments from the molecular ion and it confirmed the product formation. The mass fragmentation pattern of the (**5a**) is given in the scheme 3.12.



**Scheme 3.12: Mass Fragmentation Pattern of Compound (5a)**

The GC-MS of the *N'*-[(*E*)-thiophen-2-ylmethylidene]pyridine-4-carbohydrazide (**5b**) gave the molecular ion peak at *m/z* 231. The molecular ion lost different fragments and gave fragments at *m/z* 153, 148, 125, 106, 96 and 78 which confirms the synthesis of the product. The mass fragmentation pattern of the (**5b**) is given in the scheme 3.13.

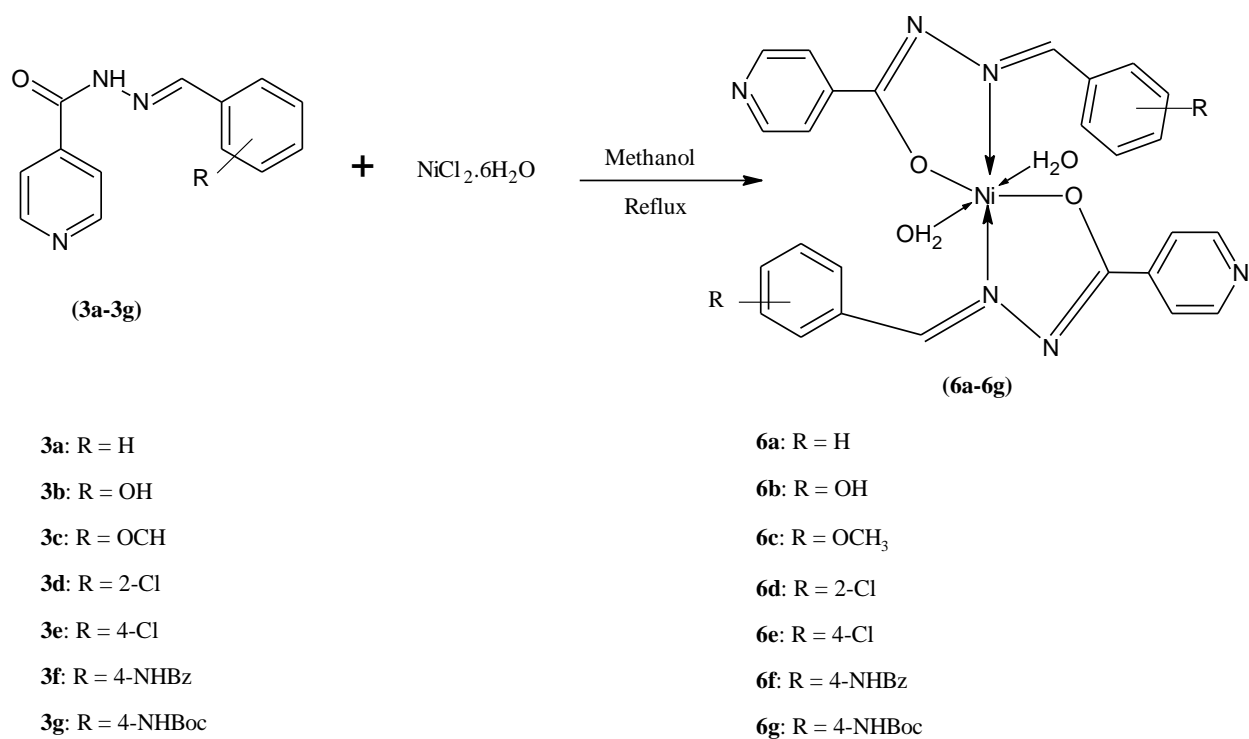


**Scheme 3.13: Mass Fragmentation Pattern of Compound (5b)**

### 3.1.3 Synthesis of Metal Complexes of Schiff Bases (6a-6g)

Ni complexes were synthesized by reacting Schiff base ligands (3a-3g) with  $NiCl_2 \cdot 6H_2O$ . The product formation was indicated through FT-IR. The physical data of the compounds is given in the table 3.5

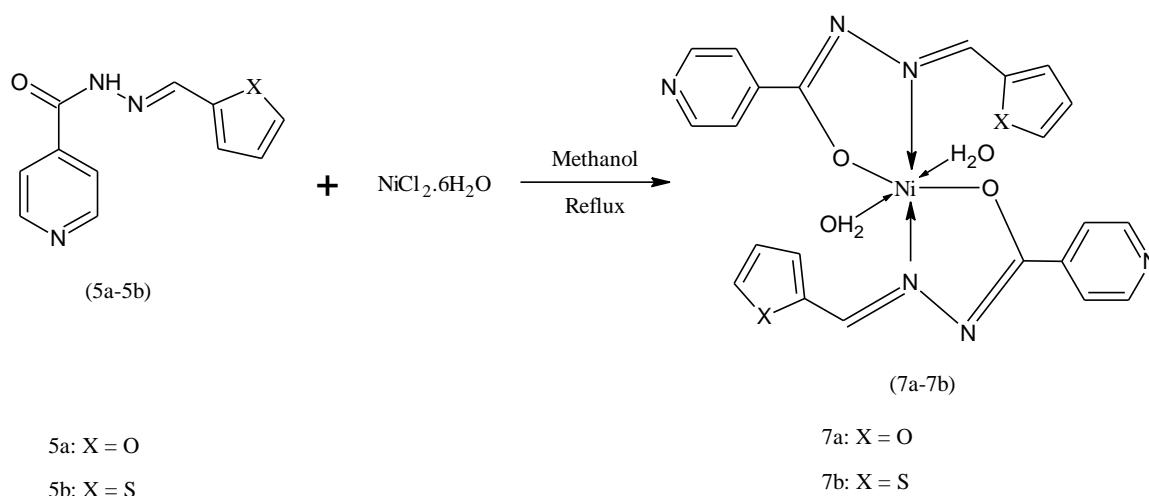




Scheme 3.14: Synthesis of Nickel Complex using Schiff Base of Aromatic Aldehyde.

Table 3.5: Physical Data of the Compounds (6a-6g)

Physical Properties	6a (R = H)	6b (R = OH)	6c (R = OCH <sub>3</sub> )	6d (R = 2-Cl)	6e (R = 4-Cl)	6f (R = 4-NHBz)	6g (R = 4-NHBoc)
Appearance	Grassy green	Mustard colour	Mustard	Pale green	Yellowish green	Brick red	Brick red
Molecular Weight (g/mol)	578	610	638	647	647	816	808
Melting Point (°C)	295	238	270	285	280	260	245
Yield (%)	73	67	65	66	69	64	63



**Scheme 3.15: Synthesis of Nickel Complexes from Compounds (5a-5b)**

The physical data of the compounds (**7a-7b**) is given in the table 3.6.

**Table 3.6: Physical Data of the Compounds (7a-7b)**

Physical Properties	7a (X=O)	7b (X=S)
Appearance	Mud green	Mustard
Molecular Weight (g/mol)	558	590
Melting Point(°C)	228	250
Yield (%)	63	71

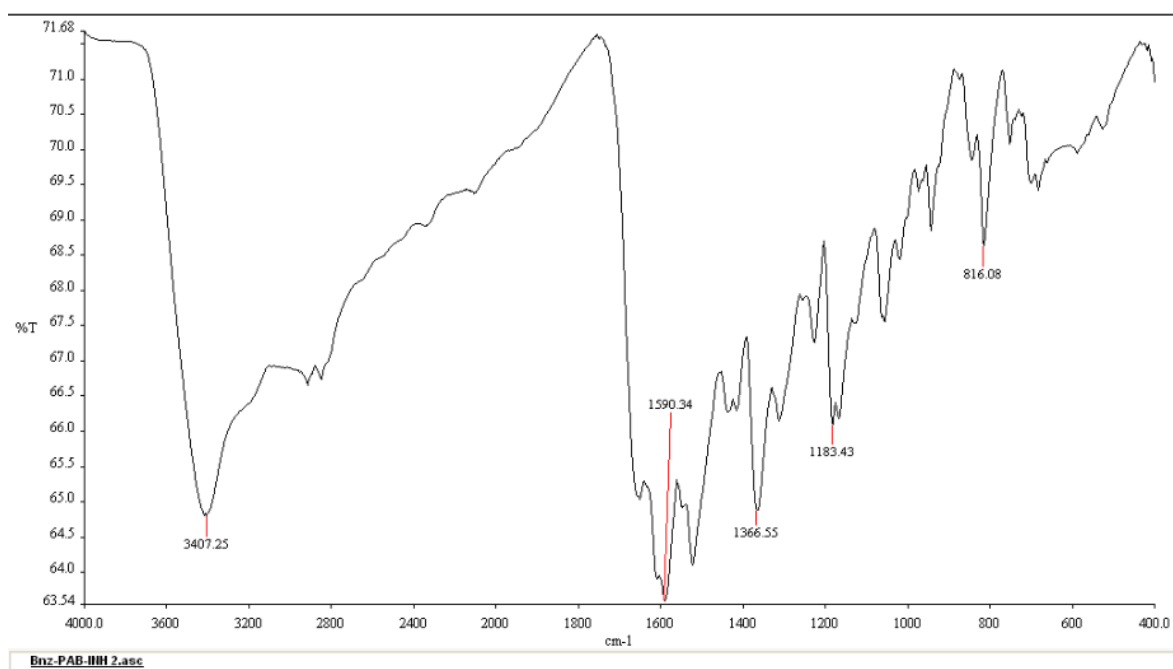
### 3.2 Infrared Spectra

Schiff bases were coordinated to the metal atom in two ways showing that the ligands were coordinated in a bidentate manner on the basis of FT-IR data. This was observed by the comparison of FT-IR spectra of Schiff bases and their metal chelates. The Schiff base ligands were first synthesized, isolated, characterized and afterward were used for complexation reaction. The FT-IR spectra of the ligands and complexes were compared to indicate the synthesis of metal complexes. The characteristic C=N band appeared in the FT-IR of the ligands at 1575-1602  $\text{cm}^{-1}$  shifted to 1542-1594  $\text{cm}^{-1}$  due to the coordination of the lone pair of nitrogen of azomethine group to the metal. Due to the presence of water molecule, the absorption band of OH group appeared around 3300  $\text{cm}^{-1}$  in all the complexes. The coordinating atoms of the ligands were N and O and the FT-IR band of the

M-N and M-O were observed at  $440\text{ cm}^{-1}$  and  $420\text{ cm}^{-1}$  respectively. The M-O band indicated that C=O (amide) coordinated to the metal atom in the form of enol [79]. The M-N band also justifies the coordination of imino group to metal ions. The FT-IR spectrum of  $[\text{Ni}(\text{Bz-PAB-INH})_2(\text{H}_2\text{O})_2]$  is shown in the Figure 3.3.

**Table 3.7: FT-IR Data of the Metal Complexes**

<b>Metal Complex</b>	<b>OH (<math>\nu\text{ cm}^{-1}</math>)</b>	<b>C=N (<math>\nu\text{ cm}^{-1}</math>)</b>	<b>M-O (<math>\nu\text{ cm}^{-1}</math>)</b>	<b>M-N (<math>\nu\text{ cm}^{-1}</math>)</b>
$[\text{Ni}(\text{INHB})_2(\text{H}_2\text{O})_2]$ <b>(6a)</b>	3177	1549	508	420
$[\text{Ni}(\text{INHS})_2(\text{H}_2\text{O})_2]$ <b>(6b)</b>	3056	1548	522	420
$[\text{Ni}(\text{INHMB})_2(\text{H}_2\text{O})_2]$ <b>(6c)</b>	3207	1552	525	420
$[\text{Ni}(\text{INH2C})_2(\text{H}_2\text{O})_2]$ <b>(6d)</b>	3230	1548	527	420
$[\text{Ni}(\text{INH4C})_2(\text{H}_2\text{O})_2]$ <b>(6e)</b>	3186	1594	542	420
$[\text{Ni}(\text{Bz-PAB-INH})_2(\text{H}_2\text{O})_2]$ <b>(6f)</b>	3407	1590	580	420
$[\text{Ni}(\text{Boc-PAB-INH})_2(\text{H}_2\text{O})_2]$ <b>(6g)</b>	3410	1591	576	420
$[\text{Ni}(\text{INHF})_2(\text{H}_2\text{O})_2]$ <b>(7a)</b>	3118	1557	566	420
$[\text{Ni}(\text{INHT})_2(\text{H}_2\text{O})_2]$ <b>(7b)</b>	3365	1542	524	420



**Figure 3.3: FT-IR Spectrum of the Compound (6f)**

### 3.3 UV-vis Spectroscopy

UV-vis spectroscopy shows different absorption bands in the range from 200-800 nm. UV ranges from 200-400 nm and the visible region from 400-800 nm. The d-d transitions of the transition metals in the visible region help to deduce the geometry of the metal complexes [68]. The UV-vis spectra of the complexes were recorded in ethanol at room temperature ranges from 200-800 nm.

**Table 3.8: UV-vis Data of the Metal Complexes**

<b>Compounds</b>	<b>Solvent</b>	<b><math>\pi</math>-<math>\pi^*</math>, and Transition (nm)</b>	<b><math>n</math>-<math>\pi^*</math> CT Transition (nm)</b>	<b>d-d Transition (nm)</b>	<b>Geometry</b>
[Ni(INHB) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(6a)</b>	Ethanol	267, 352, 438		654	Octahedral
[Ni(INHS) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(6b)</b>	Ethanol	270, 349, 447		658	Octahedral
[Ni(INHMB) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(6c)</b>	Ethanol	274, 350, 445		652	Octahedral
[Ni(INH2C) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(6d)</b>	Ethanol	277, 352, 436		653	Octahedral
[Ni(INH4C) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(6e)</b>	Ethanol	279, 348, 439		655	Octahedral
[Ni(Bz-PAB- INH) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(6f)</b>	Ethanol	286, 350, 436		656	Octahedral
[Ni(Boc-PAB- INH) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(6g)</b>	Ethanol	285, 348, 432		659	Octahedral
[Ni(INHF) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(7a)</b>	Ethanol	278, 344, 440		654	Octahedral
[Ni(INHT) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(7b)</b>	Ethanol	275, 339, 438		655	Octahedral

The absorption band of Ni (II) complexes were shown in the range of 267-286 nm which are the  $\pi$ - $\pi^*$  transitions. The  $n$ - $\pi^*$  transition of C=N were observed in the range of 339-352 nm. The charge transfer (CT) absorption of the Ni (II) complexes were observed at 432-447 nm. The d-d transitions of the complexes were observed in the visible region i.e 652-659 nm. All these absorption indicated the octahedral geometry of the complexes [80].

### 3.4 Biological Activity

The biological activity of the compounds was checked against colorectal carcinoma cell line HCT 116. All synthesized compounds and complexes were screened separately for their antitumor activity in four different concentrations including: 200  $\mu\text{g}/\mu\text{L}$ , 100  $\mu\text{g}/\mu\text{L}$ , 50  $\mu\text{g}/\mu\text{L}$  and 25  $\mu\text{g}/\mu\text{L}$ . All the ligands and complexes were active against the cell line. The ligands show % cell mortality in the range of 38.44282-76.44231 % in case of first dilution *i.e.* 100 $\mu\text{g}/\mu\text{L}$ . At first dilution complexes were active in the range of 41.60584-76.60256 %. The maximum cell mortality was shown at 1<sup>st</sup> dilution in all the cases and as the concentration decreases cell mortality rate decreases. The antitumor activity of the ligands were enhanced when they coordinate to the metal atom. This enhancement in the activity is due to the additional imine (HC=N) linkage in the metal complexes. Another reason for the increase of the activity on complexation is due to the nitrogen and oxygen donor atom present in the ligands. Due to the coordination the polarity of metal atom decreases due to sharing of charge with donor atoms and in turn lipophilicity of the metal atom increases making it more active against cell line. The case in which cell mortality rate decreases on complexation is due to the active binding sites of ligands [79, 81-82]. It is also due to solubility of the compounds. More prominence results were in the case of **INF-Ni** where % cell mortality were increased as the concentration of **INHF-Ni** complex increased such as at 200  $\mu\text{g}/\mu\text{L}$  76.60256 %, at 100  $\mu\text{g}/\mu\text{L}$  76.92308 %, at 50  $\mu\text{g}/\mu\text{L}$  71.95513 % and at 25  $\mu\text{g}/\mu\text{L}$  70.64103 % cell mortality was observed. All the ligands and their respective complexes' cell mortality rate were given in the table 3.7.

**Table 3.9: (%) Cellular Mortality (Cytotoxicity) against Colorectal Carcinoma Cell**

#### Line HCT-116

% Cellular Mortality (HCT-116 colorectal carcinoma cell line)				
Sample Name	1 <sup>st</sup> Dilution	2 <sup>nd</sup> Dilution	3 <sup>rd</sup> Dilution	4 <sup>th</sup> Dilution
INHB ( <b>3a</b> )	56.38507	50.49116	49.31238	36.14931
[Ni(INHB) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] ( <b>6a</b> )	46.71533	43.06569	30.0146	27.73723
INHS ( <b>3b</b> )	71.11984	67.5835	60.11788	59.92141

[Ni(INHS) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(6b)</b>	52.55474	45.9854	45.74209	38.68613
INHMB <b>(3c)</b>	65.22593	57.56385	59.72495	54.22397
[Ni(INHMB) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(6c)</b>	66.40472	66.40472	63.85069	63.63654
INH2C <b>(3d)</b>	55.47445	52.79805	44.28224	42.33577
[Ni(INH2C) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(6d)</b>	69.15521	68.17289	67.77996	66.79764
INH4C <b>(3e)</b>	76.44231	76.28205	64.90385	27.88462
[Ni(INH4C) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(6e)</b>	45.25547	44.52555	44.03893	44.00555
INHf <b>(5a)</b>	49.63504	35.27981	36.20438	35.06569
[Ni(INHF) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(7a)</b>	76.60256	76.92308	71.95513	70.64103
INHt <b>(5b)</b>	38.44282	30.90024	36.49635	35.44526
[Ni(INHT) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] <b>(7b)</b>	41.60584	40.14599	39.60584	35.52311

- Concentration of each sample: 200 µg/mL
- Two fold dilution was prepared
- 1<sup>st</sup> Dilution contains 200 µg/µL
- 2<sup>nd</sup> Dilution contains 100 µg/µL
- 3<sup>rd</sup> Dilution contains 50 µg/µL
- 4<sup>th</sup> Dilution Contains 25 µg/µL

### 3.5 Conclusion

In the present work, we have synthesized and characterized the nickel complexes of the Schiff base ligands derived from Isoniazid and different aldehydes. The syntheses of metal complexes were carried out in molar ratio (1:2). All the synthesized compounds were characterized by melting point, FT-IR, UV-Vis and GC-MS. The results of antitumor activity of the ligands as well as complexes were checked against colorectal carcinoma cell

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line HCT-116. All the compounds were active against cell line. The  $IC_{50}$  value of all the compounds have been shown in the range of 94-191. In comparison of activity between ligands and complexes, the activity of the complexes were enhanced in some cases where as in other ligands were more active as compared to complexes. The case in which activity of the complexes were more than ligands was due to incorporation of metal atom. The coordination of the ligands to the metal enhances the activity and also due to the increase in lipophilicity of metal atom [81-82].



# Chapter 4

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