SYNTHESIS OF METAL COMPLEXES OF SCHIFF BASES OF HALOGENATED ANILINES AND THEIR ANTIFUNGAL ACTIVITY

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

INTEGRATED MASTER OF SCIENCE (HONS.)
IN
CHEMISTRY
(Minor Subject: Biochemistry)

By
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CERTIFICATE I

This is to certify that the thesis entitled **"SYNTHESIS OF METAL COMPLEXES OF SCHIFF BASES OF HALOGENATED ANILINES AND THEIR ANTIFUNGAL ACTIVITY"** submitted for the degree of **5-year Integrated M.Sc. (Hons.) Programme,** in the subject of **Chemistry** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by Ms. Gursimran Kaur under the supervision of Dr. (Mrs.) Divya Utreja upto 07.09.2016 and under my supervision thereafter for completion of degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled **“SYNTHESIS OF METAL COMPLEXES OF SCHIFF BASES OF HALOGENATED ANILINES AND THEIR ANTIFUNGAL ACTIVITY”** submitted by **Ms. Gursimran Kaur** (Admn. No. L-2011-BS-64-M) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **5-year Integeated M.Sc. (Hons.) Programme,** in the subject of **Chemistry** (Minor subject: **Biochemistry)** has been approved by the Student’s Advisory Committee along with Head of the Department after an oral examination on the same.

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ABSTRACT

Schiff bases of halogenated anilines were synthesized from 4-chloro/4-bromo anilines and substituted aromatic aldehydes. Further, their eight metal complexes were synthesized from Cu (II), Ni (II) Cd (II) and Mn (II) chloride in 1:2 ratio. Microanalytical data, IR, ^{1}HNMR, ^{13}CNMR, CHN, mass and molar conductance measurements were used to characterize the structure of complexes. The complexes were found to be non-electrolytic on the basis of molar conductance studies. The Schiff bases and their metal complexes were screened for their antifungal activity against three test fungi *i.e. Alternaria triticina*, *Bipolaris sorokiniana* and *Ustilago segetum tritici*. Schiff bases and their metal complexes showed significant antifungal activity.

Keywords: Schiff bases, metal complexes, anilines, antifungal potential, spore germination method, phytopathogens.

Signature of Major Advisor **Signature of the Student**

ਖੋਜ ਪ੍ਰਬੰਧ ਦਾ ਸਿਰਲੇਖ	ਹੈਲੋਜੀਨੇਟਡ ਐਨੀਲੀਨਜ ਦੇ ਆਪਣੇ ਧਾਤ ਕੰਪਲੈਕਸ, ਉੱਲੀਨਾਸ਼ਕ ਦੀ ਸ਼ਿਫ ਠਿਕਾਣਾ ਅਤੇ ਸਰਗਰਮੀ ਦੇ ਸੰਸਲੇਸ਼ਣ
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ਸਾਰ-ਅੰਸ਼

ਹੈਲੋਜੀਨੇਟਡ ਐਨੀਲੀਨਜ ਦੇ ਸ਼ਿਫ ਬੇਸਜ਼, 4-ਕਲੋਰੋ / ਬਰੋਮੋ ਐਨੀਲੀਨਜ਼ ਤੇ ਸਬਸੀਟਿਊਟਡ ਐਰੋਮੈਟਿਕ ਐਲਡੀਹਾਈਡਜ਼ ਤੋਂ ਬਣਾਏ ਸਨ । ਇਸ ਤੋਂ ਇਲਾਵਾ 1:2 ਦੇ ਅਨੁਪਾਤ ਵਿੱਚ ਇਹਨਾਂ ਦੇ ਅੱਠ ਮੈਟਲ ਕੰਪਲੈਕਸਜ਼ ਜਿਵੇਂ ਕਿ ਕਾਪਰ (II), ਨਿਕਲ (II), ਕੈਡਮੀਅਮ (II) ਅਤੇ ਮੈਗਨੀਜ਼ (II) ਕਲੋਰਾਈਡ ਬਣਾਏ ਸਨ। ਕੰਪਲੈਕਸਾਂ ਦੀ ਰਚਨਾ ਦੀ ਪਹਿਚਾਣ ਮਾਈਕ੍ਰੋਐਨਾਲਾਈਸਿਜ਼ ਡਾਟਾ, ਆਈ.ਆਰ, ਪ੍ਰੋਟੋਨ ਐਨ ਐਮ ਆਰ, ਕਾਰਬਨ-13 ਐਨ ਐਮ ਆਰ, ਸੀ ਐਚ ਐਨ, ਮਾਸ ਤੇ ਮੋਲਰ ਕੰਨਡਕਟੈਂਸ ਨਾਲ ਕੀਤੀ ਗਈ। ਮੋਲਰ ਕੰਨਡਕਟੈਂਸ ਦੇ ਅਧਿਐਨ ਦੇ ਆਧਾਰ 'ਤੇ ਪਤਾ ਲੱਗਾ ਕਿ ਕੰਪਲੈਖਸ ਗ਼ੈਰ-ਇਲੈਕਟ੍ਰੋਲਿਟਿਕ ਸਨ। ਸ਼ਿਫ ਬੇਸ ਤੇ ਉਹਨਾਂ ਦੇ ਮੈਟਲ ਕੰਪਲੈਕਸਾਂ ਦੀ ਉੱਲੀਨਾਸ਼ਕ ਗਤੀਵਿਧੀ ਤਿੰਨ ਉੱਲੀਆਂ-ਆਲਟਰਨੈਰੀਆ ਟ੍ਰੀਟੀਸਿਨਾ, ਬਾਇਪੋਲੈਰਿਸ ਸੋਰੋਕਿਨੀਆਨਾ ਤੇ ਐਸਟੀਲੈਗੋ ਸੇਜੀਟਮ ਟ੍ਰੀਟੀਸਾਈ ਦੇ ਵਿਰੁੱਧ ਪਤਾ ਕੀਤਾ ਗਿਆ ਹੈ। ਸ਼ਿਫ ਬੇਸਿਜ਼ ਤੇ ਮੈਟਲ ਕੰਪਲੈਕਸਿਜ਼ ਨੇ ਪ੍ਰਭਾਵਸ਼ਾਲੀ ਉੱਲੀਨਾਸ਼ਕ ਗਤੀਵਿਧੀ ਵਿਖਾਈ ।

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ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ

CONTENTS

CHAPTER - I

INTRODUCTION

Synthesis of Schiff bases and their metal complexes having novel structural features and unusual physico-chemical properties have considerable importance in biological processes and constitute an active area of research in modern coordination chemistry. Ever since the Italian chemist, Hugo Schiff used imines to make several “metallo-imines”, numbers of variants of the condensation products of imines and aldehydes or ketones such as RCH=NR'- where R and R' are alkyl and /or aryl substituents, are popularized. They are also known as Schiff bases, anils, imines or azomethines. These have several applications in organic studies, such as for building new heterocyclic systems, for identification, detection, and determination of aldehydes and ketones, for purification of carbonyl or amino compounds, or for the protection of these groups during the complex formation or such sensitive reactions (Patai *et al* 1970). Schiff bases derived from aromatic amines and aromatic aldehyde have a wide variety of application in many fields *e.g.* biological, inorganic and analytical chemistry. Although Schiff bases have been reported to possess antifungal (Dash *et al* 1984, Manrao and Kohli 1986), antibacterial (Dhar and Taploo 1982), antimicrobial (Dehuri and Nayak 1983), nitrification inhibitor activities (Datta *et al* 2001), these are also important precursors for the synthesis of several biologically versatile heterocyclic rings, *e.g.* 1,3-benzoxazines and dithiazolidines (Shakil *et al* 2005).

It is fact that copper, iron, zinc and cobalt are essential metallic elements and exhibit great biological activity when associated with certain metal protein complexes, participating in oxygen transport, electronic transfer reactions or the storage of ions and have created enormous interest in the study of system containing these metals. The interaction of transition metal complexes with DNA has been extensively studied in the development of new tools for nanotechnology. Antioxidants are extensively studied for their capacity to protect organism and cell from damage that is induced by oxidation stress.

Wheat is a cereal grain and is a type of fruit called caryopsis. It is an important staple food and strategic cereal crop of 36% of the world population. It is an excellent source of multiple essential nutrients such as proteins, dietary fiber, manganese, phosphorous and niacin, several vitamins and other dietary minerals and contains 13% water, 71% carbohydrates content significantly. It has higher protein content than any other cereal crop.

Currently, India is the second largest producer of wheat in the world after China with about 12% share in total world wheat production. In 2013, world production of wheat was 713 tones making it third most produced cereal crop after maize and rice (Shewry 2009). The production level of wheat in India had quantum jump from 6.46 million tons from an area of

9.75 million hectares in 1950-51 to more than 93 million tons from an area of about 30 million hectares during 2011-12. Now a days the production of wheat has been affected by various bacteria, fungi and viruses *e.g.* leaf rust (*Puccinia recondita tritici*), yellow rust (*Puccinia striiformis tritici*), stem rust (*Puccinia graminis tritici*), Karnal bunt (*Tilletia indica*) foliar blights (*Bipolaris sorokiniana*, *Alternaria triticina*) powdery mildew (*Erysiphe graminis tritici*) and loose smut (*Ustilago segetum tritici*) (Roelfs and Bushnell 1985) which affects the yield and quality of seed of wheat. Pathogens have caused severe epidemics in the entire past. Pathogens easily attack crops because they wide spread by wind and they easily adapt to host resistance (Hovmoller *et al* 2002, Kolmer 2005) that make easy for pathogens to attack crop.

Foliar fungicides *e.g.* pyraclostrobin, propiconazole, tebuconazole, metaconazole *etc.*, are very effective in controlling diseases but they need proper time of application, application cost, effectiveness and the risk of developing fungicide resistance. Fungicides based on triazole (*e.g.* fluconazole, lanosterol and anvoriconazole), act by inhibiting cytochrome P450 14a-desmethylase (CYP51) and recently became the most rapidly expanding group of antifungal compounds (Sun *et al* 2007). However, their clinical value has been limited by their relative high risk of toxicity, the emergence of drug resistance, pharmacokinetic deficiencies and insufficiencies in their antifungal activities.

Development of cereal varieties that carry resistance against diseases is always a big challenge due to cost and availability of new breeds. Therefore, development of fungicides against diseases is thus a main focus for plant breeders and researchers.

Although, a number of fungicides have been synthesized by the scientists to control such diseases, but due to their cost, non-availability and environment sustainability, there is still a need to synthesize new ecofriendly and easily available fungicides. Therefore, it will be useful research effort to study the effectiveness of the Schiff bases and their metal complexes against different phytopathogenic fungi.

The work was undertaken with the following objectives:

1. To synthesize Schiff bases and their transition metal complexes.
2. To evaluate antifungal activity of synthesized Schiff bases and their respective metal complexes against *Alternaria triticina*, *Bipolaris sorokiniana* and *Ustilago segetum tritici*.

The thesis runs into several chapters, namely, review of literature, materials and methods, results and discussion followed by summary.

Since almost the entire investigation incorporated in this thesis is about synthesis of Schiff bases and their metal complexes of Cd (II), Mn (II), Ni (II) and Cu (II) and their bioefficacy against fungi of wheat *A. triticina, B. sorokiniana* and *U. segetum tritici*, a review of different Schiff bases, their metal complexes and their biological activities was thought to be appropriate. This review is given in chapter 2 of the thesis. Various methods and techniques employed and different reagents and chemicals used during investigations are given in chapter 3. In chapter 4, results of our investigations along with discussion have been described. Chapter 5 comprises the summary of the research work carried out. The references cited in the text are alphabetically arranged at the end of the thesis.

CHAPTER – II

REVIEW OF LITERATURE

A number of methods are available for introducing a carbon-nitrogen double bond in a molecule and the most convenient being condensation of an aldehyde or ketone with primary amines (Layer and Carman 1968). General reaction scheme (scheme 1) for this is as follows:

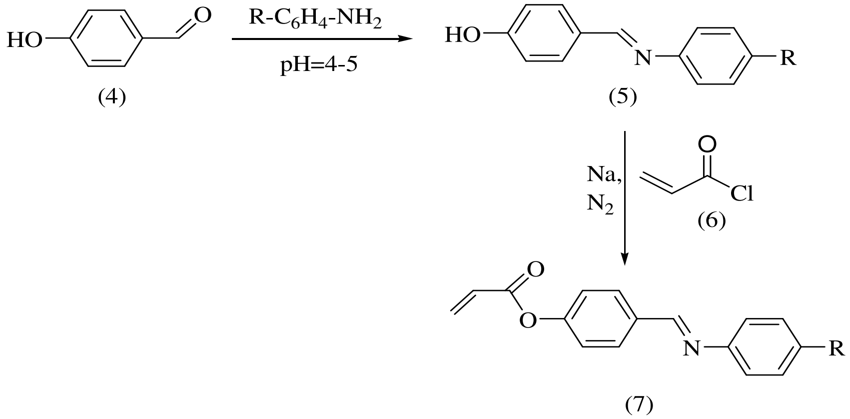
$$\underset{(1)}{RNH_2} + \underset{(2)}{R^2\overset{O}{\overset{\|}{C}}\text{-}R^1} \longrightarrow \underset{(3)}{R^2R^1C{=}N\text{-}R}$$

Amine Carbonyl compound Schiff's base

Scheme 1

Schiff bases are one of the most widely used organic compounds. They are used as pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilizers. Schiff bases have also been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic properties. The imine group present in such compounds has shown to be critical to their biological activities. In the following part, some of the different methods for synthesizing Schiff bases; their metal complexes and their biological activities have been discussed.

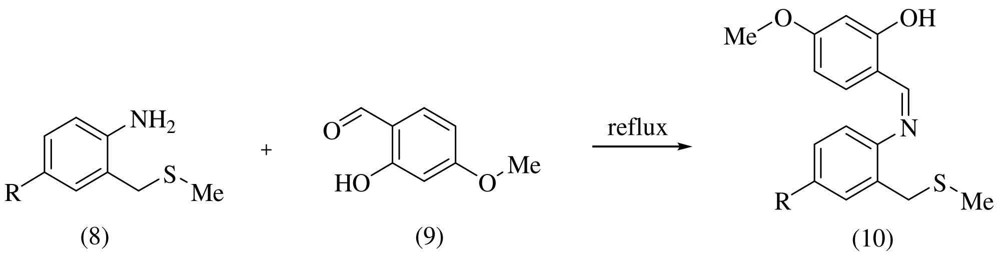
Silku *et al* (2016) synthesized novel Schiff bases of the type (5) by reacting *p*-hydroxybenzaldehyde (4) with aniline and substituted anilines, which on further reaction with Na and acrylolyl chloride (6) yielded acryloyl derivatives of Schiff bases (7) as shown in scheme 2.



R = H, *p*-NO_2, *p*-Cl, *p*-Me

Scheme 2

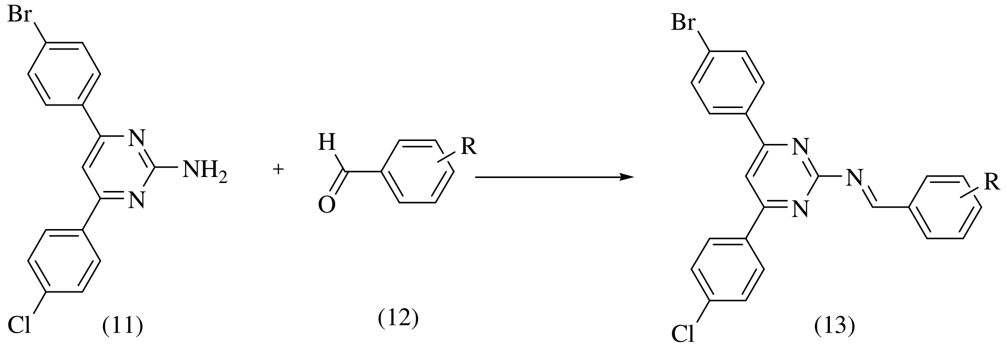
Schiff bases of the type (10) were derived by condensation reaction of (methylthiomethyl) anilines (8) and 4-methoxysalicylaldehyde (9) by Olalekan *et al* (2016) as shown in scheme 3. Further the coordination complexes of the Schiff bases were synthesized by refluxing of the Schiff base with $CuCl_2.2H_2O$.



R = H, Me, OMe, Cl, Br, NO_2

Scheme 3

Prasad *et al* (2016) synthesized Schiff bases (13) by reacting a mixture of 4-(4-bromophenyl)-6-(4-chlorophenyl)-2-aminopyrimidine (11) and substituted benzaldehyde (12) in ethanol as shown in scheme 4. The synthesized compounds were screened for antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa, Bacillus subtilis* and *Escheirchia coli* and it was found that the compound 4-(4-bromophenyl)-6-(4-chlorophenyl)-2-aminopyrimidine (11) showed good antibacterial activity against the gram positive bacteria *S. aureus, B. subtilis* and the compounds like {[4-(4-bromophenyl)-6-(4-chlorophenyl) pyrimidin-2-yl]imino}methyl]phenol showed good antibacterial activity against gram negative bacteria *P. aeruginosa* and *E. coli.*

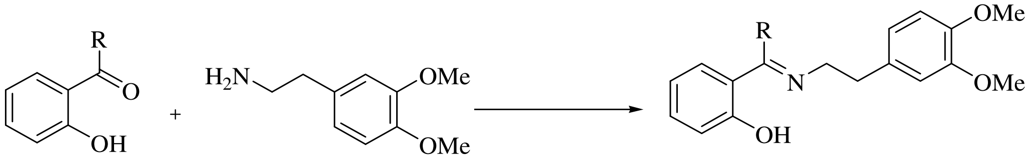


R = H, *p*-OMe, *p*-OH

Scheme 4

Schiff base ligands (16) were synthesized by Satheesh *et al* (2016) by condensation of 2-hydroxy benzaldehyde (14) and 2-(3,4-dimethoxyphenyl)ethanamine (15) as shown in scheme 5. Ni (II) and Pd (II) complexes were formed by reacting the chlorides of the metals with the Schiff bases. The Schiff bases and the metal complexes were screened for antimicrobial activity against gram negative bacteria *E. coli, Klebsiella aerogenes* and

Pseudomonas desmolyticum, gram positive bacteria *S. aureus* and two fungi *Aspergillus flavus* and *Candida albicans*. The synthesized complexes were found to be more toxic than their parent ligand.

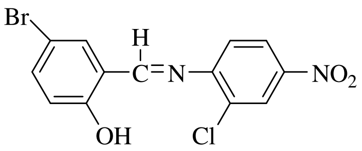


(14) (15) (16)

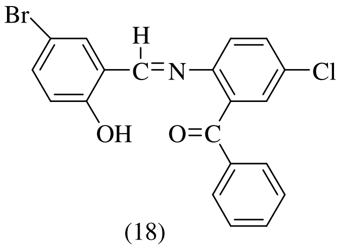
R = H, Me

Scheme 5

Jain and Mishra (2016) synthesized the coordination complexes of Cr (III), Co (II), Ni (II) and Cu (II) derived from 5-bromosalicylidene-2-chloro-4-nitroaniline (17) and 5-bromosalicylidene-2-amino-5-chlorobenzophenone (18). The Schiff bases and metal complexes showed a good activity against the gram-positive bacterium, *S. aureus* and gram-negative bacterium *E. coli* and fungi *Aspergillus niger* and *C. albicans*. Metal complexes were found to be biologically more active as compared to the parent Schiff base ligand against all pathogenic species.

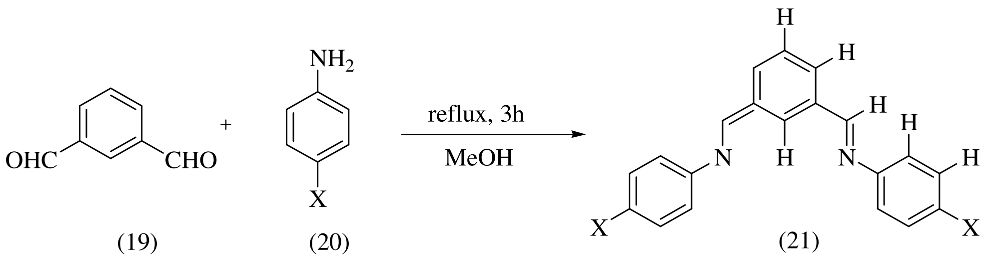


(17)



(18)

Salehi *et al* (2015) synthesized two new Schiff bases (21) by the reaction between isophthalaldehyde (19) and appropriate aniline derivatives (20) as shown in scheme 6.



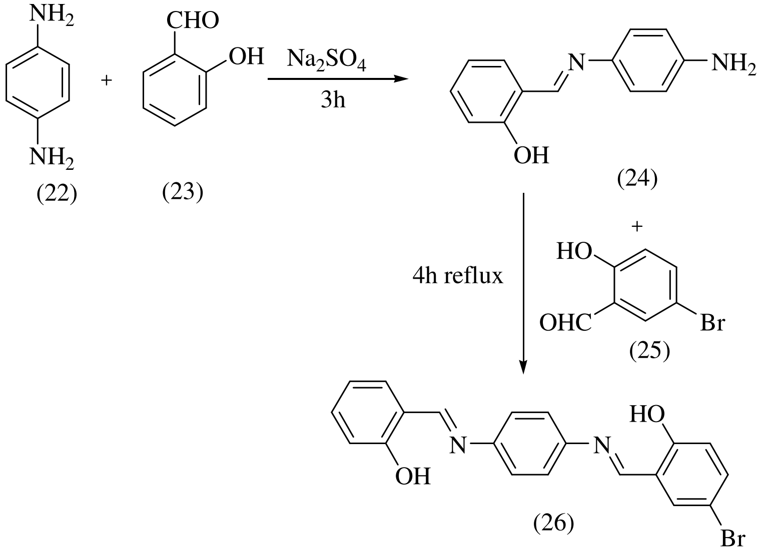
(19) (20) (21)

X= Br, O, Me

Scheme 6

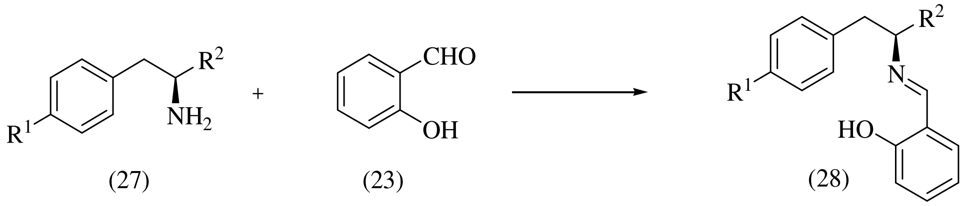
The compounds so formed (21) were screened for *in vitro* antibacterial activities against two gram positive *S. aureus* and *B. subtilis* and two gram negative bacteria *E. coli* and *Salmonella typhi* and it was found that one of the new Schiff base had effective and selective antibacterial activity against the tested bacteria.

Rajaei and Mirsattari (2015) synthesized a new asymmetrical Schiff base (24) by refluxing a mixture of *p*-phenyl diamine (22) in ethanol and a solution containing 2-hydroxybenzaldehyde (23) in 60 ml ethanol, (Scheme 7). The amino derivatives of the Schiff base (24) so obtained were reacted with substituted aromatic aldehyde (25) to yield yellow precipitate of (26) which on further reaction with copper (II) acetate monohydrate furnished Cu (II) complexes.



Scheme 7

Xia *et al* (2015) synthesized a Schiff base (28) by reaction between amidogen of amino acids (27) and 2-hydroxybenzaldehyde (23) in the presence of alkali as shown in scheme 8. The synthesized compounds were analyzed for antibacterial activity against *E. coli* and *B. subtilis* and all the compounds were found to possess good antibacterial activity.

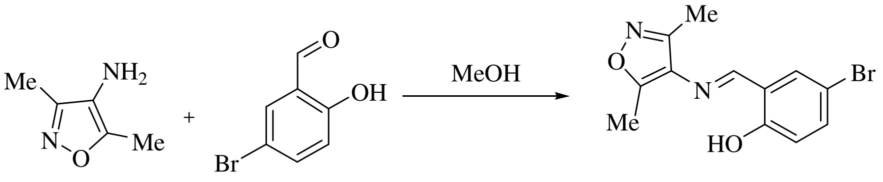


R^1 = H, OH

R^2 = COOK, COOMe

Scheme 8

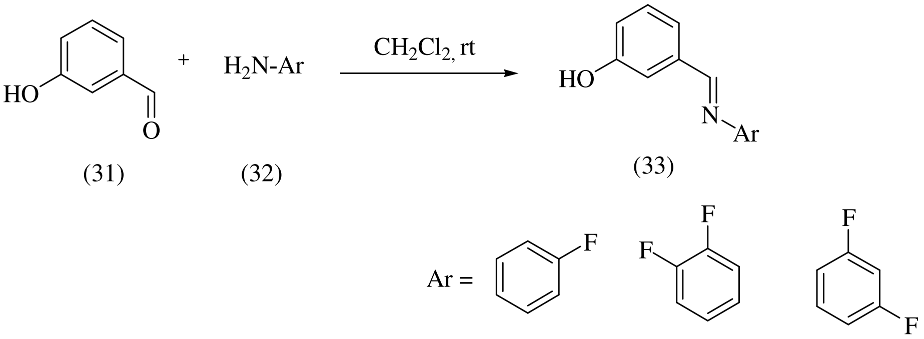
Three novel binary Cu (II) complexes were synthesized by Kumar *et al* (2015) from the synthesized Schiff bases as shown in scheme 9. One of the Schiff base (30) was derived by the reaction between 3,5-dimethyl-4-amino-isoxazole (29) and 2-hydroxy-5-bromo benzaldehyde (25). The resulting mixture was refluxed with stirring for 3 h. The dark yellow product so formed (30) was washed with petroleum and recrysrtallized from methanol. Metal complexes were formed by adding hot methanolic solution of $Cu(CH_3COO)_2{\cdot}H_2O$ to the Schiff base (30). The *in vitro* antibacterial activity of the Schiff base ligand and their complexes were tested against the gram negative bacteria *E. coli, Pseudomonas putida* and *Klebsiella pneumonia* and gram positive bacteria *B. subtilis* and *S. aureus*. The antifungal activity was tested against *A. niger* and *C. albicans* and it was evaluated that the complexes exhibited higher antibacterial activity as compared to the Schiff bases.



(29) (25) (30)

Scheme 9

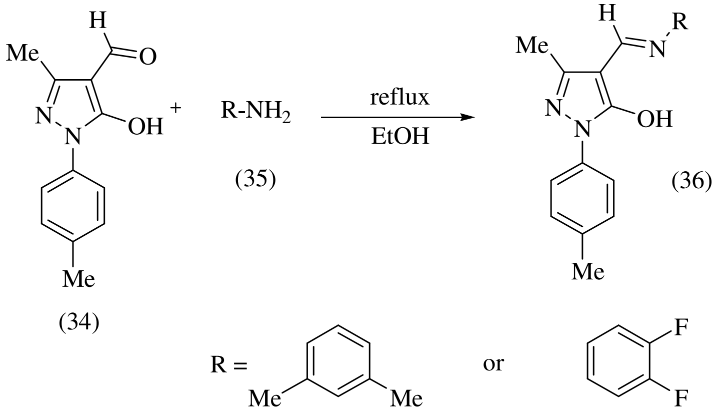
A series of fluorinated Schiff bases (33) were synthesized by Sorrosa *et al* (2015) by the condensation reaction of 3-hydroxybenzaldehyde (31) with different fluorinated anilines (32) as shown in scheme 10. The series of fluorinated Schiff bases were obtained as microcrystalline solids in good yield. The antibacterial activity of the imino fluorinated compounds were evaluated against both gram positive, *S. aureus* and *B. subtilis* and against gram negative bacteria *E. coli* and *K. pneumonia* and it was found that the compound 3-((*E*)-(2,4,6-trifluorophenylimino)methyl)phenol exhibited slight activity towards *E. coli*, moderate activity in case of *K. pneumonia* and no activity against all gram positive organisms. The best activity results for both the type of organisms *i.e.* (gram positive and gram negative) were shown by the compounds which contained either -F or $–CF_3$ substituents.



(31) (32) (33)

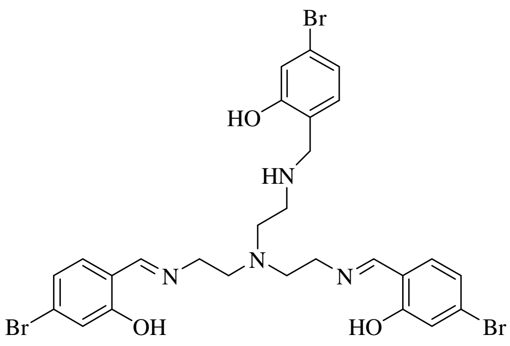
Scheme 10

Two new Cu (II) complexes of pyrazolone based Schiff base ligands were synthesizd by Joseph *et al* (2015). The Schiff base ligands (36) were synthesized by adding 4,5-dihydro-5-hydroxy-3-methyl-1-*p*-tolyl-1H-pyrazole-4-carbaldehyde (34) dissolved in hot absolute ethanol to 2,4-dimethylaniline and 3,4-difluoroaniline (35) as shown in scheme 11. The reaction mixture was refluxed for 12 h, cooled, filtered and dried in vaccum. Cu (II) complexes were prepared by reacting the corresponding metal salts with the Schiff base and the reaction mixture was refluxed for 4 h. The Schiff bases and their metal complexes were evaluated for their antibacterial activity against *E. coli, B. subtilis*, *S. aureus* and *P. aeuroginosa* by using the following standards *e.g.* Levofloxacin, Moxifloxacin, Amoxicillin, Cefprozil, Ciprofloxacin *etc.,* and from the results it was found that the Cu (II) complexes showed good inhibition against the bacterial species than the Schiff base ligand due to the inhibition of enzyme activity of the bacterial system.

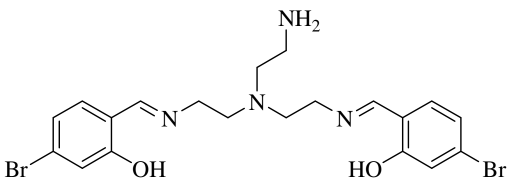


Scheme 11

Sang *et al* (2015) prepared a novel phenolato-and peroxo-bridged dinuclear cerium (IV) complex with tripodal Schiff bases (37) and (38). The complex was prepared by the reaction of 4-bromosalicylaldehyde, *tris*(2-aminoethyl)amine, and cerium (III) trifluoromethanesulfonate in methanol. Antibacterial activities of the tripodal Schiff bases and the complex were tested *in vitro* against *B. subtilis, S. aureus*, *E. coli* and *Pseudomonas fluorescens* and the complex was found to have effective activity against the tested micro-organisms.

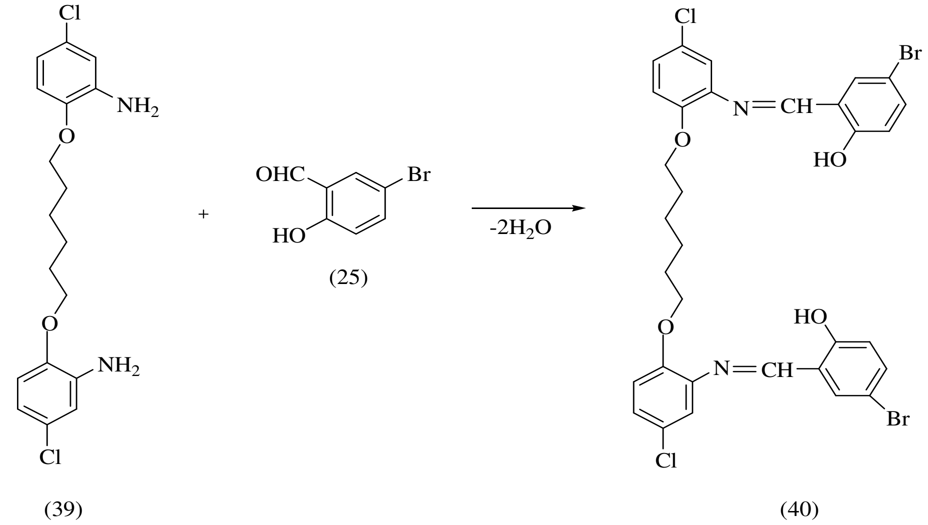


(37)



(38)

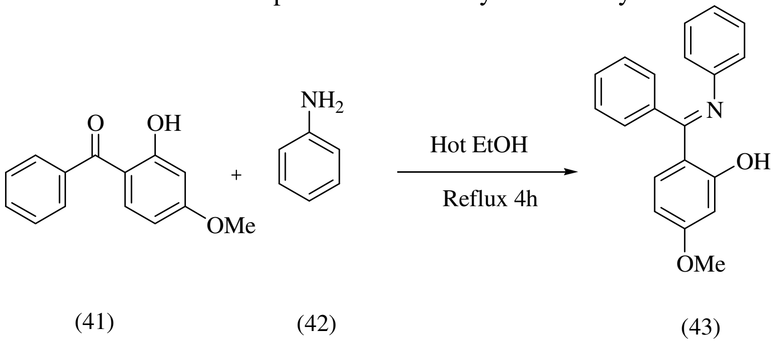
Iihan *et al* (2014) synthesized a new Schiff base (40) by the reaction of 5-bromosalicylaldehyde (25) with equivalent amount of 1,6-*bis*(4-chloro-2-aminophenoxy) hexane (39) in ethanol medium as shown in scheme 12.



Scheme 12

The reaction was continued for 2 h and the mixture was cooled to room temperature. Solution of metal salts Cu (II), Zn (II) Ni (II) and Co (II) were reacted with the Schiff base (40) in the ratio 1:1 to form the complexes. The reaction mixture was stirred for 2 h and the content was cooled to the room temperature. The compounds were analyzed for their antibacterial activity and antimicrobial activity against *E. coli, B. subtilis* and *S. aureus* and it was found that except Cu (II) complexes, all the compounds showed slightly weak antibacterial activity against *B. subtilis*. Ni (II) complexes showed the highest antibacterial activity against *E. coli*.

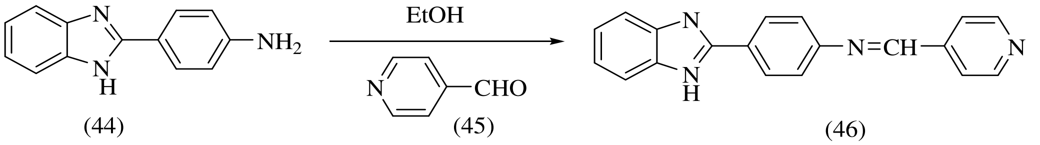
Subbaraj *et al* (2014) synthesized a new bidendate NO type Schiff base (43) by reacting 2- hydroxyl-4-methoxy-phenyl phenylmethanone (41) with aniline (42) as shown in scheme 13 and further its metal complexes have been synthesized by reaction with different



Scheme 13

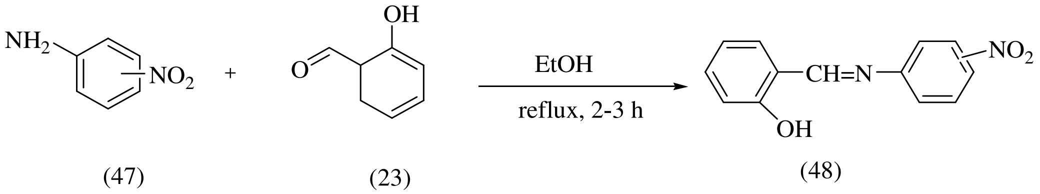
metal salts like Mn (II), Co (II), Ni (II), Cu (II) and Zn (II). The Cu (II) complexes were found to show higher *in vitro* antimicrobial activity than the other complexes against different microorganisms.

Zhang *et al* (2014) synthesized an asymmetrical Schiff base ligand 4-(1H-benzimidazol-2-yl)-phenyl]-pyridin-4-methyl amine (46) by the reaction between 4-(2-benzimidazolyl) aniline (44) and 4-pyridine formaldehyde (45) as shown in scheme 14. Single crystals of Mn (II) and Cd (II) complexes were obtained by the diffusion of diethyl ether into the mixture of the ligand and Mn (II) and Cd (II) salts in methanol. The Schiff base (46) and the metal complexes were evaluated for their antibacterial activity against *E. coli, P. aeruginosa* and *Sporosarcina ureae*, and it was found that Mn (II) complex showed better activity against *E. coli* and Cd (II) complex was a good antibacterial agent against *P. aeruginosa*.



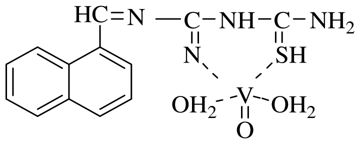
Scheme 14

Ekta *et al* (2014) synthesized ligands 2-((3-nitrophenylimino) methyl) phenol and 2-(4-nitrophenylimino) methyl) phenol (48) by refluxing of (47) and (23) as shown in scheme 15. Further six metal complexes of Cd (II), Mn (II) and Zn (II) were synthesized from these ligands. The Schiff bases and their metal complexes were screened for their antinemic activity against root knot nematode, *Meloidogyne incognita*. Metal complexes were found to show more significant activity than their corresponding Schiff bases.



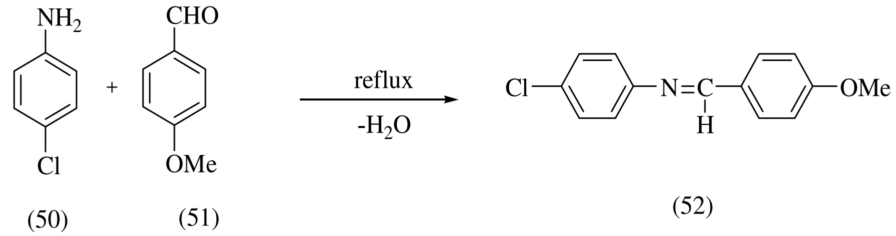
Scheme 15

The co-ordination complexes of VO (II) (49) were synthesized by Shukla and Mishra (2014) from the Schiff base which was derived by reaction between methanolic solution of aldehyde or ketone with methanolic solution of amine in 1:1 equimolar ratio. The reaction mixture was refluxed for 5-8 h, the colored solid so obtained was filtered and recrsytallized from ethanol. The VO (II) complexes were prepared by mixing methanolic solution of $VOSO_4.nH_2O$ with the methanolic solution of Schiff base in 1:1 and 1:2 M ratio.



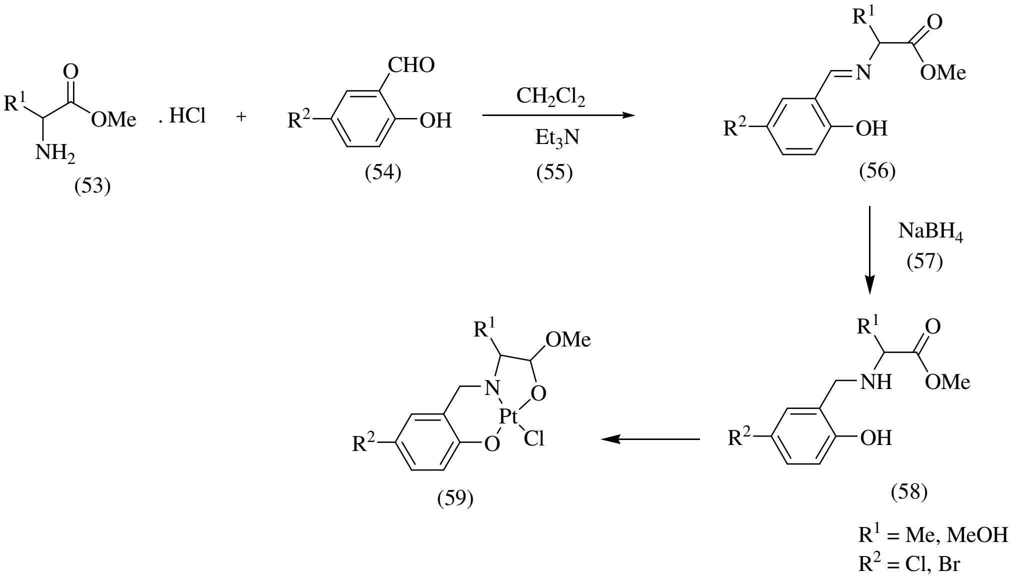
(49)

4-Chloro-4-methoxy benzylideneaniline (52), an optical material, was synthesized by Leela *et al* (2014) by the condensation reaction between *p*-chloroaniline (50) and *p*-methoxy benzaldehyde (51) in equimolar ratio as shown in scheme 16. The reaction mixture was refluxed for about 8 h and the solution was filtered using the Whatman filter paper and the resulting product of 4-chloro-4-methoxy benzylideneaniline (52) was obtained.



Scheme 16

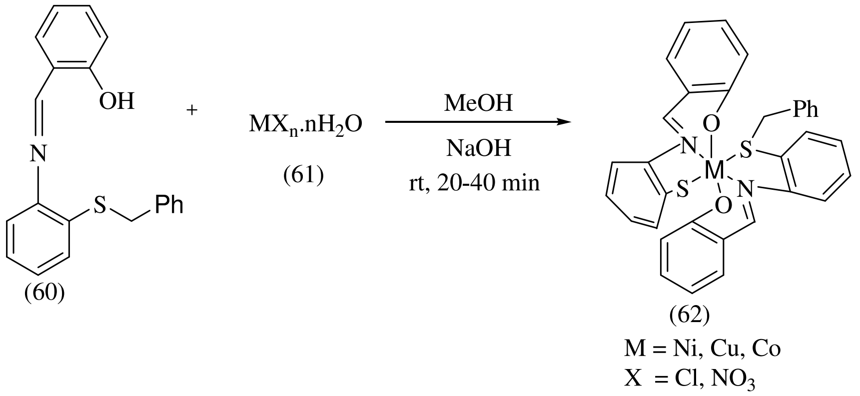
Li *et al* (2014) synthesized a series of platinum (II) complexes (59) with reduced amino acid ester Schiff bases as ligands (56) as shown in scheme 16. Amino acid esters Schiff bases (56) were prepared by adding amino acid ester (53) to a round bottom flask containing an aromatic aldehyde (54) dichloromethane, triethylamine (55), and a certain amount of



Scheme 17

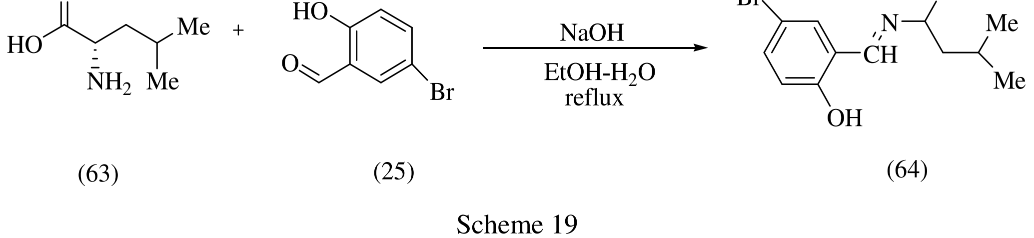
anhydrous magnesium sulfate, the reaction mixture was stirred for 1h at room temperature, salicyladehyde solution was added to the dichloromethane solution which was stirred for another 36 h, further to this mixture methanolic solution containing sodium borohydride (57) was added. The reduced amino acid Schiff bases (58) formed were purified by column chromatography. Further, Pt (II) complexes (59) were synthesized from (58). The *in vitro* antitumour activity of the complexes have been validated and the complex Pt[methyl 2-(5-chloro-2-hydroxybenzylamino)-4-methylpentanoate]Cl was found to be the most effective agent among the complexes.

Kalita *et al* (2014) synthesized a Schiff base (60) by the reaction between (2-benzylthio)aniline and 5-bromo 2-hydroxy benzaldehyde. The solution was refluxed for 30 min and the color of the solution changed to orange red which was kept undisturbed for 6 h at room temperature. Series of Ni (II), Co (II) Cu (II) and Pd (II) complexes (62) were formed by reacting metal salts (61) with the Schiff base (60) as shown in scheme 18. The ligand and the complexes were tested for their *in vitro* antibacterial activities against the gram positive and gram negative bacteria *S. aureus* and *K. pneumonia* and it was observed that Cu complex with lower stock concentration was active against both bacterial strains.



Scheme 18

Lekha *et al* (2014) synthesized several new lanthanide complexes Pr (III), Sm (III), Gd (III), Tb (III), Er (III) and Yb (III) with the sodium salt of the Schiff base (64) which was

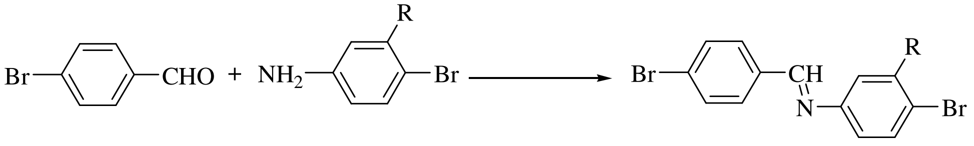


Scheme 19

derived from leucine (63) and 5-bromosalicylaldehyde (25) as shown in scheme 19. The Gd

(III) Schiff base complex was found to be an efficient catalyst for the oxidation of anilines and substituent anilines under mild condition.

The synthesis of (4-bromobenzylidene)-(4-bromo-phenyl)-imine (67) was carried out by Marin *et al* (2013) by the acid catalyzed reaction of 4-bromobenzaldehyde (65) with 4-bromoaniline (66) as shown in scheme 20. The obtained mixture was gently refluxed overnight and was kept undisturbed for 24 h and the obtained crystals were filtered washed and recrystallized from ethanol.

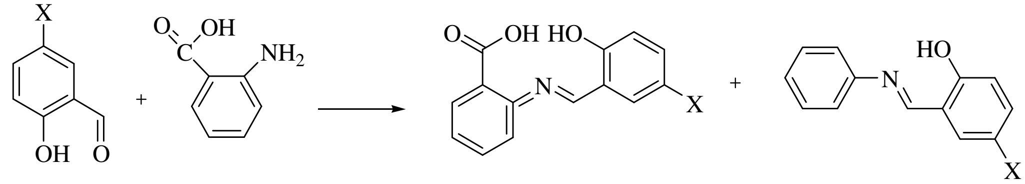


(65) (66) (67)

R= H, Me

Scheme 20

Five Cu (II) complexes have been prepared by Zhu *et al* (2013) from tridentate (70) and bidentate Schiff bases (71) which were synthesized from an equimolar solution of 5-X-salicyaldehyde (68) and anthranilic acid or aniline (69) as shown in scheme 21. The Cu (II) complexes were further synthesized by the reaction of Schiff base with $Cu(OAc)_2.2H_2O$ in water or ethanol. One of the Cu (II) complexes with a ratio of 2:1 ligand showed very strong inhibition against protein tyrosine phosphatase pathway.



(68) (69) (70) (71)

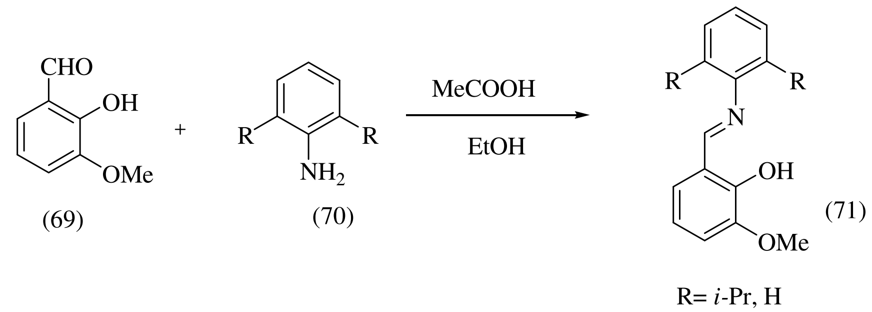
X = Br, Cl, NO_2

X = Br, Cl, NO_2

X = Cl, NO_2

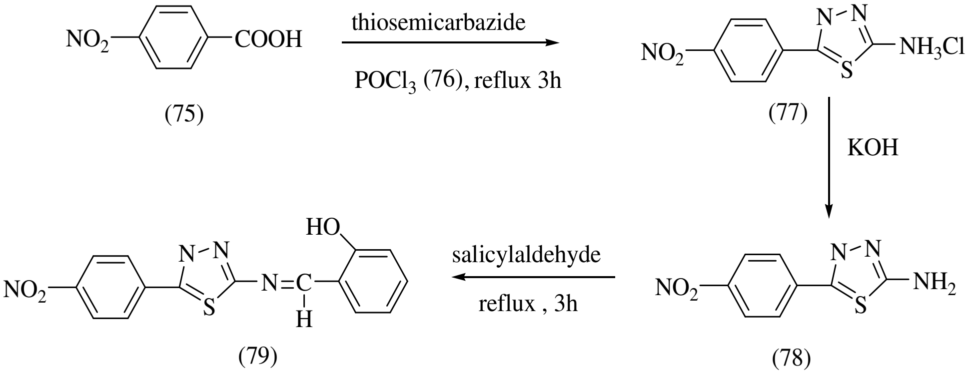
Scheme 21

Upadhyay *et al* (2013) synthesized square planar complexes of Cu (II) and Ni (II) from the Schiff base (74). The Schiff base was synthesized by the reaction between 2-hydroxy-3-methoxy benzaldehyde (72) which was dissolved in 25 ml of ethanol with equimolar amount of 2,6 di-isopropylaniline or aniline (73) as shown in scheme 22. The contents were heated under reflux for 24 h. The reaction between the deprotonated ligand with the chloride precursors of Cu (II) and Ni (II) offered the corresponding metal complexes.



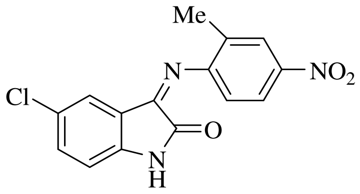
Scheme 22

A total of new metal complexes of 2N-salicylidene-5-(*p*-nitrophenyl)-1,3,4-thiadiazole, with the metal ions VO (II), Co (II), Rh (III), Pd (II) and Au (III) have been successfully prepared in alcoholic medium by Yousif *et al* (2013). The Schiff base (79) was prepared by refluxing the mixture of 4-nitrobenzoic acid (75), thiosemicarbazide and phosphorous oxychloride (76) for 3 h, the obtained filtrate (77) was neutralized with potassium hydroxide, then the precipitate was washed, filtered and recrystallized using ethanol to obtain 5-(4-nitrophenyl)-1,3,4,-thiadiazol-2-amine (78). Further, (78) was heated under reflux for 3 h to obtain the yellow precipitate of (79) as shown in scheme 23. The synthesized complexes and the Schiff base were screened for their *in vitro* antibacterial activity against *S. aureus, S. typhi* and *E. coli* and it was found that all the complexes showed moderate activity against the tested bacterial strains and a slightly higher activity as compared to the Schiff base.



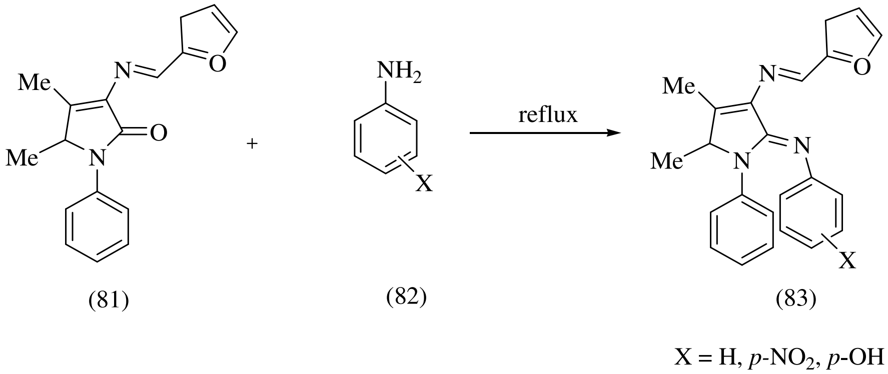
Scheme 23

Cu (II), Co (II), Ni (II) and Zn (II) complexes of 5-chloroisatin Schiff base (80) were synthesized by Rao *et al* (2013). The metal complexes were prepared by adding solution of metal salts in 50 ml absolute ethanol to 100 ml of an ethanolic solution of the Schiff base (80) in the ratio 1:1 followed by heating under reflux conditions for about 2-4 h. The reaction mixture so formed was cooled and crystallized metal salts were then filtered and dried under vacuum over fused calcium chloride. The Schiff base and its metal complexes were screened for antibacterial activities against bacteria *S. aureus* and *E. coli etc*., and antifungal activity against fungi *A. niger* and *A. flavous etc*. and it was found that the ligand, Schiff base and its metal complexes were highly active at higher concentrations.



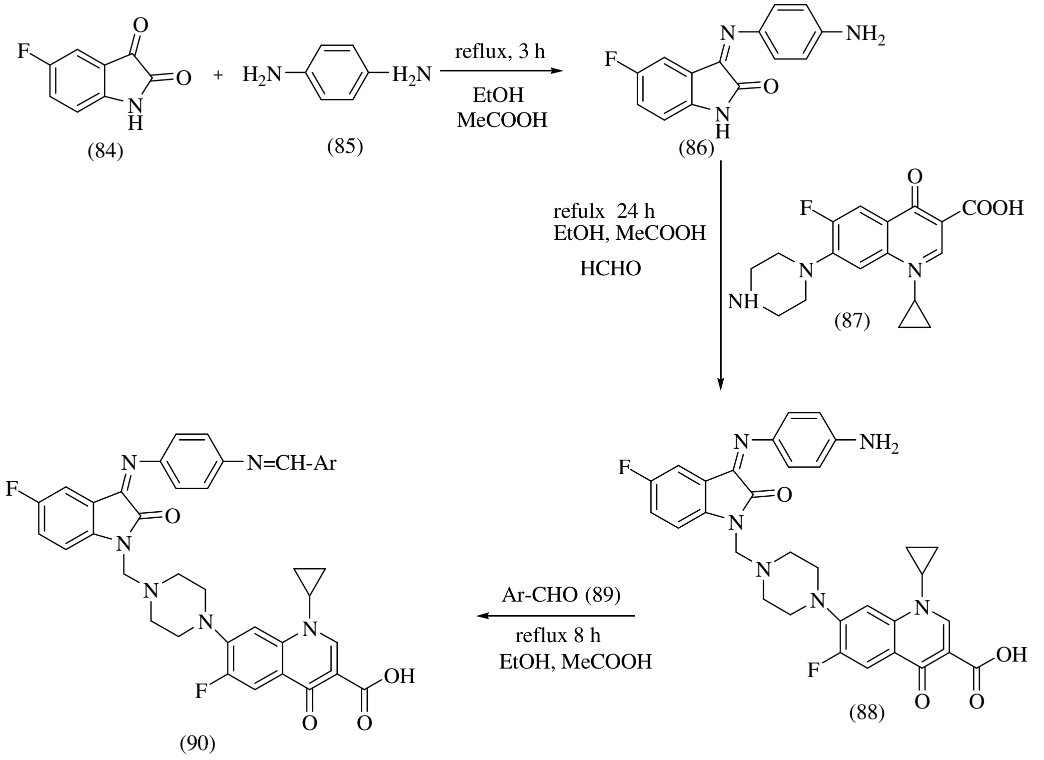
(80)

Cu (II) complexes have been synthesized from the Schiff base ligands (83) by Joseph *et al* (2013) which have been derived from furfurylidene-4-aminoantipyrine (81) and aniline and substituted anilines (82) as shown in scheme 24. The synthesized compounds were screened for their *in vitro* biological activity against the bacterial species *S. aureus, E. coli, K. pneumonia, Proteus vulgaris* and *P. aeruginosa* and fungal species, *A. niger, Rhizopus stolonifer, A. flavus, Rhizoctonia bataicola* and *C. albicans*, and from the results it was evaluated that the Cu (II) complexes were proved as active bactericidal and fungicidal agents as compared to the uncomplexed Schiff base ligands.



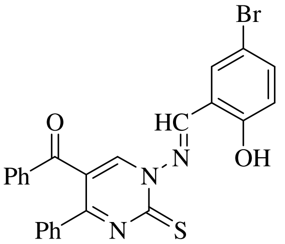
Scheme 24

Prakash and Raja (2013) synthesized a series of novel ciprofloxacin methylene isatin derivatives by incorporating different aromatic aldehydes. Initially, 5-fluoro isatin (84) and 4-amino aniline (85) were used as starting material to synthesize 3-(4-aminophenylimino)-5-fluoroindolin-2-one (86) by a condensation reaction. Further, this compound (86) was treated with ciprofloxacin (87) to give 7-(4-((3-(4-aminophenylimino)-5-fluoroindolin-1-yl)methyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihyroquinoline-3-carboxylic acid (88). This mannich base (88) was treated with different aromatic aldehydes (89) in the presence of glacial acetic acid to isolate the Schiff base (90) as shown in scheme 25. All the compounds were evaluated for their antimicrobial activity against *S. aureus* and *P. aeruginosa* and antifungal activity against *A. niger and* it was found that the derivatives with electron withdrawing groups like nitro and chloro showed least activity against the tested microorganisms.



Scheme 25

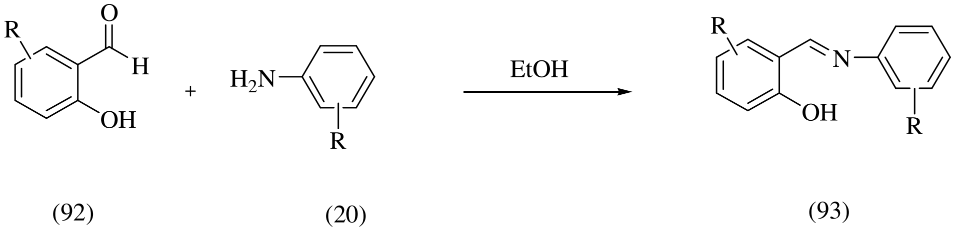
Gulcan *et al* (2012) synthesized a new Schiff base {1-[5-bromo-2-hydroxy-benzylidene)-amino]-4-phenyl-2-thioxo-1,2-dihydro-pyrimidin-5-yl}-phenyl-methanone (91) which was prepared by the condensation of 1-amino-5-benzoyl-4-phenyl-1*H* pirimidine-2-thione with 5-bromosalicyaldehyde by refluxing the mixture for 3h. The precipitated ligand so formed was filtered and recrystallized from ethanol. Metal complexes were prepared from the acetate salts of Cu (II), Ni (II), Co (II), Pt (II), and Pd (II), and $PtCl_2$ in methanol. All the compounds were evaluated for their antimicrobial activity against *S. aureus* and *E. coli* and



(91)

for antifungal activity against *C. albicans* and it was found that the Co (II) and Cu (II) complexes possess effective and selective antibacterial activity against *S. aureus* and *E. coli.*

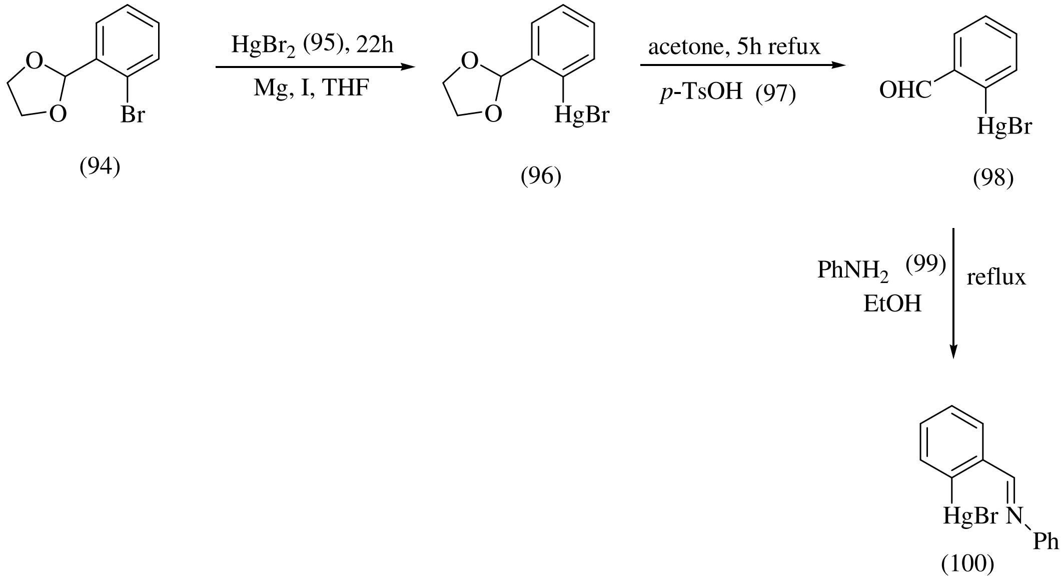
A set of substituted Schiff bases (93) were synthesized by Altmann *et al* (2012) by reacting the solution of salicyaldehyde derivative (92) in ethanol with a solution of aniline derivative (20) which was dissolved in ethanol as shown in scheme 26. The mixture was refluxed after stirring for 1h at room temperature and the obtained Schiff bases were purified by recrystallization. All the Schiff bases formed were reacted with methyltrioxorhenium to form stable complexes.



R = Cl, Br, F, NO_2, CF_3

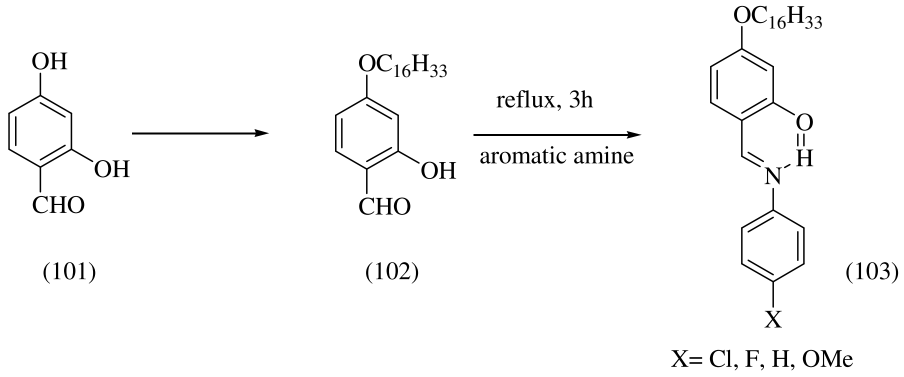
Scheme 26

Baligar *et al* (2011) synthesized Schiff bases (100) and ther derivatives by the reaction between the precursors (94), and (95) which were prepared from 2-bromobenzaldehyde. The reaction of the intermediate, (2-(1,3-dioxolan-2-yl)phenyl)magnesium bromide obtained from (94) with 1 equivalent of $HgBr_2$ (95) yielded (96) which on subsequent reaction with *p*-toluenesulfonic acid monohydrate (97) gave (98) as showm in scheme 27. The condensation reaction of (98) with different monoamines (99) led to the synthesis of the mono lewis acidic Schiff bases.



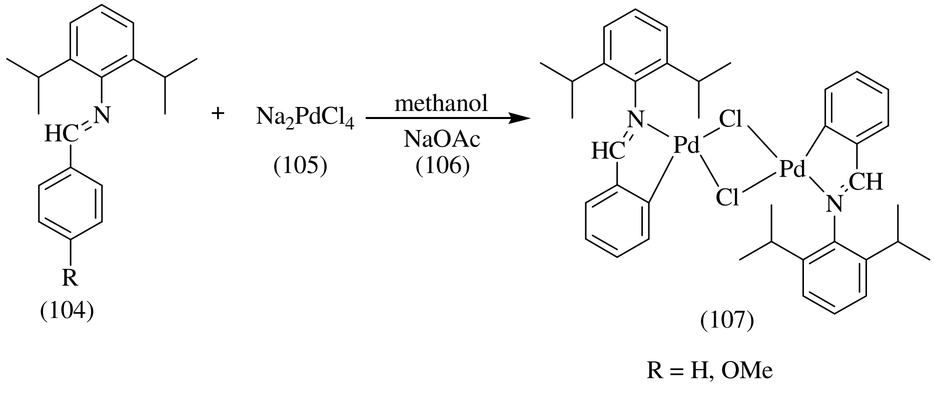
Scheme 27

Chakraborty *et al* (2011) synthesized Co (II), VO (IV) and Gd (III) complexes which were derived from polar Schiff base (103). Schiff bases were synthesized by refluxing a mixture of ethanolic solution (1 mmol) of the 4-*n*-hexadecyloxysalicylaldehyde (102) with 1 mmol of appropriate amine and few drops of glacial acetic acid as catalyst for 3 h as shown in Scheme 28.



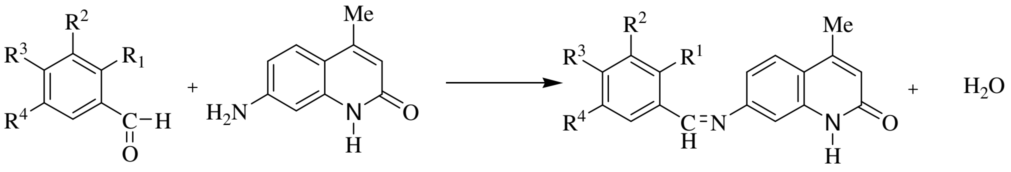
Scheme 28

Jiang *et al* (2011) synthesized μ-chloro bridged dinuclear cyclometallated Pd (II) complexes (107) by reacting Na_2PdCl_2 (105) with benzylidene anilines (104). The Schiff-base ligands 2,6-diisopropyl-N-(benzylidene)aniline (104) was prepared by condensation of 2,6-diisopropylaniline with the corresponding aldehyde in dried alcohol as solvent. On treatment of the Schiff base ligand (104) with Na_2PdCl_2 (105) in methanol in the presence of NaOAc (106) at room temperature yielded *μ*-chloro bridged dinuclear cyclometallated Pd (II) complex (107) as shown in scheme 29.



Scheme 29

Quinolin-2(1H)-one derived Schiff bases (110) and its copper (II) complexes were prepared by Crevan *et al* (2010). All the Quinolin-2(1H)-one derived Schiff bases were prepared by equimolar condensation reaction between substituted aromatic aldehyde (108)



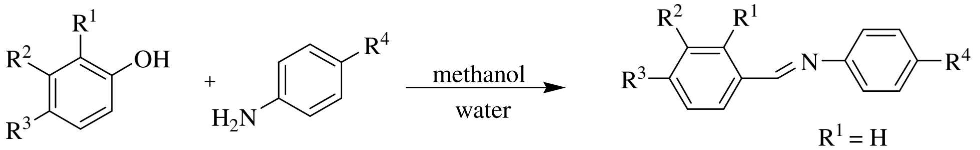
(108) (109) (110)

R^1 = OH, H
R^2 = H, OMe
R^3 = OH, H, OMe
R^4 = H, Br, Cl, F, I

Scheme 30

and 7-amino-4-methylquinolin-2(1H)-one (109) in hot ethanol and the mixture was refluxed for 4 h as shown in scheme 30. On cooling at room temperature coloured precipitates of the ligand were formed in good yield. All the copper (II) complexes were synthesized by reacting Cu (II) acetate or Cu (II) perchlorate hexahydrate, which was dissolved in methanol and was added drop wise to hot methanolic solution of the appropriate ligand. The resulting mixture was refluxed with stirring for 5 h until a precipitate was formed. Quinolinone ligands and their Cu (II) complexes were evaluated for their antibacterial activity against *C. albicans* and it was found that the ligand and its Cu (II) complexes showed considerable activity against the bacteria *C. albicans*. Synthesized compounds were also screened for their *in vitro* anticancer potential using the human hepatic carcinoma cell line, and all the compounds except a few showed moderate cytotoxicity.

Cheng *et al* (2010) synthesized eight hydroxyl-substituted Schiff bases (111) by the reaction between the corresponding aromatic aldehyde (108) and aniline (42) in small amount of water or methanol as shown in scheme 31. The synthesized Schiff bases were bio-evaluated for their antioxidant and antiproliferative activities in pursuit of more active antioxidant and cancer chemopreventive agents. From the structure–activity relationship analysis it was found that *o*-diphenolic groups and 4-hydroxyl group attached to the aromatic ring were vital for their antioxidant and antiproliferative activities.

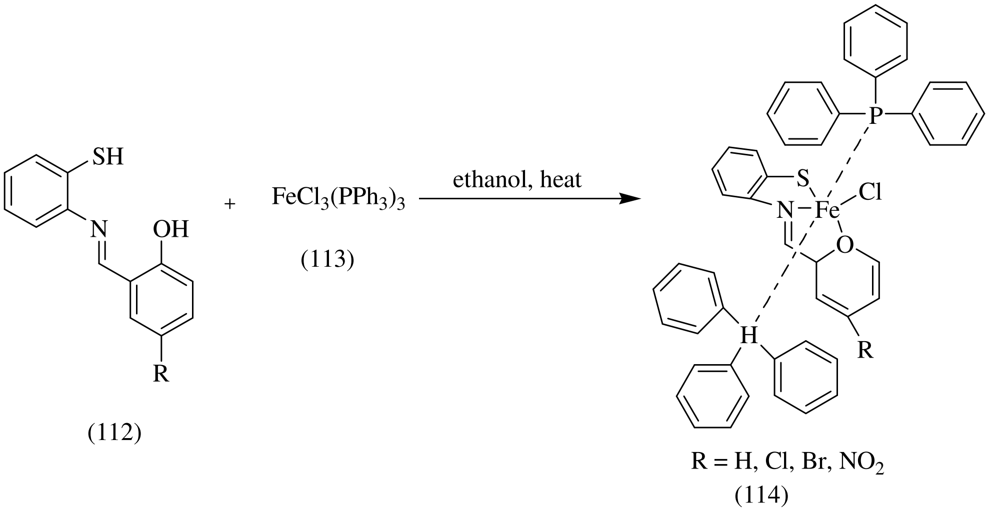


(108) (42) (111)

R^1 = H
R^2 = OH, H
R^3 = H, OH
R^4 = OMe

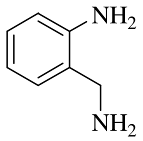
Scheme 31

Rani and Bhat (2010) synthesized Fe (III) complexes (114) containing Schiff base and triphenylphosphine ligands as shown in scheme 32. The Schiff base ligands were prepared by the condensation between salicylaldehyde, its derivatives or 2-hydroxy-1-naphthaldehyde and 2-mercaptoaniline in ethanol and the complexes (114) were prepared by stirring the solution of ligands (112) and $FeCl_3(PPh_3)_3$ (113) in ethanol.



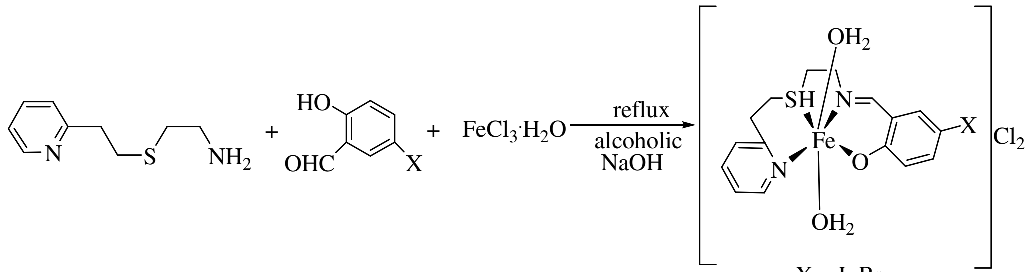
Scheme 32

Ni (II) and Zn (II) complexes of 2-aminomethylaniline (115) have been reported by Mushtapa *et al* (2010). The complexes derived from 2-aminomethylaniline were easily prepared by reacting the ligand directly with relevant nitrate of Ni (II), Cu (II) and Zn (II). The Ni complex was prepared by reacting the solution mixture of 2-aminomethylaniline and $Ni(NO_3)_2.6H_2O$ in methanol. Both the solutions were mixed and the resulting purple mixture was refluxed which led to the formation of light purple micro-crystalline powder which was collected and washed with methanol and dried with diethyl ether.



(115)

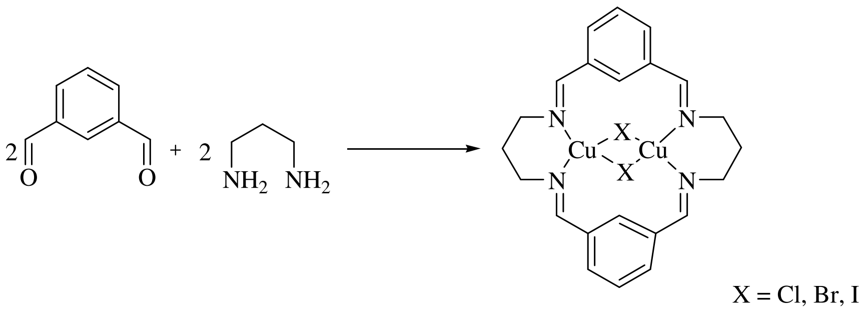
The synthesis and characterization of two new iron (III) complexes (118), $[Fe(pythsalI)]Cl_2$ and $[Fe(pythsalBr)]Cl_2$ with the NSNO-donor tetradentate Schiff base ligands pythsal HX [(5-X-*N*- (2pyridylethylsulfanylethyl)salicylideneimine] (X = I, Br) obtained from the inserted condensation of 1-(2-pyridyl)-3-thia-5-aminopentane (116) with the respective derivative of salicylaldehyde (117) in 1:1 molar ratio has been reported by Shabani *et al* (2010) as shown in scheme 33. These new compounds have shown antitumor activity against two kinds of cancer cells that are K562 (human chronic myeloid leukemia) and Jurkat (human T lymphocyte carcinoma).



(116) (117) (118)

Scheme 33

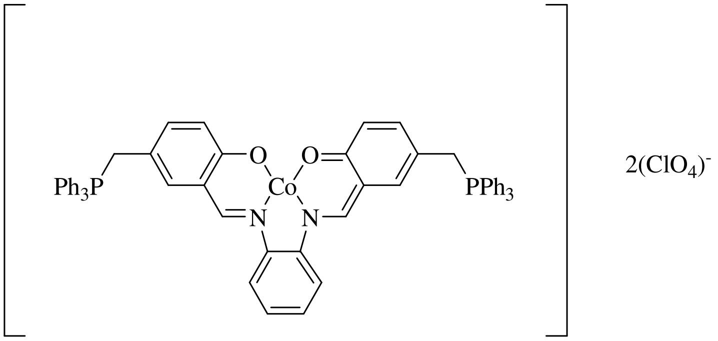
Khandar *et al* (2010) synthesized dinuclear macrocyclic Schiff base and its Cu (I) complexes (121). The crystalline copper (I) complexes were prepared by cyclic condensation reactions with a 1:2 ligand : metal stoichiometry using benzene-1,3-dicarboxaldehyde (119) with 1,3-diaminopropane in (120) the presence of CuX (X = Cl, Br, I) salts as shown in scheme 34.



(119) (120) (121)

Scheme 34

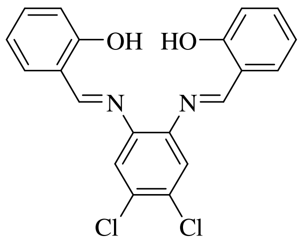
Water soluble Co (II) complexes (122) of a Schiff base were synthesized by Shahabadi *et al* 2010. The Co (II) complex was prepared by mixing 3-formyl-4 hydroxybenzyl-triphenyl phosphonium chloride, Co (II) *o*-acetate and phenylenediamine. It was found from the results that Schiff base has more affinity to bind to DNA than its Co (II) complex which suggests that the complexation changes the mode of binding, because



(122)

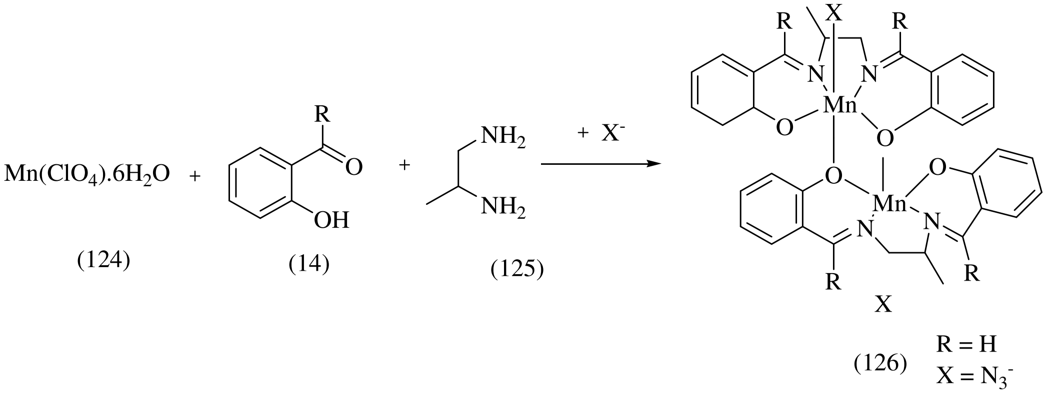
complexation with Co (II) induces stiffening of the ligand. Therefore, from the experimental results, it was concluded that the CH_2-PPH_3 group of SF ligand binds with the nucleotide phosphate unit of the DNA backbone.

Ayman and Aziz (2010) prepared $Cr(CO)_6$ and $Mn(CO)_6$ complexes of novel Schiff base N,NO-*bis*(salicylidene)4,5-dichloro-1,2-phenylenediamine (123). The Schiff base was prepared by adding salicyaldehyde to a hot methanolic solution of 4,5-dichloro-1,2-phenylenediammine. The yellow solution obtained was refluxed for 2 h and after cooling the precipitate of Schiff base was collected from filtration and was recrystallized from methanol. The $Cr(CO)_6$ complex was prepared by adding Schiff base to $Cr(CO)_6$ which was dissolved in dry benzene. The reaction mixture was heated for 5 h resulting in change of the solution colour from yellow to brown. The synthesized compounds were tested for their *in vitro* antimicrobial screening against bacteria *S. aureus* and *E. coli* bacteria and fungi *A. flavus* and *A. niger* and it was found that the complexes were more active antibacterial agents as compared to the parent ligand.



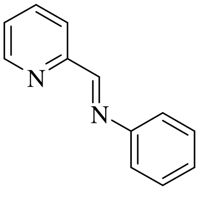
(123)

Mandal *et al* (2010) synthesized four new phenoxo-bridged binuclear Mn (III) complexes (126) by reacting manganese perchlorate (124) with salicylaldehyde (14) and 1,2-diaminopropane (125) as shown in scheme 35. Stirring was continued for 20 min and the pure crystals were obtained after 5 days.

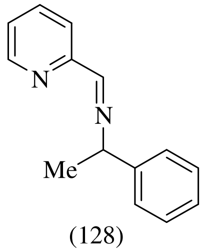


Scheme 35

Mn (II) complexes were synthesized by Sasi *et al* (2010) from Schiff bases (127) and (128) were obtained by the condensation of pyridine-2-carbaldehyde with aniline and its derivatives. The complexes were prepared by refluxing the mixture of pyridine-2-carbaldehyde and aniline in methanol for about 2 h. $Mn(ClO_4)_2.6H_2O$ dissolved in MeOH, was added and stirred to the mixture followed by the slow addition of an aqueous solution of NaN_3. The solution was stirred for 15 min to obtain orange coloured precipitates.

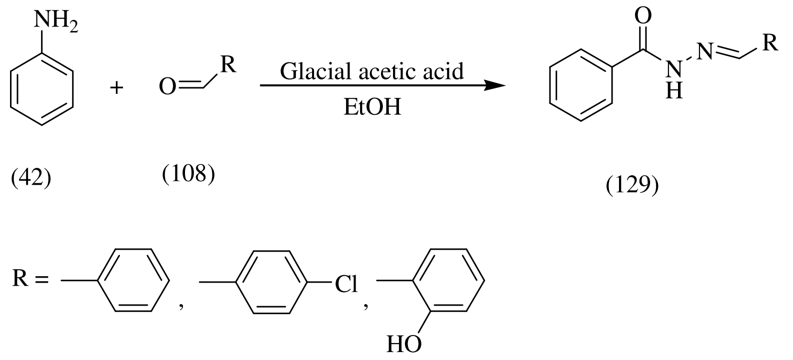


(127)



(128)

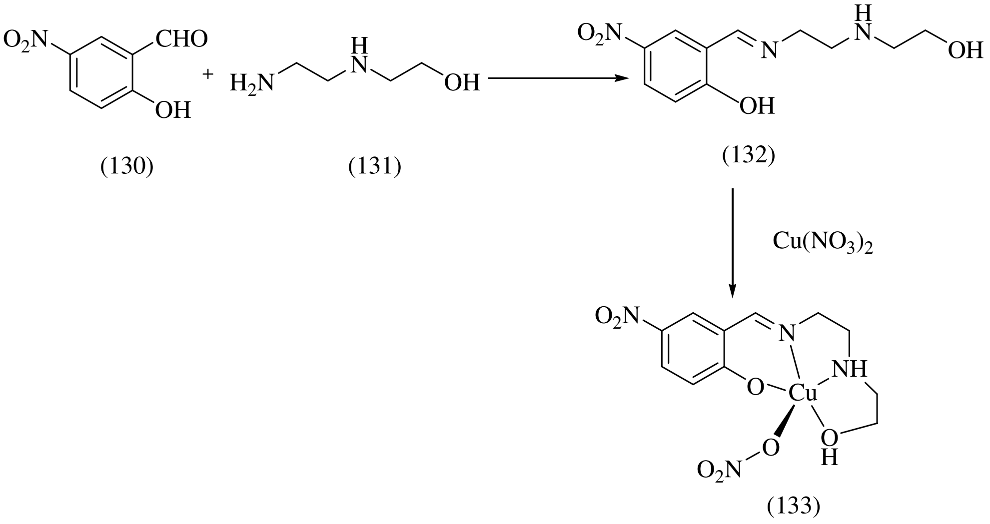
Niazi *et al* (2010) reported the synthesis of a series of twelve different Schiff bases (129) in four sets each with different linkers and substitutions which were synthesized by condensing appropriate aromatic amines (42) with different aromatic aldehydes (108) in the presence of glacial acetic acid at pH 4-6 (scheme 36) and evaluated the synthesized compounds for their antimicrobial activities (*A. niger*, *E. coli* and *C. albicans*) by measuring zone of inhibition using agar diffusion method. The results of the antimicrobial activity showed that the compound (amide linker with *p*-chlorophenyl substitution) and (acetoxy amide linker with*p*-chlorophenyl substitution) had logPvalues of 3.77 and 3.74, respectively, and these were equivalent to the logP values of standard Ketoconazole and showed significant antimicrobial activity.



Scheme 36

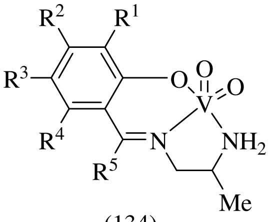
Mononuclear complexes of Cu (II) and Zn (II) were synthesized by You *et al* (2010) from Schiff base 2-{[2-(2-hydroxyethylamino)ethylimino]methyl}-4-nitrophenol (132) which was synthesized by the reaction between the equimolar quantities of 5-nitrosalicylaldehyde (130) with N-(2-hydroxyethyl)ethylenediamine (131) in a methanol solution (scheme 37). All the metal complexes were synthesized by the reaction between the methanolic solution of the Schiff base ligand with the corresponding metal salts (copper nitrate, copper chloride and zinc acetate). Urease inhibitory activity of the Schiff base ligand

and the complexes was evaluated and it was found that the urease inhibitory activity of Cu (II) complexes (133) was found to be superior than the Schiff base ligand (132).



Scheme 37

Romanowski and Wera (2010) synthesized a series of vanadium (V) complexes (134) by the single condensation of R(-)-1,2-diaminopropane with salicylaldehyde and its derivatives. For the preparation of complexes a solution of R(-)-1,2-diaminopropane in toluene was added, with stirring, to a freshly filtered solution of triethyl vanadate in ethanol which resulted in the production of a dark coloured intermediate. After refluxing, the separated solids were filtered, washed over ethanol, and recrystallized using DMSO.

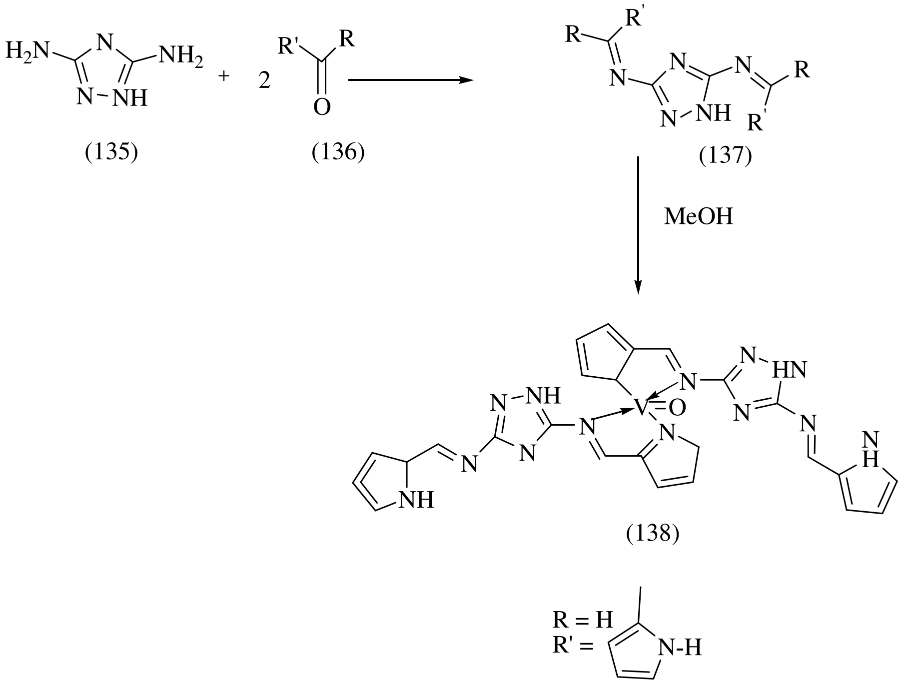


(134)

R^1 = H, OMe
R^2 = H, OMe
R^3 = H, OMe, Br
R^4 = H, Me

A series of oxovanadium (IV) complexes were synthesized by Chohan *et al* (2010) from triazole Schiff bases. Triazole-derived Schiff bases (137) were formed by the reaction 3,5-diamino-1,2,4-triazole (135) with a variety of aldehydes such as; 2-hydoxy-1-naphtahldehyde, pyrrole-2-carboxaldehyde, pyridine-2-carboxaldehyde and acetyl pyridine-2-carboxaldehyde as shown in scheme 38. All the Schiff bases along with their oxovanadium (IV) complexes (138) were screened for their *in vitro* antibacterial activity against four Gram-negative *E. coli, Shigella flexenari, P. aeruginosa* and *S. typhi* and two Gram-positive *S.*

aureus and *B. subtilis* bacterial strains and for antifungal activity against *Trichophyton longifucus, C. albican, A. flavus, Microscopum canis, Fusarium solani and Candida glaberata* and it was concluded from the results that the Schiff bases showed varied antibacterial and antifungal activity against one or more strains and their activity generally enhanced upon co-ordination with the vanadium (IV) co-ordination.



Scheme 38

Conclusions

Thus from this brief review of literature, it has been concluded that synthesis of Schiff bases and their metal complexes having novel structural features and their unusual physico-chemical properties have considerable importance in biological processes. Although different biological studies have been carried out, still their antifungal potential against wheat fungi *Alternaria triticina*, *Bipolaris sorokiniana* and *Ustilago segetum tritici* is need to explore furthermore. Therefore, in this thesis, in the following chapters, the results of our investigations in this direction have been reported.

CHAPTER - III

MATERIALS AND METHODS

Keeping in view the objectives mentioned in chapter I, this chapter provides information regarding the chemicals and reagents used, experimental procedure followed during the course of study and the characterization of the synthesized compounds using analytical instruments and chromatographic techniques.

3.1 Materials

3.1.1 Chemicals and Reagents

The chemicals and reagents used were of AR grades. List of chemicals and reagents used are given below:

1. Cupric chloride - Loba Chemie Pvt. Ltd, Mumbai.
2. 2-Hydroxybenzaldehyde - Loba Chemie Pvt. Ltd, Mumbai.
3. 4-Chloroaniline - Loba Chemie Pvt. Ltd, Mumbai.
4. 4-Bromoaniline - Loba Chemie Pvt. Ltd, Mumbai.
5. Cadmium chloride - Loba Chemie Pvt. Ltd, Mumbai.
6. Nickel Chloride - Loba Chemie Pvt. Ltd, Mumbai.
7. Calcium chloride - Loba Chemie Pvt. Ltd, Mumbai.
8. Manganese chloride - Loba Chemie Pvt. Ltd, Mumbai.
9. Diethyl ether - S.D. Fine Chemicals Ltd, Mumbai.
10. Ethanol, AR - Bengal Chemicals & Pharmaceuticals Ltd, Kolkata.
11. Ethyl acetate - Samir Tech-Chem Pvt. Ltd, Vadodara.
12. Hexane - SISCO Research Laboratries Ltd, Mumbai.
13. Sulfuric acid - S.D. Fine Chemicals Ltd, Mumbai.
14. Silica gel G - Loba Chemie Pvt. Ltd, Mumbai.
15. Tween 20 - S.D. Fine Chemicals Ltd, Mumbai.
16. Acetone - S.D. Fine Chemicals Ltd., Mumbai.
17. Chloroform - S.D. Fine Chemicals Ltd., Mumbai.
18. DMSO - S.D. Fine Chemicals Ltd., Mumbai.

3.2 Instruments used

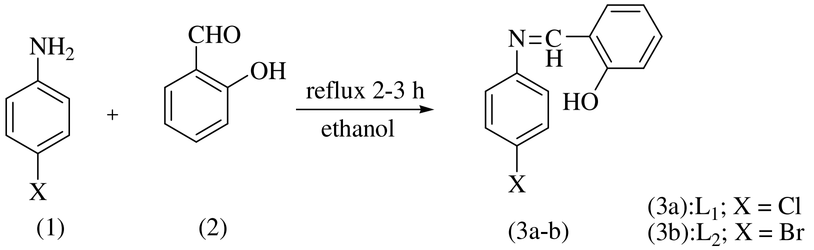
Melting points were recorded in open capillaries and are uncorrected. The ^{1}HNMR and ^{13}CNMR spectra of the Schiff bases were recorded in $CDCl_3$ and DMSO on a BRUKER 400 MHz and 100 MHz spectrometer respectively, at room temperature using TMS as an internal standard and chemical shifts are given in δ. Elemental analysis (C,H,N) were recorded on Euro Vecto elemental analyser. IR spectra of the Schiff base ligands and their Cd (II) and Ni (II) complexes were recorded on a Perkin Elmer Spectrum Two-IR Fourier-

Transform spectrophotometer in the range 400-4000 cm^{-1} using KBr pellets. The electronic spectra of the complexes were recorded on a SHIMADZU 1601 PC spectrophotometer in the region 200-1100 nm, with quartz cuvette (path length, 1 cm) and studies were performed in AR grade dimethyl sulfoxide. Mass spectra were recorded on Waters LC-MS-MS Quattro micro^{TM} API. Molar conductivity measurements were recorded on conductivity bridge type 305 with a cell having constant 1. Mass spectra, UV-visible and molar conductance analysis were recorded at Department of Chemistry, Punjab Agricultural University, Ludhiana. ^{1}HNMR, ^{13}CNMR spectra from SAIF, Panjab University, Chandigarh. Infrared spectra were recorded from SAIF, Deen Bhandu Chota Ram University, Moorthal. Elemental analyses were recorded from SAIF, CSIR-Central Drug Research Institute, Lucknow.

3.3 Synthesis of Schiff bases (3a-b) and their metal complexes (4-11)

3.3.1 General procedure for the synthesis of Schiff base (3a-b)

Equimolar mixture of 4-chloroaniline/4-bromoaniline (1) and salicyaldehyde (2) were refluxed in absolute alcohol (40.00 ml) until the reaction was completed (2-3 h, TLC) (Scheme 1). The solid products formed in the reaction mixture were filtered, washed with diethyl ether and recrystallized from absolute alcohol to obtain pure products (3a-b).



Scheme 1

3.3.2 General procedure for the synthesis of metal complexes (4-11)

To the hot solution of Schiff bases (3a-b) (2.00 mmol) in the absolute alcohol (20.00 ml), drop wise addition of metal salt (1.00 mmol) ($CdCl_2$, $NiCl_2$, $CuCl_2$, $MnCl_2$) (2:1) in hot alcoholic solution was made with constant stirring. Stirring was continued until the completion of reaction (1-2 h, solid formation, color change). The metal complexes (4-11) thus formed were filtered and washed with absolute alcohol to remove unreacted ligands and were dried in vacuum over fused $CaCl_2$. Table 3.1 show the list of synthesized Schiff bases (3a-b) and metal complexes (4-11).

3.4 Antifungal activity of Schiff bases (3a-b) and their metal complexes (4-11)

The synthesized compounds were screened *in vitro* for their antifungal potential against two test fungi *viz. Alternaria triticina*, *Bipolaris sorokiniana* and *Ustilago segetum tritici*. The results have been expressed in terms of ED_{50} values and ED_{90} *i.e.* the effective dose at which 50 and 90 percent spore germination inhibition occurred.

Table 3.1 List of synthesized Schiff bases (3a-b) and their metal complexes (4-11)

Entry	Compounds
(3a)	$C_{13}H_{10}ClNO$ (L_1)
(3b)	$C_{13}H_{10}BrNO$ (L_2)
(4)	$C_{26}H_{18}Cl_2CuN_2O_2$
(5)	$C_{26}H_{18}Cl_2NiN_2O_2$
(6)	$C_{26}H_{18}Cl_2CdN_2O_2$
(7)	$C_{26}H_{18}Cl_2MnN_2O_2$
(8)	$C_{26}H_{18}Br_2CuN_2O_2$
(9)	$C_{26}H_{18}Br_2NiN_2O_2$
(10)	$C_{26}H_{18}Br_2CdN_2O_2$
(11)	$C_{26}H_{18}Br_2MnN_2O_2$

3.4.1 Collection, isolation and maintenance of cultures

3.4.1.1 Source

The spores/cultures of the three test fungi were obtained from Department of Plant Pathology, Punjab Agricultural University, Ludhiana.

Alternaria triticina	-	Foliar blights of wheat
Bipolaris sorokiniana	-	Foliar blights of wheat
Ustilago segetum tritici	-	Loose smut of wheat

3.4.2 Preparation of stock solution

The stock solution (2000 μg/ml) of each compound and standard fungicide *i.e.* Raxil 60 FS (Tebuconazole) and Tilt 25 EC (Propiconazole) were prepared separately by dissolving the chemical (20 mg) in 1 ml of Tween 20 (Polyoxyethylene sorbitan) and volume was made 10 ml with sterilized distilled water. The stock solution of 2000 μg/ml of the compound thus prepared on active ingredient basis was kept in refrigerator till use. The required dilutions of 1000, 500, 200, 100, 50 and 20 10, 5 and 1μg/ml were subsequently made from the stock solution by adding distilled water as and when required.

3.4.3 Spore germination method

All the test compounds were screened against three phytopathogenic fungi, *viz. U. segetum tritici*, *B. sorokiniana* and *A. triticina* by using spore germination method (Nene and Thapliyal 1993). Spore suspension was made by adding sterilized distilled water to the fresh cultures of *A. triticina*, *B. sorokiniana* and *U. segetum tritici*. Suspension was filtered through three layers of sterilized muslin cloth in order to remove mycelia under aseptic conditions. Small droplets (0.02 ml) of test solution and spore suspension in equal amount were seeded in the cavity of the cavity slides. These slides were placed in Petri plates lined with moist filter paper and were incubated for 24 h at 25±1°C for *A. triticina*, 24 h at 25±1°C for *B. sorokiniana* and 72 h at 15±1°C for *U. segetum tritici*. Observations on spore germination were taken at 2 h interval and percent spore germination inhibition was calculated using following formula devised by Vincent (1947).

Percent spore germination inhibition (% SGI)

$$\%\ \text{SGI} = \frac{\text{Spore germination in control - Spore germination in treatment}}{\text{Spore germination in control}} \times 100$$

The ED_{50} and ED_{90} values for each fungicide were calculated by plotting per cent inhibition in spore germination against different concentrations of different compounds.

CHAPTER – IV

RESULTS AND DISCUSSION

4.1 General discussion

All the synthesized compounds *i.e.* Schiff base and their metal complexes of Cu (II), Ni (II), Cd (II) and Mn (II) were coloured, stable in air and non-hygroscopic. They were sparingly soluble in common organic solvents; however, these complexes were soluble in DMF and DMSO. All the complexes were obtained in good yield. The molar conductance values at 10^{-3} M concentration were too low to account for any dissociation of the complexes in acetone. Hence, the Cu (II), Ni (II), Cd (II) and Mn (II) complexes may be regarded as non electrolytes. The spectral and analytical data showed the formula of the Cd (II), Mn (II) and Ni (II) complexes as [ML_2] where M = Cu (II), Ni (II), Cd (II) and Mn (II) and L= $C_{13}H_{10}ClNO$. From spectroscopic studies and elemental analysis data, it was concluded that the Cu (II), Ni (II), Cd (II) and Mn (II) complexes had tetra co-ordinated geometry.

4.1.1 Physical appearance

The physical appearance of the compounds was noted by visual observation. The compounds obtained were colored and crystalline solids. The colour, molecular weights and yield of the Schiff bases and their Cu (II), Ni (II), Cd (II) and Mn (II) complexes are listed in Table 4.1.

4.1.2 Melting point determination

Melting point of the ligands and their metal complexes were determined by taking small amount of compound in a capillary tube closed at one end and placed in the melting point apparatus and the temperature at which compounds melts were noted. Melting points are given in Table 4.1.

Table 4.1 Physical characteristics of compounds (3a-b,4-11)

Entry	Compounds	Color	Molecular weight	Yield (%)	M.pt. (°C)
(3a)	$C_{13}H_{10}ClNO$ (L_1)	Orange	231.5	85	109
(3b)	$C_{13}H_{10}BrNO$ (L_2)	Yellow	275.9	87	111
(4)	$C_{26}H_{18}Cl_2CuN_2O_2$	Dark green	598.44	60	>250
(5)	$C_{26}H_{18}Cl_2NiN_2O_2$	Drak yellow	592.59	64	>250
(6)	$C_{26}H_{18}Cl_2CdN_2O_2$	Yellow	646.31	55	>250
(7)	$C_{26}H_{18}Cl_2MnN_2O_2$	Light yellow	588.83	60	>250
(8)	$C_{26}H_{18}Br_2CuN_2O_2$	Black	686.24	55	>250
(9)	$C_{26}H_{18}Br_2NiN_2O_2$	Light yellow	681.39	68	>250
(10)	$C_{26}H_{18}Br_2CdN_2O_2$	Yellow	735.11	54	>250
(11)	$C_{26}H_{18}Br_2MnN_2O_2$	Lemon	677.63	56	>250

4.1.3 ^{1}H and ^{13}CNMR spectra of Schiff bases (3a-b)

The ^{1}HNMR spectra (DMSO) of the Schiff bases (3a) exhibited signals at δ 8.58 ppm due to the azomethine protons which indicated the formation of Schiff base. Aromatic protons exhibited multiplets at δ 6.92–7.40 ppm for ligand (3a). A singlet due to the phenolic OH proton appeared at δ 13.00 for the Schiff base (3a) which exchanged with D_2O.(Annexure II)

The ^{1}HNMR spectra (DMSO) of the Schiff bases (3b) exhibited signals at δ 8.59 ppm due to the azomethine protons which indicated the formation of Schiff base. Aromatic protons exhibited multiplets at δ 6.92-7.55 ppm for ligand (3b). A singlet due to the phenolic OH proton appeared at δ 12.98 for the Schiff base (3b) which exchanged with D_2O.(Annexure VI)

The peaks in ^{13}CNMR (DMSO) spectrum for ligand (3a) appeared at δ 117.3, 119.0, 119.2, 122.5, 129.5, 132.4, 132.5, 133.4, 147.0, 161.1 and 163.0 ppm.(Annexure III)

The peaks in ^{13}CNMR (DMSO) spectrum for ligand (3b) appeared at 117.3, 119.0, 119.2, 120.3, 122.8, 132.4, 133.4, 147.4, 161.1 and 163.0 ppm. (Annexure VII)

4.1.4 Elemental analysis

Elemental analysis helps in determination of the ratio of C, H and N in the synthesized compound. Compound (3a) having molecular formula $C_{13}H_{10}ClNO$, the calculated value of carbon was 67.38 per cent and the observed value found to be 67.09 per cent which was very close to the required value. Similarly calculated value of hydrogen in (3a) was 4.31 per cent and observed was 4.34 per cent; for nitrogen calculated value was 6.04 per cent and observed was 6.01 per cent.

Compound (3b) having molecular formula $C_{13}H_{10}BrNO$, the calculated value of carbon was 56.54 per cent and the observed value found to be 55.35 per cent which was close to the required value. Similarly calculated value of hydrogen in (3b) was 3.62 per cent and observed was 3.71 per cent; for nitrogen calculated value was 5.07 per cent and observed was 4.85 per cent.

Compound (4) having molecular formula $C_{26}H_{18}Cl_2CuN_2O_2$, the calculated value of carbon was 59.49 per cent and the observed value found to be 59.90 per cent which was close to the required value. Similarly calculated value of hydrogen in (4) was 3.46 per cent and observed was 3.91 per cent; for nitrogen calculated value was 5.34 per cent and observed was 5.49 per cent.

All other compounds also showed the required results as shown in Table 4.2. From elemental analysis data, the molecular formula of the complex (5) was found to be $C_{26}H_{18}Cl_2NiN_2O_2$ and the carbon, hydrogen and nitrogen per cent was noted.

Table 4.2 Elemental analysis of compounds (3a-b,4-11)

Compound No.	Compounds	C(%) Found (Cal.)	H(%) Found (Cal.)	N(%) Found (Cal.)
(3a)	$C_{13}H_{10}ClNO$ (L_1)	67.09 (67.38)	4.34 (4.31)	6.01 (6.04)
(3b)	$C_{13}H_{10}BrNO$ (L_2)	55.35 (56.54)	3.71 (3.62)	4.85 (5.07)
(4)	$C_{26}H_{18}Cl_2CuN_2O_2$	59.90 (59.49)	3.91 (3.46)	5.49 (5.34)
(5)	$C_{26}H_{18}Cl_2NiN_2O_2$	60.10 (60.05)	3.62 (3.49)	5.21 (5.39)
(6)	$C_{26}H_{18}Cl_2CdN_2O_2$	54.38 (54.43)	3.54(3.16)	4.97 (4.88)
(7)	$C_{26}H_{18}Cl_2MnN_2O_2$	60.30 (60.49)	3.56 (3.51)	5.21 (5.43)
(8)	$C_{26}H_{18}Br_2CuN_2O_2$	50.68 (50.88)	2.89 (2.96)	4.31 (4.56)
(9)	$C_{26}H_{18}Br_2NiN_2O_2$	51.12(51.28)	3.21 (2.98)	4.71 (4.60)
(10)	$C_{26}H_{18}Br_2CdN_2O_2$	47.43 (47.13)	2.54 (2.74)	4.32 (4.23)
(11)	$C_{26}H_{18}Br_2MnN_2O_2$	51.20 (51.60)	3.30 (3.00)	4.56 (4.63)

4.1.5 IR spectral studies

The important IR frequencies of the Schiff bases and their Cu (II), Ni (II), Cd (II) and Mn (II) complexes are presented in Table 4.4. In order to study the binding mode of the Schiff base to metal ions, the IR spectrum of the free Schiff base was compared with the IR spectra of the metal complexes.

A strong band was observed for the free Schiff base (3a) around 1608 cm^{-1}, characteristic band of the azomethine *v*(C=N) group. A highly intense band was present around 1456 cm^{-1}, which was assigned to phenolic *v*(C-O) stretching vibrations. A broad band was observed around 3000 cm^{-1}, which was due to *v*(OH) group and a band at 753.67 cm^{-1} was observed which represented *v*(C-Cl). (Annexure I)

For ligand (3b), azomethine group showed strong band at 1613 cm^{-1}. Stretching vibration for phenolic group was observed at 1479 $cm^{-1}$and a band at 677.86 cm^{-1} was observed which represented *v*(C-Br).(Annexure V)

For Cu (II) complex of ligand (3a), stretching vibrations for (C=N) group increases to higher frequency *i.e.* 1610 cm^{-1}, similarly for Cd (II) complex of ligand (3a) this band shifted to higher frequency *i.e.* 1610 cm^{-1}, suggesting coordination of the azomethine nitrogen atom to the metal ion (Sharma 2007). Similarly, for other metal complexes of ligand (3b), bands are given in Table 4.4. In the Cu complex and Ni complex of ligand (3a) the band was observed at 1485 cm^{-1} (4) which was shifted from 1456 cm^{-1}, on complexation and is indicative of bonding through phenolic oxygen (Mounika *et al* 2010). Similarly for other metal chelates of ligand (3b), bands are given in Table 4.4. A Broad band was observed at 3000 cm^{-1} because of metal chelates of ligand (3a), the broadening of the band was due to intermolecular H-bonding between OH groups. Vibrations in the regions in between 516-527 cm^{-1} have been assigned to *v*(M-N) and 698-827 cm^{-1} have been assigned to *v*(M-O) bands. (Annexure IX-XVI)

4.1.6 Mass spectra

The mass spectra of the Schiff bases (3a-b) showed a molecular ion peak at *m/z* 232 (M^{+}+1) and *m/z* 276 (M^{+}+1) which is equal to molecular weights of ligands. Mass spectra of Schiff bases (3a-b) are shown in (Annexure IV and VIII)

4.1.7 Molar conductance

The molar conductance values at 10^{-3} M concentration were too low to account for any dissociation of the complexes in acetone. Hence, the Cu (II), Ni (II), Cd (II) and Mn (II) complexes may be regarded as non electrolytes. Molar conductance values have been complied in Table 4.3.

Table 4.3 Molar conductance values of Schiff bases (3a-b) and their metal complexes (4-11)

Entry	Compounds	Molar Conductance (Λ_m) S m^2 mol^{-1} × 10^{-6}
(3a)	$C_{13}H_{10}ClNO$ (L_1)	21
(3b)	$C_{13}H_{10}BrNO$ (L_2)	18
(4)	$C_{26}H_{18}Cl_2CuN_2O_2$	10
(5)	$C_{26}H_{18}Cl_2NiN_2O_2$	19
(6)	$C_{26}H_{18}Cl_2CdN_2O_2$	14
(7)	$C_{26}H_{18}Cl_2MnN_2O_2$	13
(8)	$C_{26}H_{18}Br_2CuN_2O_2$	12
(9)	$C_{26}H_{18}Br_2NiN_2O_2$	14
(10)	$C_{26}H_{18}Br_2CdN_2O_2$	16
(11)	$C_{26}H_{18}Br_2MnN_2O_2$	20

4.1.6 Electronic spectral studies

The absorption spectra of synthesized ligands and their metal complexes with Cd (II) and Ni (II) were recorded in DMSO. The ligand (3a) absorbed at 240 nm (ε = 57300 l/mol/cm) giving a sharp band corresponding to π-π^* transitions and a broad band at 261.50 nm (ε = 56000 l/mol/cm) corresponding to n-π^* transition involving molecular orbitals of C=N chromophore and benzene ring. The ligand (3b) showed absorption bands corresponding to π-π^* transition at 274.50 nm (ε = 119500 l/mol/cm). Band due to n-π^* transition appeared at 311 nm (ε = 51200 l/mol/cm).

Table 4.4 Infra red frequencies (in cm^{-1}) of Schiff bases (3a-b) and their metal complexes with Cu (II), Zn (II) Cd (II) and Mn (II) (4-11)

Entry	Compounds	*ν*(C=N)	*ν*(OH)	*ν*(C-O)	*ν*(C-Cl)	*ν*(C-Br)	*ν*(M-N)	*ν*(M-O)
(3a)	$C_{13}H_{10}ClNO$ (L_1)	1608.14 (s)	3000 (br)	1456.26 (m)	753.67 (s)	697.46 (s)	-	-
(3b)	$C_{13}H_{10}BrNO$ (L_2)	1613.62 (s)	3100 (br)	1479.65 (m)	708.36 (s)	677.86 (s)	-	-
(4)	$C_{26}H_{18}Cl_2CuN_2O_2$	1610.79 (s)	3435.49 (br)	1485.31 (m)	757.95 (s)	-	516.76 (w)	698.45 (s)
(5)	$C_{26}H_{18}Cl_2NiN_2O_2$	1610.52 (s)	3434.71 (br)	1485.08 (m)	757.83 (s)	-	516.04 (w)	698.05 (s)
(6)	$C_{26}H_{18}Cl_2CdN_2O_2$	1610.85 (s)	3435.68 (br)	1485.30 (m)	757.9 (s)	-	516.90 (w)	699.52 (s)
(7)	$C_{26}H_{18}Cl_2MnN_2O_2$	1610.53 (s)	3434.71 (br)	1485.16 (m)	757.8 (s)	-	516.60 (w)	698.84 (s)
(8)	$C_{26}H_{18}Br_2CuN_2O_2$	1616.29 (s)	3430.59 (br)	1481.75(m)	-	753.33 (s)	527.85 (w)	827.80 (s)
(9)	$C_{26}H_{18}Br_2NiN_2O_2$	1616.55 (s)	3412.82 (br)	1481.66 (m)	-	753.30 (s)	527.87 (w)	827.83 (s)
(10)	$C_{26}H_{18}Br_2CdN_2O_2$	1616.93 (s)	3413.25 (br)	1481.82 (m)	-	753.80 (s)	523.41(w)	827.80 (s)
(11)	$C_{26}H_{18}Br_2MnN_2O_2$	1616.09 (s)	3435.32 (br)	1481.44 (m)	-	753.28 (s)	523.43 (w)	827.53 (s)

(m = medium, s = strong, br = broad, w = weak)

In the metal complexes of the ligands, there is increase in ε value was observed. In case of (4), (5), (6) and (7) bands appeared at 284.5 nm (ε = 66900 l/mol/cm); 260.5 nm (ε = 140800 l/mol/cm); 270 nm (ε = 59300 l/mol/cm) and 261 nm (ε = 138600 l/mol/cm) respectively, due to π-π* transitions. In case of (8), (9), (10) and (11) bands corresponding to π-π* transition appeared at 231 nm (ε = 157400 l/mol/cm); 269 nm (ε = 111900 l/mol/cm) 257.50 nm (ε = 11700 l/mol/cm) and at 269 nm (ε = 131900 l/mol/cm) in complexes, respectively.

n-π* transitions of ligands on complexation with metal had shown an appreciable change. In complex (4) and (5) showed a band at 312.5 nm (ε = 90500 l/mol/cm) and at 284.5 nm (ε = 122700 l/mol/cm), whereas in complex (6) and (7) showed a broad band at 290.5 nm (ε = 49900 l/mol/cm) and at 312.5 nm (ε = 128900 l/mol/cm). Similarly, complexes (8) and (9) also showed broad bands at 283.5 nm (ε = 177200 l/mol/cm) and at 290 nm (ε = 99900 l/mol/cm). In complexes (9) and (11) band appeared at 288 nm (ε = 4100 l/mol/cm) and at 290 nm (ε = 119900 l/mol/cm). The electronic spectra of Schiff bases and metal complexes have been shown in Figure 4.1.

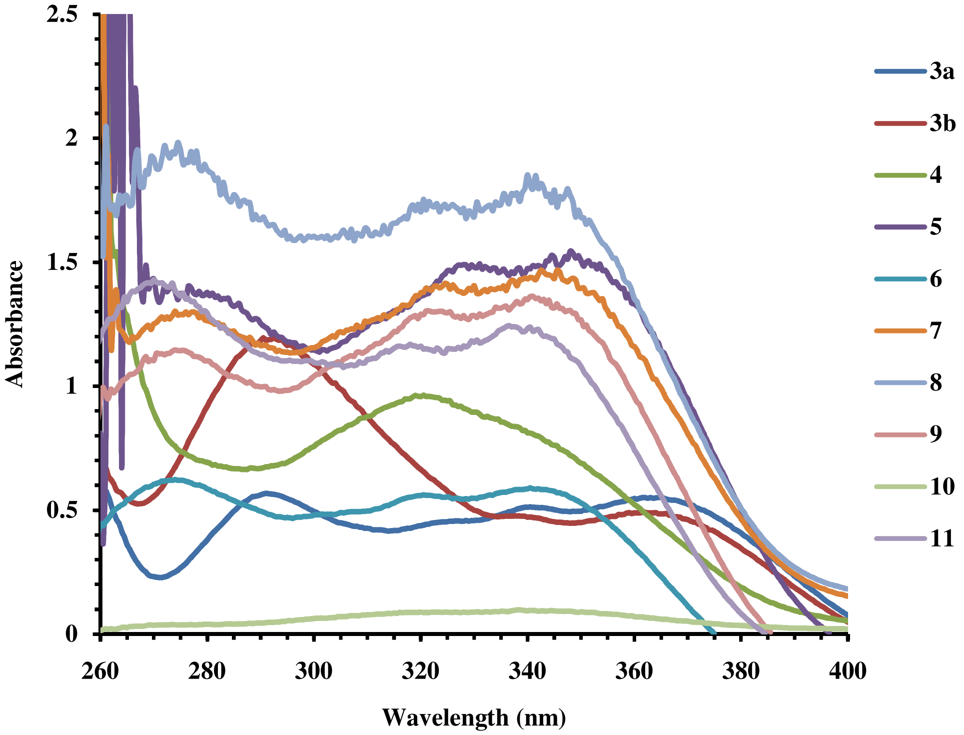


Figure 4.1: UV spectra of Schiff bases (3a-b) and their metal complexes (4-11)

4.4 Antifungal activity of Schiff's bases (3a-b) and their metal complexes (4-11)

Schiff's bases and their metal complexes were screened *in vitro* for their antifungal potential against *Alternaria triticina*, *Bipolaris sorokiniana* and *Ustilago segetum tritici* by spore germination inhibition technique (Vincent 1947) at different concentration levels *viz.*

1000, 500, 200, 100, 50, 10 20, 5 and 1 μg/ml. The results for percentage inhibition are given in tables 4.4, 4.5 and 4.6 for *A. triticina*, *B. sorokiniana* and *U. segetum tritici* respectively. The results have also been expressed in terms of ED_{50} and ED_{90} values *i.e.* the effective dose at which 50 and 90 percent inhibition has occurred. Graphical representations for ED_{50} values of synthesized compounds (3a-b and 4-11) are given in Figures 4.2, 4.3 and 4.4 for *A. triticina*, *B. sorokiniana* and *U. segetum tritici* fungi respectively.

4.4.1 Antifungal activity of Schiff bases (3a-b) and their metal complexes (4-11) against *Alternaria triticina*

In vitro antifungal potential of the synthesized Schiff bases and their metal complexes was carried against *A. triticina* by spore germination method (Figure 4.1 A) at different concentration *viz.* 1000, 500, 200, 100, 50, 20, 10 5 and 1 μg/ml. Percent spore germination inhibition at different concentration was calculated and results are given in Table 4.5. Direct correlation between concentration and percent spore germination inhibition was found to occur. The compounds showed minimum spore germination inhibition about 5-10 percent at concentration 1, 5 and 10 μg/ml. From the analyzed data it was found that with decrease in concentration of the compounds the inhibition also decreases. Maximum spore germination inhibition about 90-95 percent was shown by Schiff base (3b) and it was found that Cu (II) complex of (3b) showed maximum spore germination inhibition at 1000 μg/ml. The compounds were found to more effective at higher concentration as compared to low concentration. ED_{50} and ED_{90} was calculated for all the compounds and the compounds showed ED_{50} value less than 280 μg/ml and ED_{90} of less than 1700 μg/ml. The Schiff base of (3a) have ED_{50} equal to 70 μg/ml which is somewhat close to the standard Tilt 25 EC 30 μg/ml and ED_{90} equal to nearly 800 μg/ml which is near to the value of standard Tilt 25 EC 700 μg/ml. The Cu (II) complex (8) has ED_{50} value 120 μg/ml. So, it was found from the analysis that the Schiff base (3b) showed good antifungal activity with ED_{50} equal to 70 μg/ml followed by the compound (3a) which has ED_{50} value of 80 μg/ml and out of the metal complexes, Cu (II) complex (8) showed more antifungal potential as compared to other metal complexes. All other compounds possessed moderate antifungal activity. Graphical representation of ED_{50} and ED_{90} values are given in the Figure 4.2

4.4.2 Antifungal activity of Schiff bases (3a-b) and their metal complexes (4-11) against *Bipolaris sorokiniana*

In vitro antifungal potential of the synthesized Schiff bases and its metal complexes was carried against *B. sorokiniana* by spore germination method at different concentration *viz.* 1000, 500, 200, 100, 50, 20, 10, 5 and 1 μg/ml. Percent spore germination inhibition at different concentration was calculated and results are given in Table 4.6. Direct correlation between concentration and percent spore germination inhibition was found. The compounds showed minimum spore germination inhibition about 5-20 percent at concentration 1, 5 and

10 µg/ml and maximum spore germination inhibition at 1000 µg/ml. From the analyzed data it was found that with decrease in concentration of the compounds the inhibition also decreases. Maximum spore germination inhibition about 90-95 percent was shown by Schiff base (3a) and it was found that the Mn (II) complex (7) and Ni (II) complex (9) of showed maximum spore germination inhibition at 1000 µg/ml. The compounds were found to more effective at higher concentration as compared to lower concentrations. ED_{50} and ED_{90} was calculated for all the compounds and the compounds showed ED_{50} value less than 240 µg/ml and ED_{90} of less than 1700 µg/ml. The Schiff base (3a) have ED_{50} equal to 70 µg/ml which is somewhat close to the standard Tilt 25 EC µg/ml and ED_{90} equal to nearly 700 µg/ml which is near to the value of standard Tilt 25 EC (650 µg/ml). ED_{50} values for the complex (7) and (9) are 160 µg/ml and it was found from the analysis that the Schiff base (3a) and the complexes (7) and (9) showed good antifungal activity as compared to other complexes. Metal complexes possessed less antifungal potential as compared to the Schiff bases. All other compounds showed moderate antifungal activity. The ED_{50} and ED_{90} values are given in the Figure 4.3.

4.4.3 Antifungal activity against *Ustilago segetum tritici*

In vitro antifungal potential of the synthesized Schiff bases and its metal complexes was carried against *U. segetum tritici* by spore germination method (Figure 4.1 B) at different concentration *viz.* 1000, 500, 200, 100, 50, 20, 10, 5 and 1 µg/ml. Percent spore germination inhibition at different concentration was calculated and results are given in Table 4.7. Direct correlation between concentration and percent spore germination inhibition was found. The compounds showed minimum spore germination inhibition about 5-20 percent at concentration 1, 5 and 10 and maximum spore germination inhibition at 1000 µg/ml. From the analyzed data it was found that with decrease in concentration of the compounds the inhibition also decreases. Maximum spore germination inhibition about 90-95 percent was shown by Schiff bases (3a) and (3b) and it was found that Cu (II) complex (4) and (8) showed maximum spore germination inhibition at 1000 µg/ml. The compounds were found to more effective at higher concentration as compared to lower concentration. ED_{50} and ED_{90} was calculated for all the compounds and the compounds showed ED_{50} value less than 240 µg/ml and ED_{90} of less than 1600 µg/ml. The Schiff base (3a) and (3b) have ED_{50} equal 70 and 80 µg/ml which is somewhat close to the standard Raxil 60 FS 20 µg/ml and ED_{90} equal to 800 and 900 µg/ml which is near to the value of standard Raxil 60 FS 700 µg/ml Cu (II) complexes (4) and (8) have ED_{50} equal to 140 and 120 µg/ml. So, it was found for the analysis the Schiff base (3a) and (3b) showed good antifungal activity with ED_{50} equal to 70 and 80 µg/ml and metal complexes (4) and (8) showed moderate antifungal potential as compared to the Schiff bases. All other compounds possessed moderate antifungal activity. The ED_{50} and ED_{90} values are given in the Figure 4.4.

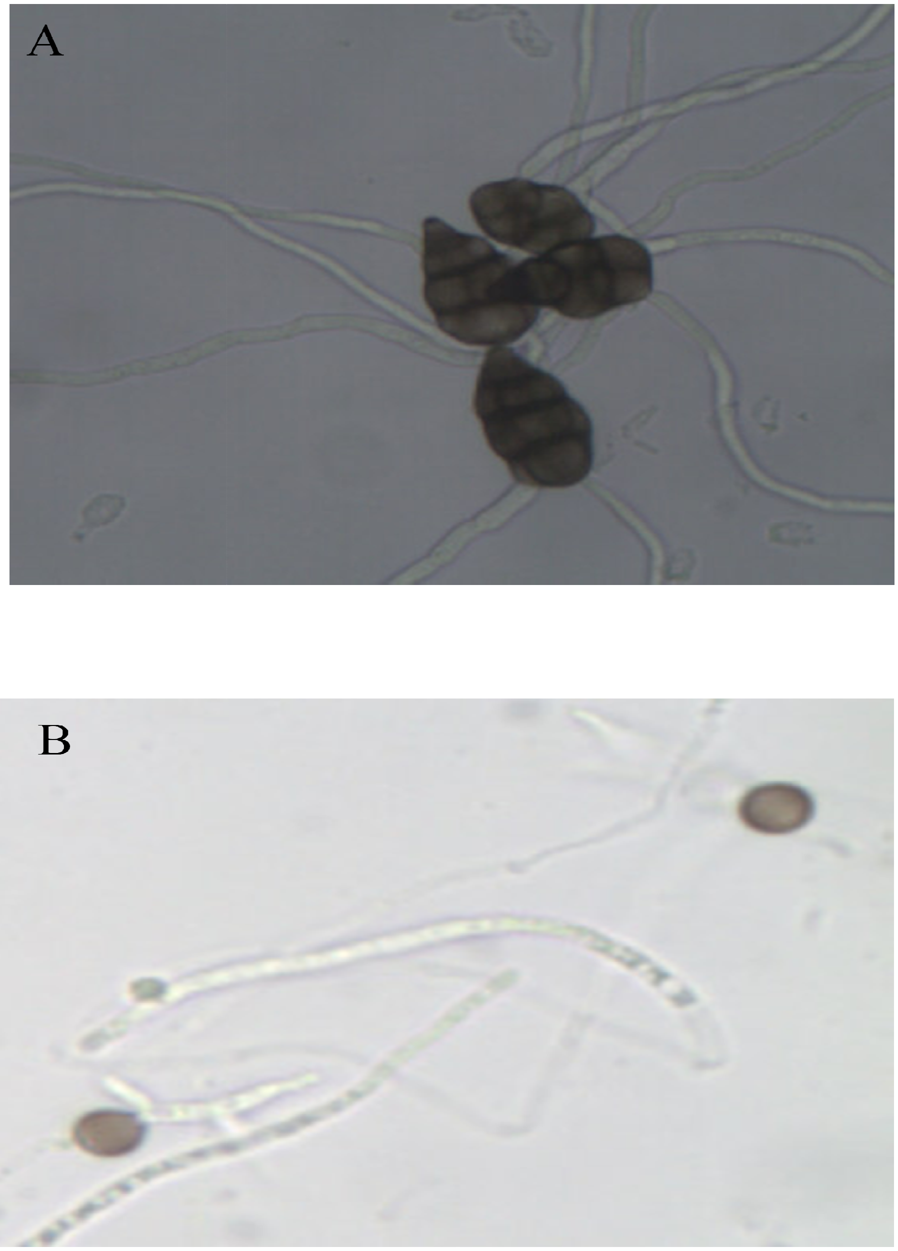


Figure 4.1: (A) Germinated spores of *A. triticina*

(B) Germinated spores of *U. segetum tritici*

Table 4.5 Effect of different concentrations of Schiff's bases (3a-b) and their metal complexes (4-11) on spore germination of *A. triticina*.

Compounds	**Concentrations (µg/ml)**									**Mean**
	1000	**500**	**200**	**100**	**50**	**20**	**10**	**5**	**1**	
(3a)	90.45 (73.26)	78.43 (62.29)	66.66 (54.42)	56.14 (48.51)	35.08 (36.29)	21.05 (27.18)	10.52 (18.52)	3.51 (8.85)	0.00 (0.06)	38.98^{b}
(3b)	93.33 (75.21)	77.19 (61.47)	77.19 (61.47)	64.91 (53.67)	36.84 (37.30)	15.78 (23.20)	5.26 (10.74)	1.75 (4.46)	0.00 (0.06)	41.36^{b}
(4)	70.17 (56.89)	64.91 (53.74)	54.38 (47.50)	42.10 (40.56)	21.05 (27.18)	7.01 (15.14)	3.51 (8.85)	1.75 (4.46)	0.00 (0.06)	29.43^{de}
(5)	70.17 (56.89)	59.64 (50.55)	50.87 (45.48)	38.59 (38.33)	31.57 (34.96)	21.05 (27.18)	12.27 (20.14)	3.51 (8.85)	0.00 (0.06)	31.96^{cd}
(6)	77.19 (61.67)	61.40 (51.61)	49.12 (44.46)	31.57 (34.12)	28.06 (31.06)	14.03 (21.90)	3.51 (8.85)	0.00 (0.06)	0.00 (0.06)	29.43^{de}
(7)	78.94 (62.78)	63.15 (52.64)	42.10 (40.42)	33.33 (35.23)	21.05 (27.18)	7.01 (15.14)	7.01 (15.14)	0.00 (0.06)	0.00 (0.06)	28.07^{e}
(8)	82.63 (65.38)	68.42 (55.83)	57.89 (49.54)	45.61 (42.41)	40.34 (39.26)	17.54 (24.70)	0.00 (0.06)	0.00 (0.06)	0.00 (0.06)	34.71^{c}
(9)	73.68 (59.18)	66.66 (54.72)	52.63 (46.49)	38.59 (38.33)	26.31 (29.98)	17.54 (24.70)	7.01 (15.14)	1.75 (4.46)	0.00 (0.06)	31.57^{cde}
(10)	78.94 (62.77)	64.91 (53.66)	56.14 (48.51)	36.84 (37.32)	29.82 (33.06)	19.29 (27.07)	7.01 (15.14)	0.00 (0.06)	0.00 (0.06)	32.55^{cd}
(11)	77.19 (61.48)	63.15 (52.64)	45.61 (42.42)	45.61 (42.46)	21.05 (27.18)	21.05 (27.18)	7.01 (15.14)	1.75 (4.46)	0.00 (0.06)	31.38^{cde}
Propiconazole (Tilt 25 EC)	94.36 (76.28)	84.65 (66.92)	80.85 (63.46)	77.13 (61.42)	65.93 (54.30)	48.67 (44.22)	41.45 (40.41)	35.53 (36.57)	14.78 (22.59)	62.93^{a}
Mean	77.37^{A}	66.93^{B}	56.17^{C}	44.12^{D}	30.44^{E}	17.77^{F}	8.72^{G}	3.95^{H}	1.14^{H}	

All the values followed by the same letter are non significant (p=0.05) according to Tukey multiple range test.
Values are in parentheses arc sine transformations.
C.D. (p=0.05) Compounds: 1.137
Concentrations: 0.6963
Interaction: 2.78

Table 4.6 Effect of different concentrations of Schiff's bases (3a-b) and their metal complexes (4-11) on spore germination of *B. sorokiniana*.

Compounds	**Concentrations (µg/ml)**									**Mean**
	1000	**500**	**200**	**100**	**50**	**20**	**10**	**5**	**1**	
(3a)	93.19 (73.47)	83.93 (63.00)	66.66 (54.72)	59.64 (50.56)	42.10 (40.42)	28.06 (31.70)	15.78 (23.20)	5.26 (10.78)	0.00 (0.06)	40.74^{b}
(3b)	90.67 (70.96)	83.92 (63.00)	62.64 (52.85)	50.10 (45.42)	42.10 (40.42)	28.06 (31.96)	15.78 (23.20)	3.51 (8.85)	0.00 (0.06)	38.20^{bc}
(4)	86.14 (68.22)	70.17 (56.89)	50.89 (45.48)	42.10 (40.42)	26.31 (30.77)	10.69 (19.90)	7.01 (15.14)	3.51 (8.85)	0.00 (0.06)	32.98^{defg}
(5)	70.17 (56.89)	60.82 (52.64)	50.87 (45.48)	36.84 (37.32)	26.31 (30.77)	17.54 (24.70)	12.27 (20.41)	5.26 (10.78)	0.00 (0.06)	29.16^{gh}
(6)	73.68 (60.78)	64.91 (53.66)	52.63 (46.49)	33.33 (35.23)	26.31 (30.77)	17.54 (24.70)	8.77 (17.03)	3.51 (8.85)	0.00 (0.06)	31.18^{efgh}
(7)	78.94 (62.58)	63.15 (52.64)	52.63 (46.49)	33.33 (35.23)	21.05 (27.18)	14.03 (21.90)	8.77 (17.03)	0.00 (0.06)	0.00 (0.06)	30.21^{fgh}
(8)	86.31 (68.29)	70.17 (56.94)	51.89 (45.76)	40.61 (39.49)	33.33 (35.23)	24.56 (29.66)	12.28 (20.41)	0.00 (0.06)	0.00 (0.06)	36.68^{bcd}
(9)	75.43 (62.29)	66.66 (55.83)	52.63 (46.49)	38.59 (38.33)	26.31 (30.77)	14.03 (21.90)	7.01 (15.14)	0.00 (0.06)	0.00 (0.06)	31.18^{efgh}
(10)	78.94 (62.78)	68.68 (56.56)	50.14 (45.46)	38.10 (38.02)	26.31 (30.77)	26.31 (30.77)	14.03 (21.90)	0.00 (0.06)	0.00 (0.06)	34.72^{cde}
(11)	73.68 (59.18)	63.15 (52.64)	47.36 (43.46)	33.33 (35.23)	17.54 (24.70)	7.01 (15.14)	7.01 (15.14)	1.75 (4.46)	0.00 (0.06)	27.87^{h}
Propiconazole (Tilt 25 EC)	95.64 (77.99)	85.68 (67.84)	80.22 (63.57)	73.37 (58.91)	65.51 (54.02)	49.48 (44.68)	44.61 (41.89)	35.41 (36.51)	15.17 (23.05)	63.75^{a}
Mean	79.67^{A}	68.25^{B}	57.27^{C}	43.29^{D}	31.21^{E}	20.82^{F}	12.87^{G}	4.61^{H}	1.17^{I}	

All the values followed by the same letter are non significant ($p=0.05$) according toTukey multiple range test.

Values are in parentheses arc sine transformations.

C.D. ($p=0.05$) Compounds: 1.395

Concentrations: 0.8545

Interaction: 3.418

Table 4.7 Effect of different concentrations of Schiff's bases (3a-b)and their metal complexes (4-11) on spore germination of *U. segetum trtici*.

Compounds	Concentrations (µg/ml)									Mean
	1000	500	200	100	50	20	10	5	1	
(3a)	90.15 (70.06)	82.68 (63.18)	61.40 (51.58)	56.14 (48.51)	45.10 (42.42)	31.57 (34.12)	21.05 (27.18)	8.77 (16.63)	0.00 (0.06)	41.91^{b}
(3b)	90.14 (70.05)	75.43 (60.29)	63.15 (52.64)	56.14 (48.51)	42.10 (40.42)	24.55 (29.47)	12.27 (20.41)	3.51 (8.89)	0.00 (0.06)	40.37^{b}
(4)	75.43 (60.29)	63.15 (52.64)	54.38 (47.50)	43.85 (41.45)	33.33 (35.23)	21.05 (27.18)	10.52 (18.52)	3.51 (8.89)	0.00 (0.06)	33.91^{cd}
(5)	70.17 (56.89)	57.89 (49.54)	50.87 (45.48)	38.59 (38.33)	28.06 (31.96)	21.05 (27.18)	8.77 (17.03)	5.26 (13.25)	0.00 (0.06)	31.18^{de}
(6)	75.43 (60.36)	63.15 (52.64)	47.36 (43.67)	31.57 (34.17)	29.82 (33.06)	14.03 (21.32)	7.01 (15.14)	0.00 (0.06)	0.00 (0.06)	29.82^{ef}
(7)	78.94 (62.78)	63.14 (52.64)	47.36 (43.67)	31.57 (34.17)	21.05 (27.18)	21.05 (27.18)	7.01 (15.14)	0.00 (0.06)	0.00 (0.06)	30.01^{ef}
(8)	80.70 (63.96)	73.68 (59.18)	59.64 (50.55)	45.61 (42.46)	33.33 (35.23)	22.80 (28.48)	5.26 (13.25)	5.26 (13.25)	0.00 (0.06)	36.25^{c}
(9)	75.43 (60.29)	63.15 (52.64)	52.63 (46.49)	38.59 (38.33)	26.31 (30.77)	15.78 (23.20)	7.01 (15.14)	5.26 (13.25)	0.00 (0.06)	31.35^{de}
(10)	78.94 (62.78)	64.91 (53.66)	54.38 (47.50)	36.84 (37.32)	31.57 (34.17)	21.05 (27.18)	7.01 (15.14)	0.00 (0.06)	0.00 (0.06)	32.74^{cde}
(11)	75.43 (60.29)	63.15 (52.64)	47.36 (43.67)	31.57 (34.17)	17.54 (24.70)	7.01 (15.14)	5.26 (13.25)	0.00 (0.06)	0.00 (0.06)	27.48^{fg}
Tebuconazole (Raxil 60 FS)	95.36 (77.90)	85.64 (67.72)	78.69 (62.49)	72.97 (58.65)	62.97 (52.50)	57.62 (49.36)	40.78 (39.67)	35.90 (36.79)	15.87 (23.46)	66.46^{a}
Mean	79.66^{A}	69.09^{B}	57.03^{C}	44.14^{D}	33.54^{E}	22.38^{F}	10.96^{G}	5.59^{H}	1.22^{I}	

All the values followed by the same letter are non significant (p=0.05) according to Tukey multiple range test.

Values are in parentheses arc sine transformations.

C.D. (p=0.05) Compounds: 1.895

Concentrations: 1.161

Interaction: 4.644

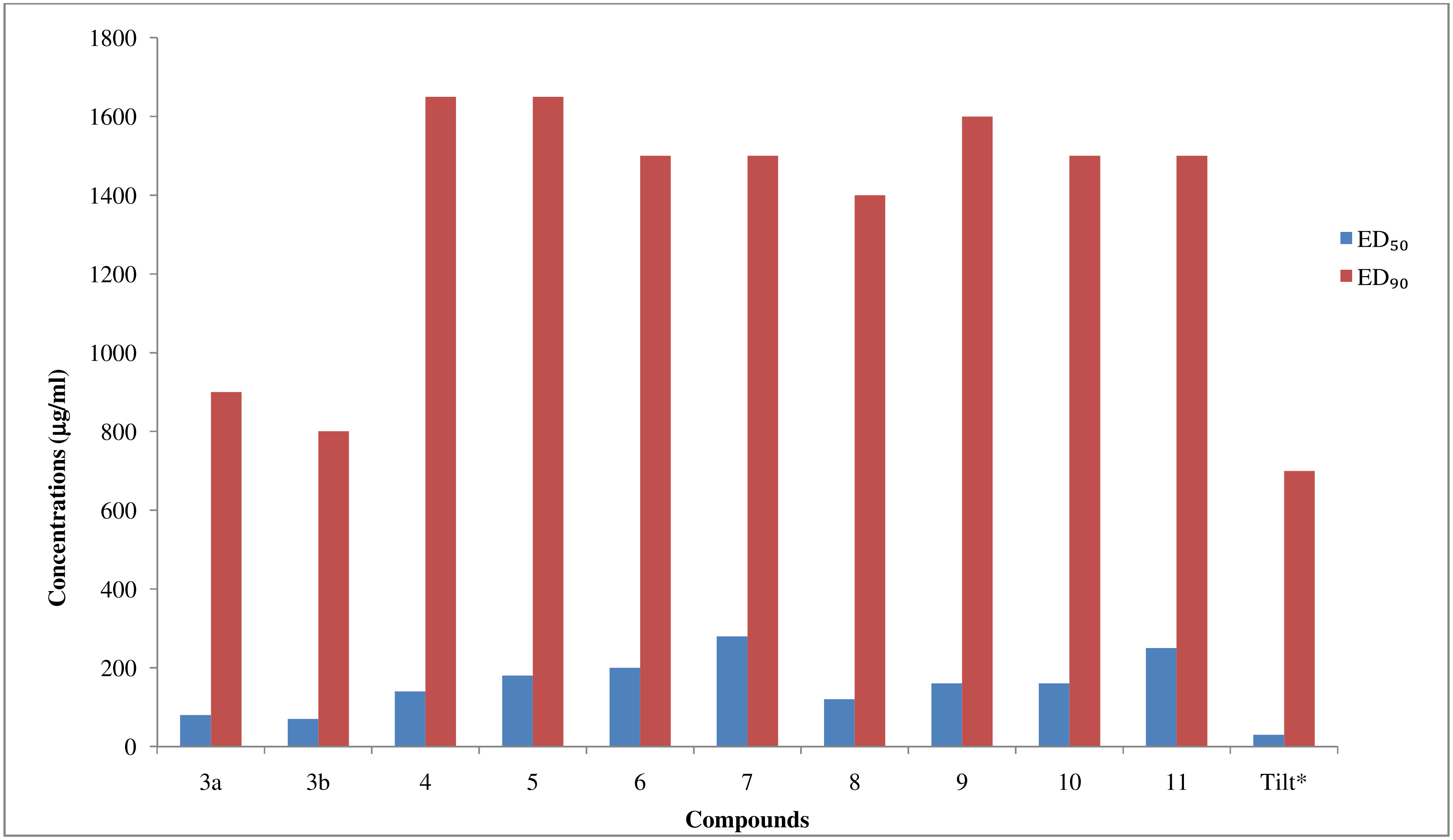


Figure 4.2: ED_{50} and ED_{90} values of all tested compounds against *A. triticina*

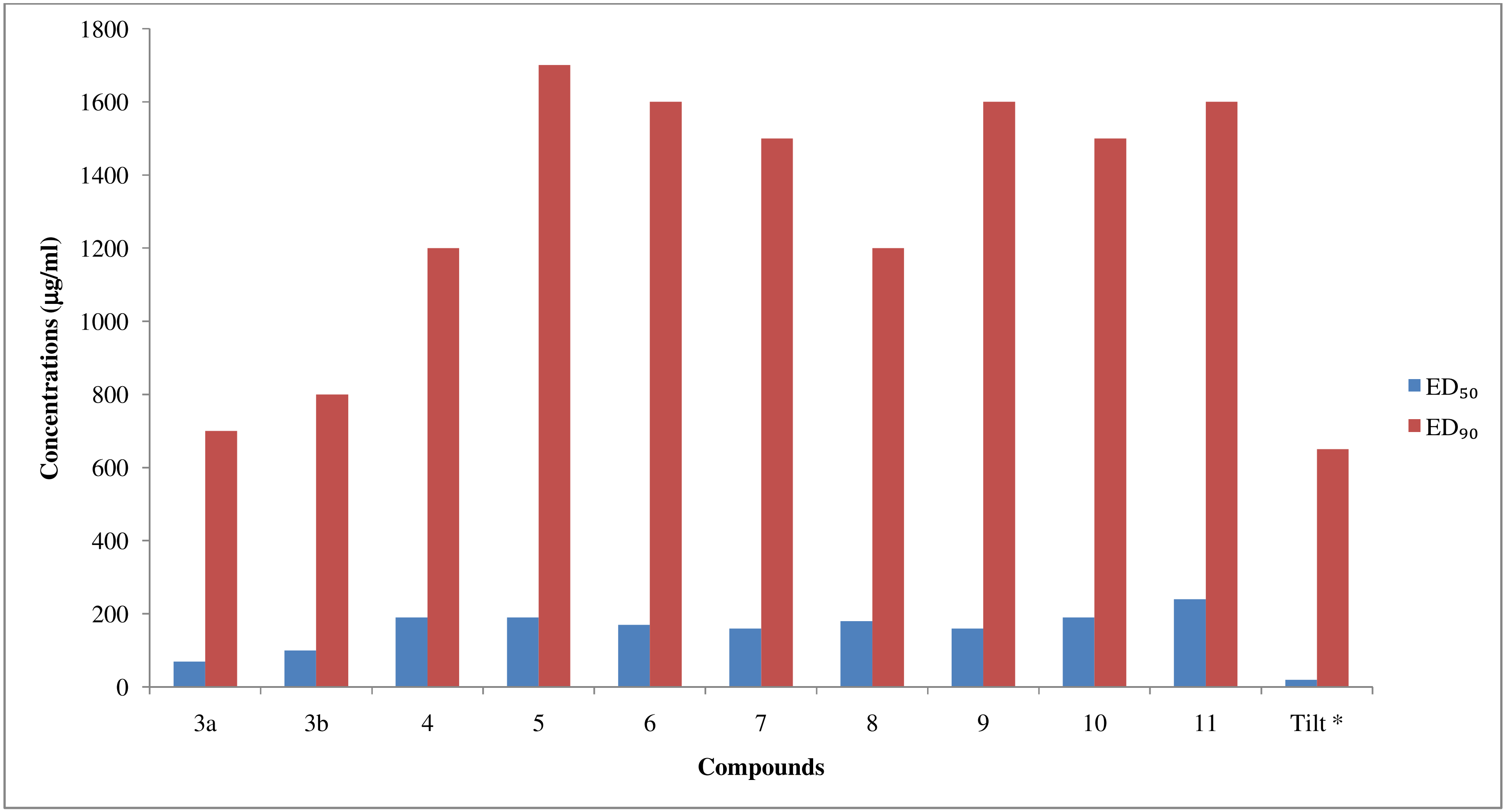


Figure 4.3: ED_{50} and ED_{90} values of all tested compounds against *B. sorokiniana*

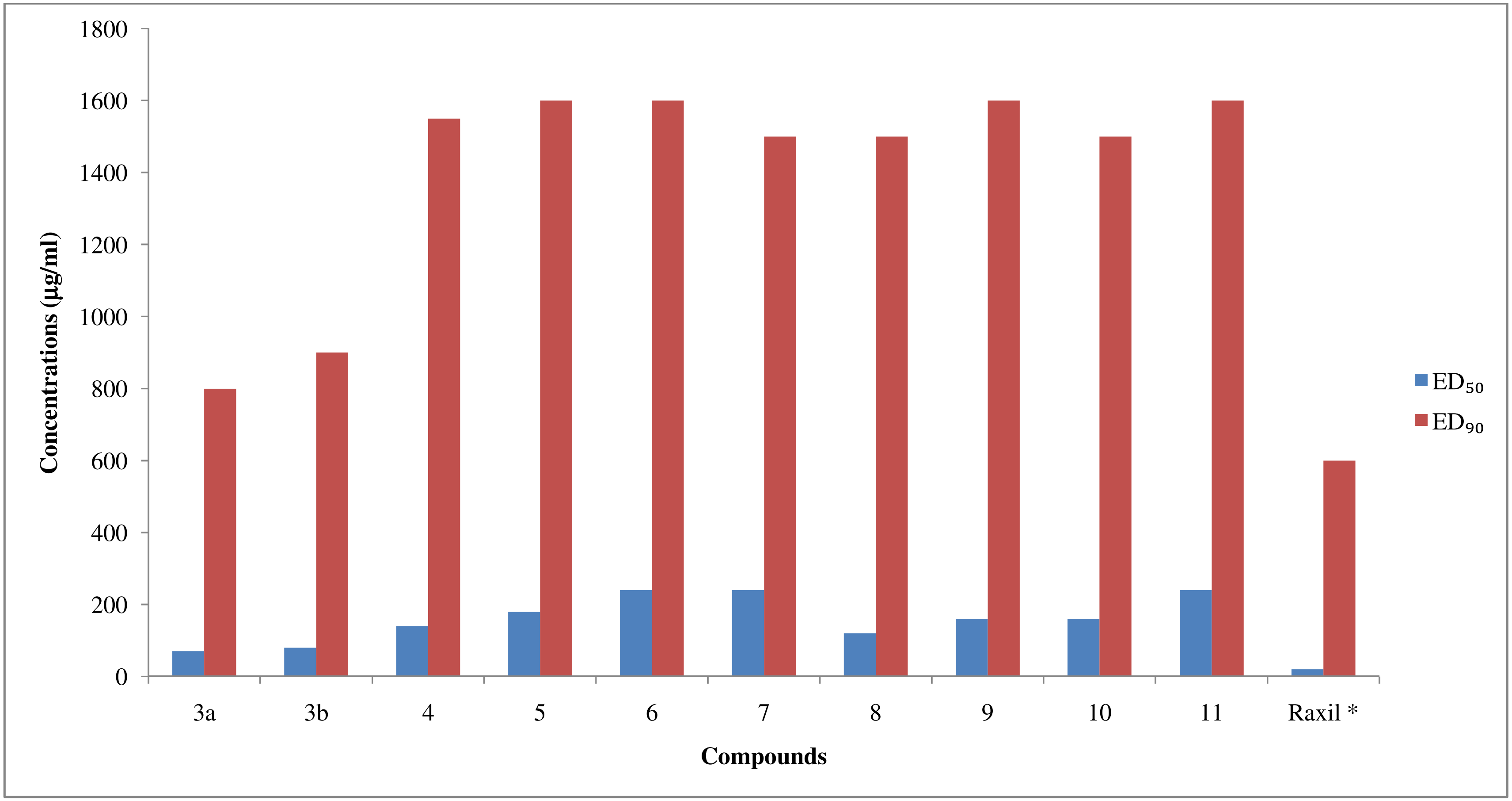


Figure 4.4: ED_{50} and ED_{90} values of all tested compounds against *U. segetum tritici.*

4.3 Conclusions

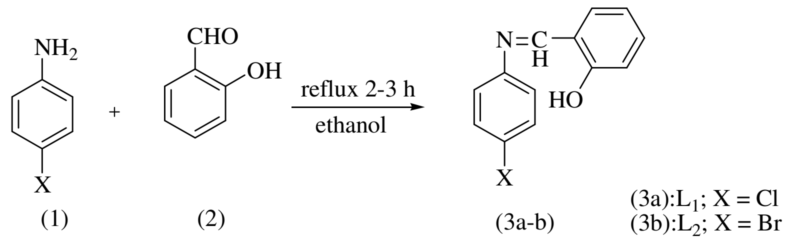
The Schiff bases (3a-b) and their derivatives (4-11) have been synthesized in good yields. All the synthesized compounds were screened *in vitro* for their antifungal potential against *A. triticina, B. sorokiniana* and *U. segetum tritici*. In most of the cases Schiff bases were found to be more active than their corresponding metal complexes at a particular concentration. Statistical analysis of the Schiff bases (3a-b) and their derivatives (4-11) was carried out and critical difference was calculated for the compounds. The results of interaction data showed that different compounds behave significantly different at different concentrations. In case of *A. triticina* (3b) and (8) showed promising antifungal activity with ED_{50} value 70 µg/ml and 120 µg/ml. In case of *B. sorokiniana* compounds (3a), (7) and (9) were found to be active even at low concentrations. In case of *U. segetum tritici* compounds (3a), (3b), (4) and (8) showed good antifungal activity. Thus, it was concluded that the compounds (3a), (3b), (4), (7) and (8) were effective against *A. triticina*, *B. sorokiniana* and *U. segetum tritici* whereas other compounds showed moderate to promising activity against test fungi.

CHAPTER - V

SUMMARY

Wheat is a cereal grain and is a type of fruit called caryopsis. It is the important staple food and strategic cereal crop of 36% of the world population. It is an excellent source of multiple essential nutrients such as proteins, dietary fiber, manganese, phosphorous and niacin, several vitamins and other dietary minerals and contain 13% water, 71% carbohydrates content significantly. It has higher protein content than any cereal crop. Development of cereal varieties that carry resistance against diseases is always a big challenge due to cost and availability of new breeds. Therefore, development of fungicides against diseases is thus a main focus for plant breeders and researchers.

Equimolar mixture of 4-chloro/4-bromoaniline (1) and salicyaldehyde (2) were refluxed in absolute alcohol (40.00 ml) until the reaction was completed (2-3 h, TLC) (Scheme 1). The solid products formed in the reaction mixture were filtered, washed with diethyl ether and recrystallized from absolute alcohol to obtain pure products (3a-d).



Scheme 1

To the hot solution of Schiff bases (3a-b) (2.00 mmol) in the absolute alcohol (20.00 ml), drop wise addition of metal salt (1.00 mmol) ($CdCl_2$, $NiCl_2$, $CuCl_2$, $MnCl_2$) (2:1) in hot alcoholic solution was made with constant stirring. Stirring was continued until the completion of reaction (1-2 h, solid formation, color change). The metal complexes (4-11) thus formed were filtered and washed with absolute alcohol to remove unreacted ligands and were dried in vacuum over fused $CaCl_2$.

All synthesized compounds were characterized by IR, ^{1}HNMR, ^{13}CNMR, mass, elemental analysis and molar conductance measurements. ^{1}HNMR and ^{13}CNMR spectra confirmed the formation of Schiff bases (3a-b). The ^{1}HNMR spectra (DMSO) of the Schiff bases (3a) exhibited signals at δ 8.58 ppm due to the azomethine protons which indicated the formation of Schiff base. Aromatic protons exhibited multiplets at δ 6.92–7.40 ppm for ligand (3a). A singlet due to the phenolic OH proton appeared at δ 13.00 for the Schiff base (3a) which exchanged with D_2O.

The ^{1}HNMR spectra (DMSO) of the Schiff bases (3b) exhibited signals at δ 8.59 ppm due to the azomethine protons which indicated the formation of Schiff base. Aromatic protons exhibited multiplets at δ 6.92-7.55 ppm for ligand (3b). A singlet due to the phenolic OH proton appeared at δ 12.98 for the Schiff base (3b) which exchanged with D_2O.

The peaks in ^{13}CNMR (DMSO) spectrum for ligand (3a) appeared at δ 117.3, 119.0, 119.2, 122.5, 129.5, 132.4, 132.5, 133.4, 147.0, 161.1 and 163.0 ppm. The peaks in ^{13}CNMR (DMSO) spectrum for ligand (3b) appeared at 117.3, 119.0, 119.2, 120.3, 122.8, 132.4, 133.4, 147.4, 161.1 and 163.0 ppm.

IR studies also justified the structure predicted for Schiff bases (3a-b) and their metal complexes (4-11). Bands of azomethine group were present around 1608 cm^{-1} in Schiff bases (3a,b) which shifts to slightly higher frequency side on co-ordination with metal ion. Vibrations in the regions in between 516-527 cm^{-1} have been assigned to *v*(M-N) and 698-827 cm^{-1} have been assigned to *v*(M-O) bands, which confirmed the formation of metal complex. Bands at 753.67 cm^{-1} and 677.86 cm^{-1} were observed due to *v*(C-Cl) and *v*(C-Br).

Elemental analysis helps in determination of the ratio of C, H and N in the synthesized compound. Compound (3a) having molecular formula $C_{13}H_{10}ClNO$, the calculated value of carbon hydrogen and nitrogen were 67.38, 4.31 and 6.04 per cent and the observed values were found to be 67.09, 4.34 and 6.01 per cent which were close to the required value. Similarly calculated value of carbon, hydrogen and nitrogen in (3b) having the molecular formula $C_{13}H_{10}BrNO$, were 56.54, 3.62 and 5.07 per cent and observed values were 55.34, 3.71 and 4.85 per cent. Elemental analysis confirmed 1: 2 geometry of the metal complexes ($M:L_2$).

Mass spectra of Schiff bases (3a-b) showed a molecular ion peak at *m/z* 232 and 276 (M^++1) respectively. Molar conductance data revealed that all the synthesized compounds are non-electrolytes as all are having very low values of molar conductance in acetone.

The absorption spectra of synthesized ligands and their metal complexes with Cu (II), Ni (II), Cd (II) and Mn (II) were recorded in DMSO. The ligand (3a) absorbed at 240 nm (ε = 57300 l/mol/cm) giving a sharp band corresponding to π-π^* transitions and a broad band at 261.50 nm (ε = 56000 l/mol/cm) corresponding to n-π^* transition involving molecular orbitals of C=N chromophore and benzene ring. The ligand (3b) showed absorption bands corresponding to π-π^* transition at 274.50 nm (ε = 119500 l/mol/cm). Band due to n-π^* transition appeared at 311 nm (ε = 51200 l/mol/cm).

Antifungal activity of synthesized Schiff bases (3a-b) and their metal complexes against *Alternaria triticina*, *Bipolaris sorokiniana* and *Ustilago segetum tritici* was done by spore germination inhibition method. Concentrations used for these tests were 1000, 500, 200, 100, 50, 10, 5 and 1 μg/ml and Tween 20 was used as emulsifier.

Pathogens were obtained from the infected wheat leaves showing the characteristic symptoms. The cultures were obtained by suitable method and maintained by further sub culturing on Potato Dextrose Agar (PDA) slants and keeping them in the refrigerator. The stock solution (2000 µg/ml) of each compound and standard fungicide *viz.* Raxil 60 FS (Tebuconazole) and Tilt 25 EC (Propiconazole) was prepared. The required dilutions of 1000, 500, 200, 100, 50, 10, 5 and 1 µg/ml were subsequently made from the stock solution by addition of distilled water.

The effect of concentration on percent spore germination inhibition was calculated. Statistical analysis showed that the compounds behaved significantly different from each other at different concentrations. Most of the compounds were found to be significantly different at higher concentrations, but at lower concentrations data showed that most of the compounds were not significantly different. Also, ED_{50} and ED_{90} values have been calculated from the plot of percent spore germination inhibition against concentration. The activity data revealed that ligands and the metal complexes were effective antifungal agents (Neelakantan *et al* 2010). In case of *A. tritcina* compound (3b) and (8) showed 90-95% inhibition at 1000 µg/ml concentration. In case of *B. sorokiniana* compound (3a), (7) and (9) showed maximum inhibition at 1000 µg/ml. In case of *U. segetum tritici* compounds (3a), (3b), (4) and (8) showed good antifungal activity. Thus it was concluded that compounds (3a), (3b), (4), (7) and (8) were most effective antifungal agents against all test fungi *i.e. Alternaria triticina*, *Bipolaris sorokiniana* and *Ustilago segetum tritici*. Other compounds showed moderate to promising antifungal activity.

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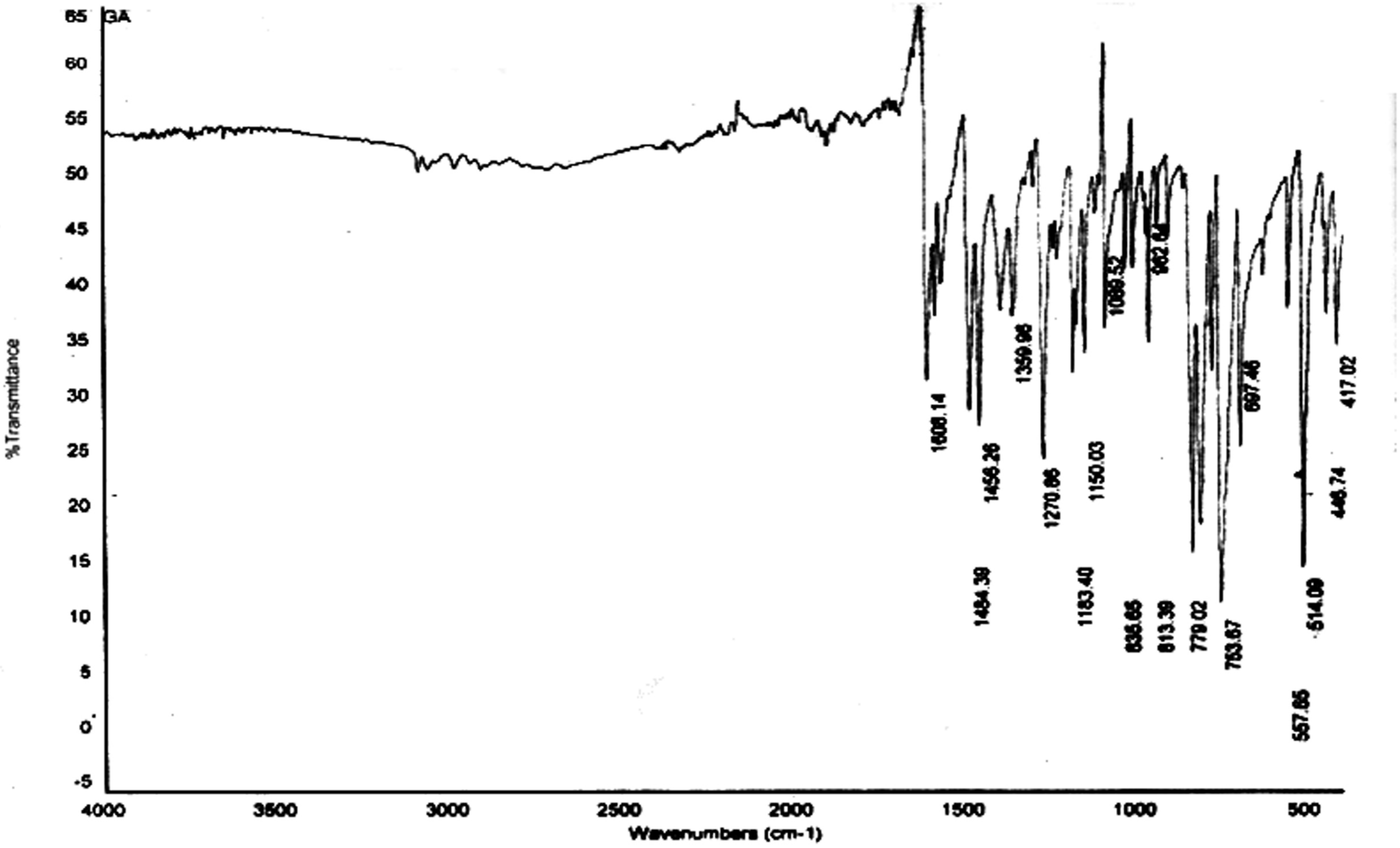
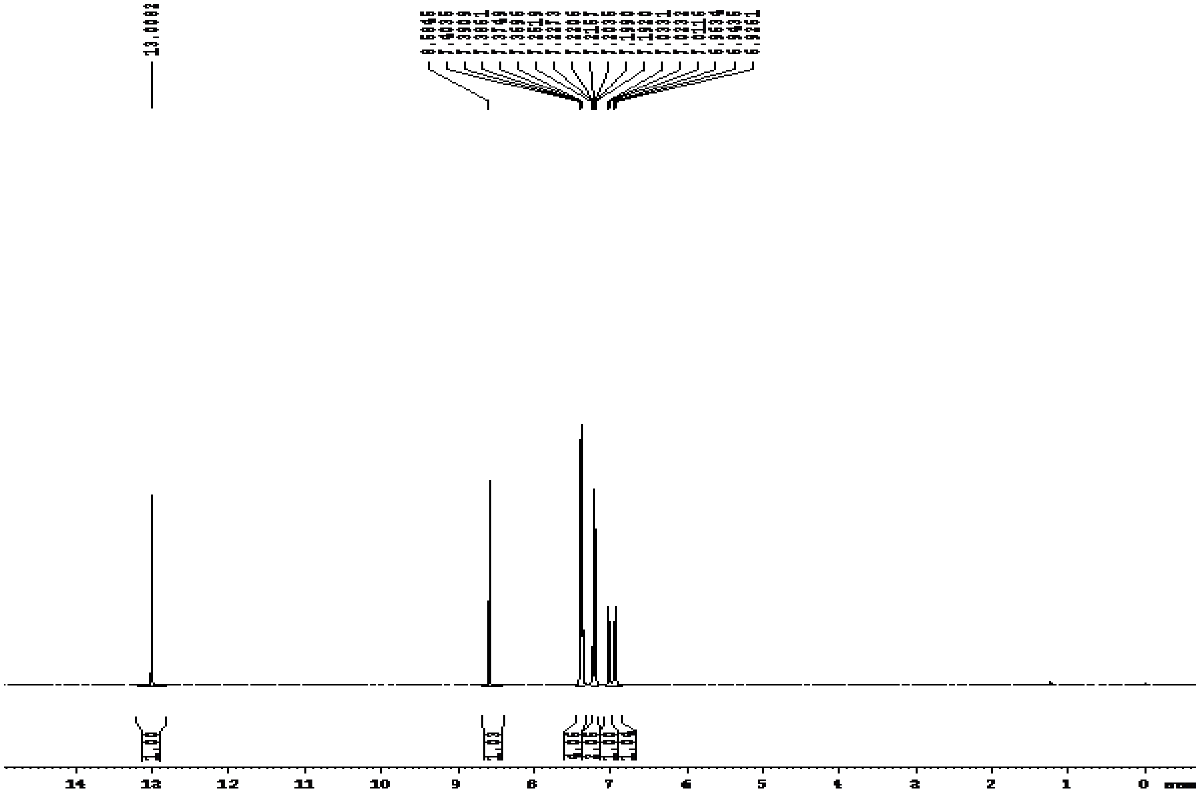
ANNEXURE I



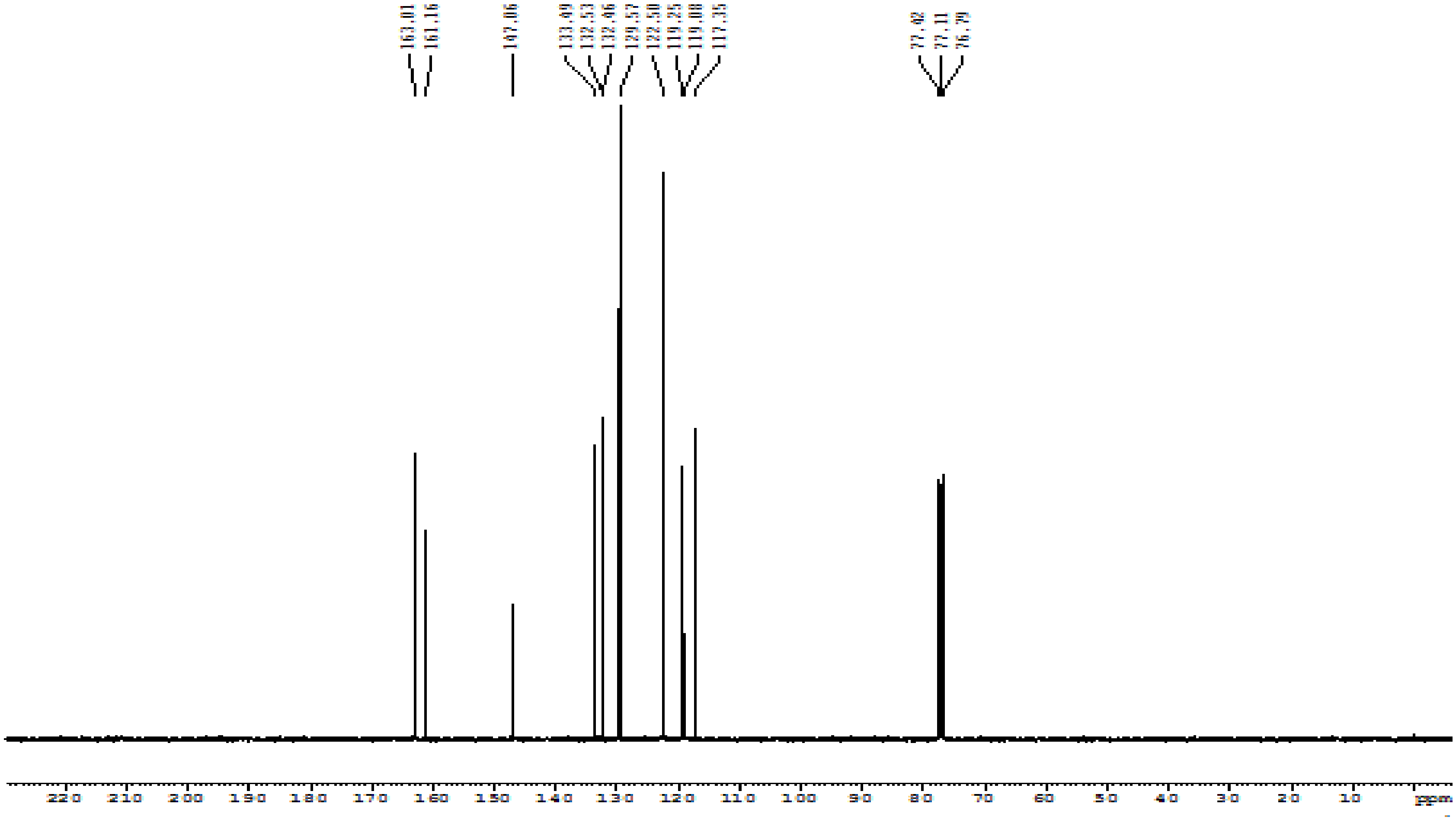
IR spectrum of compound (3a)

ANNEXURE II



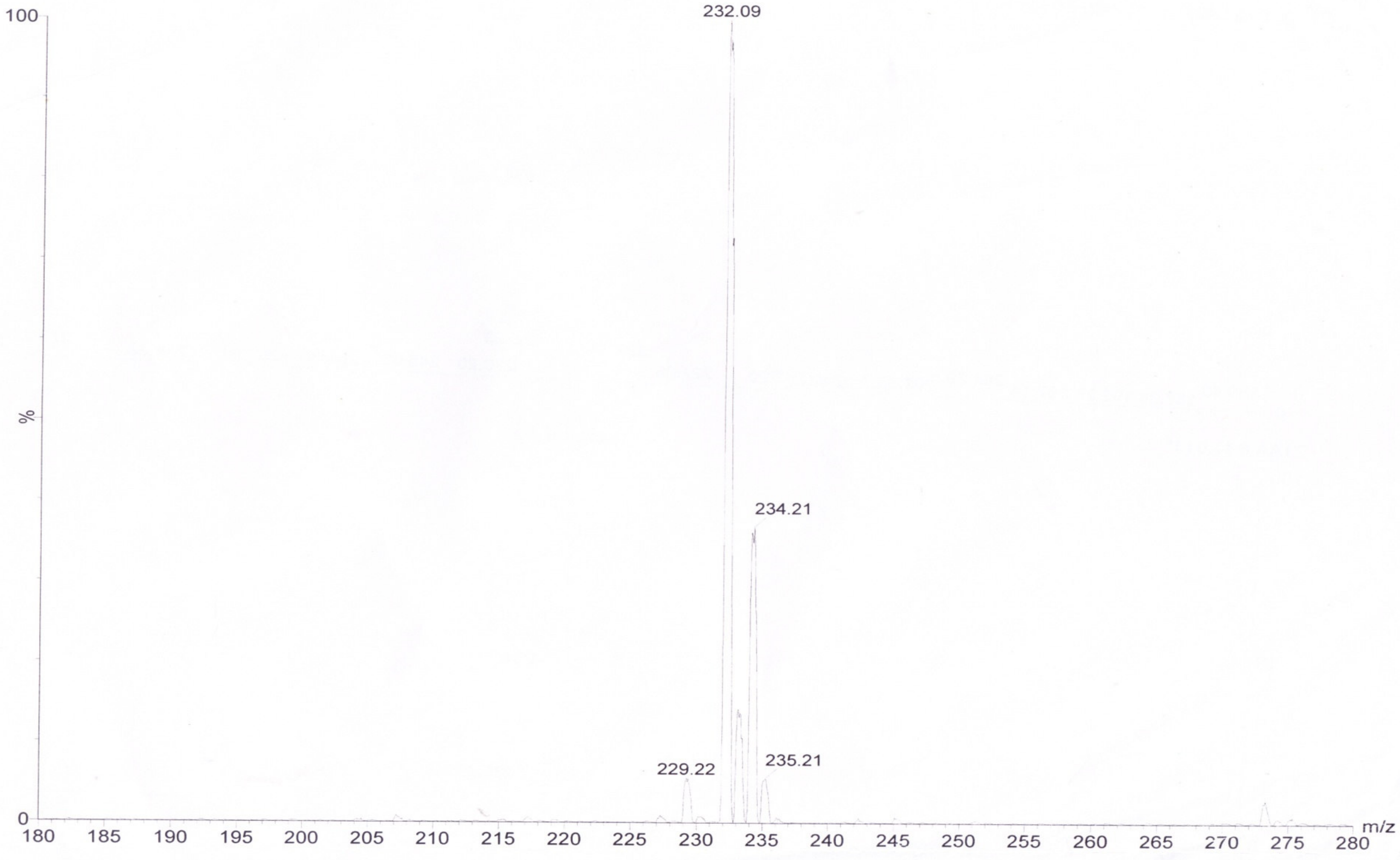
^{1}HNMR of compound (3a)

ANNEXURE III



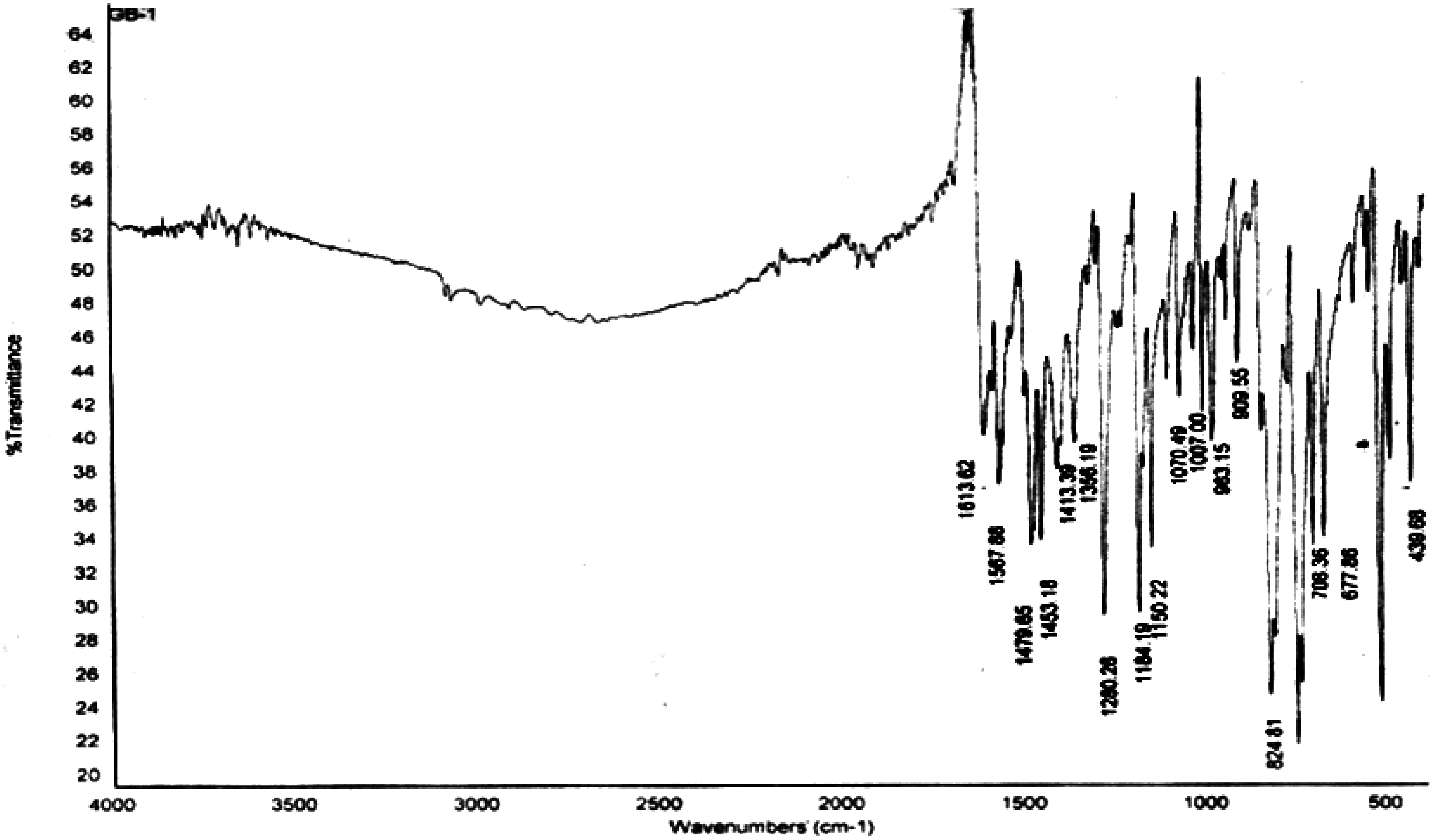
^{13}CNMR spectrum of compound (3a)

ANNEXURE IV



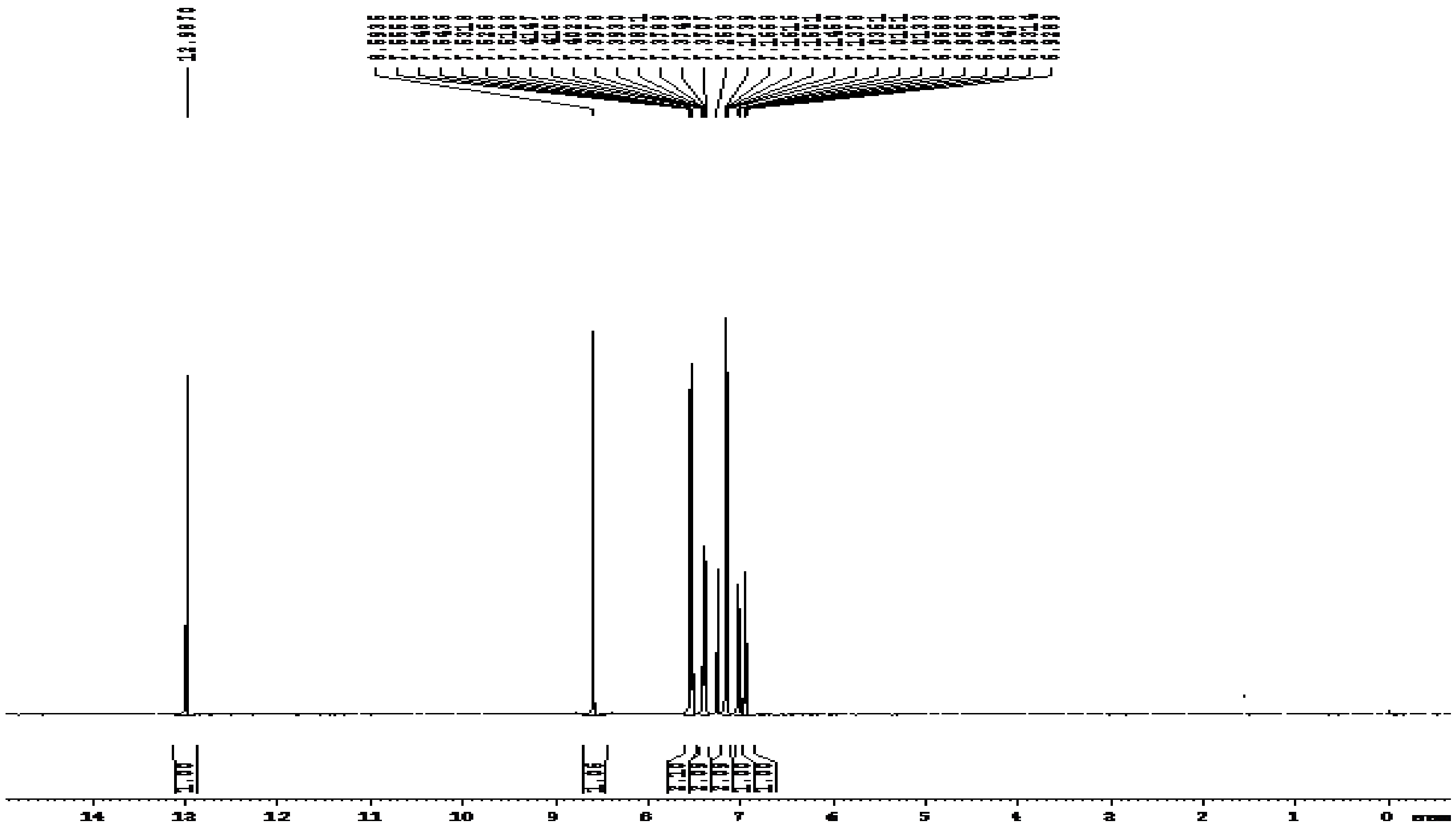
Mass Spectra of compound (3a)

ANNEXURE V



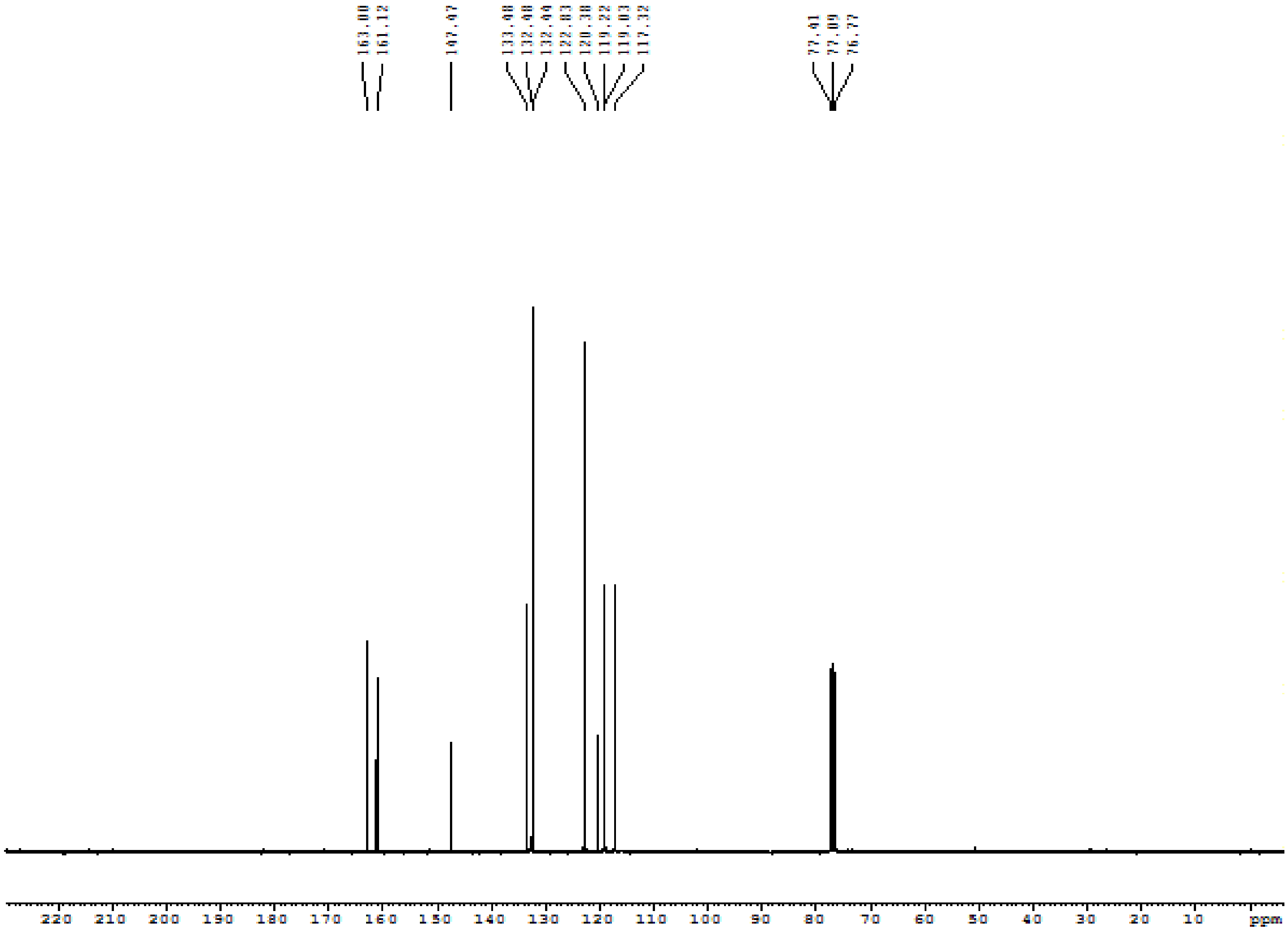
IR Spectrum of compound (3b)

ANNEXURE VI



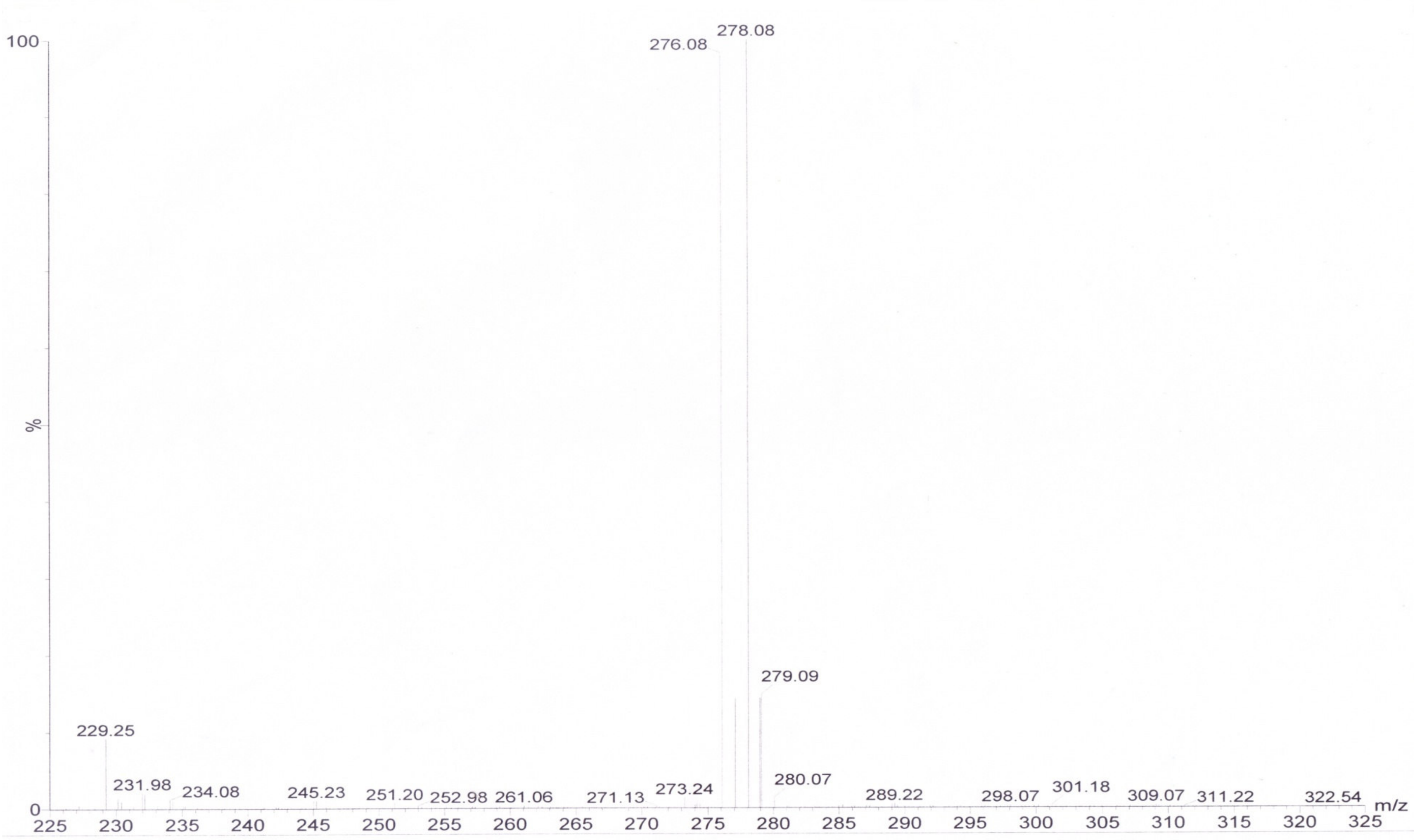
^{1}HMR of compound (3b)

ANNEXURE VII



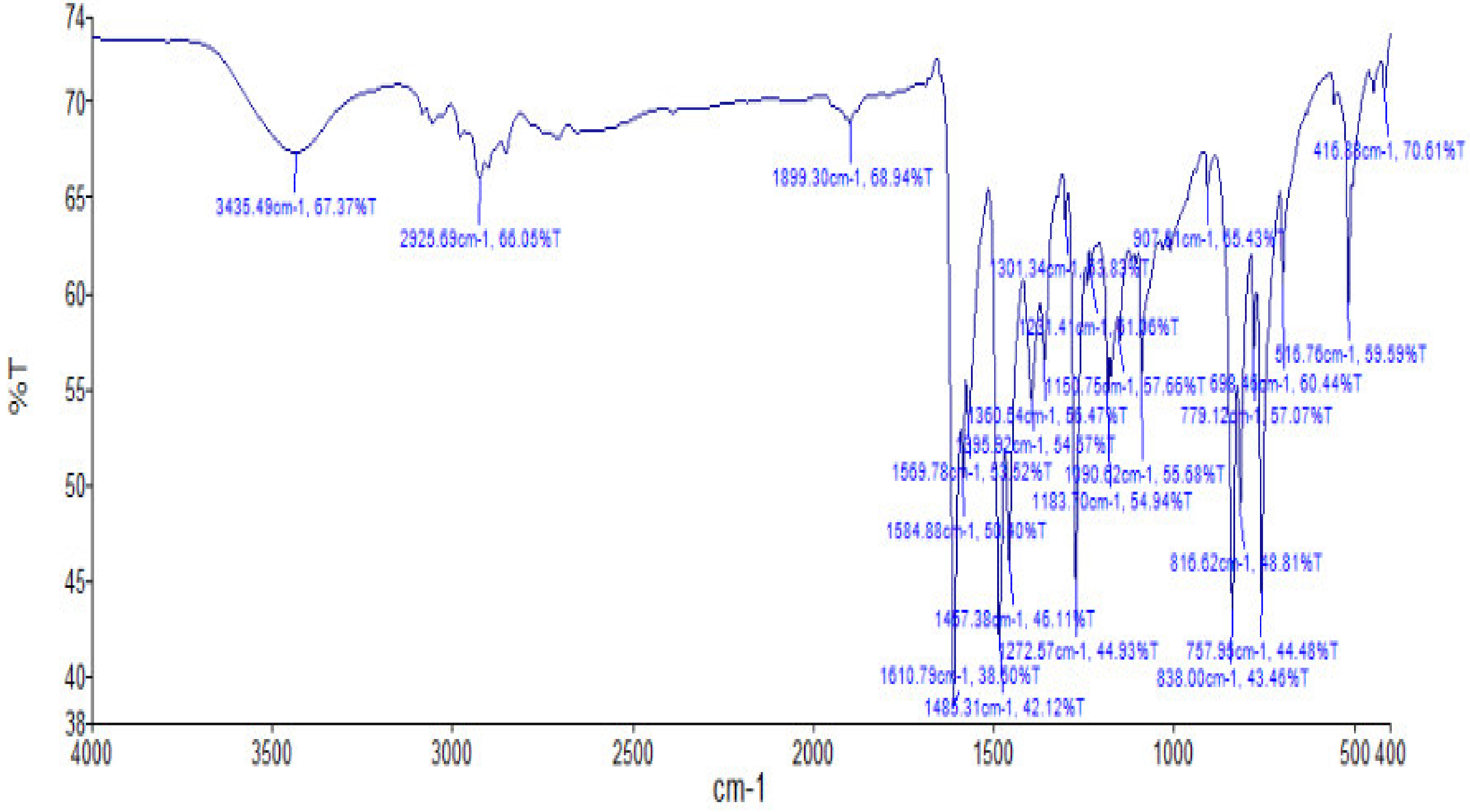
^{13}CNMR spectrum of compound (3b)

ANNEXURE VIII



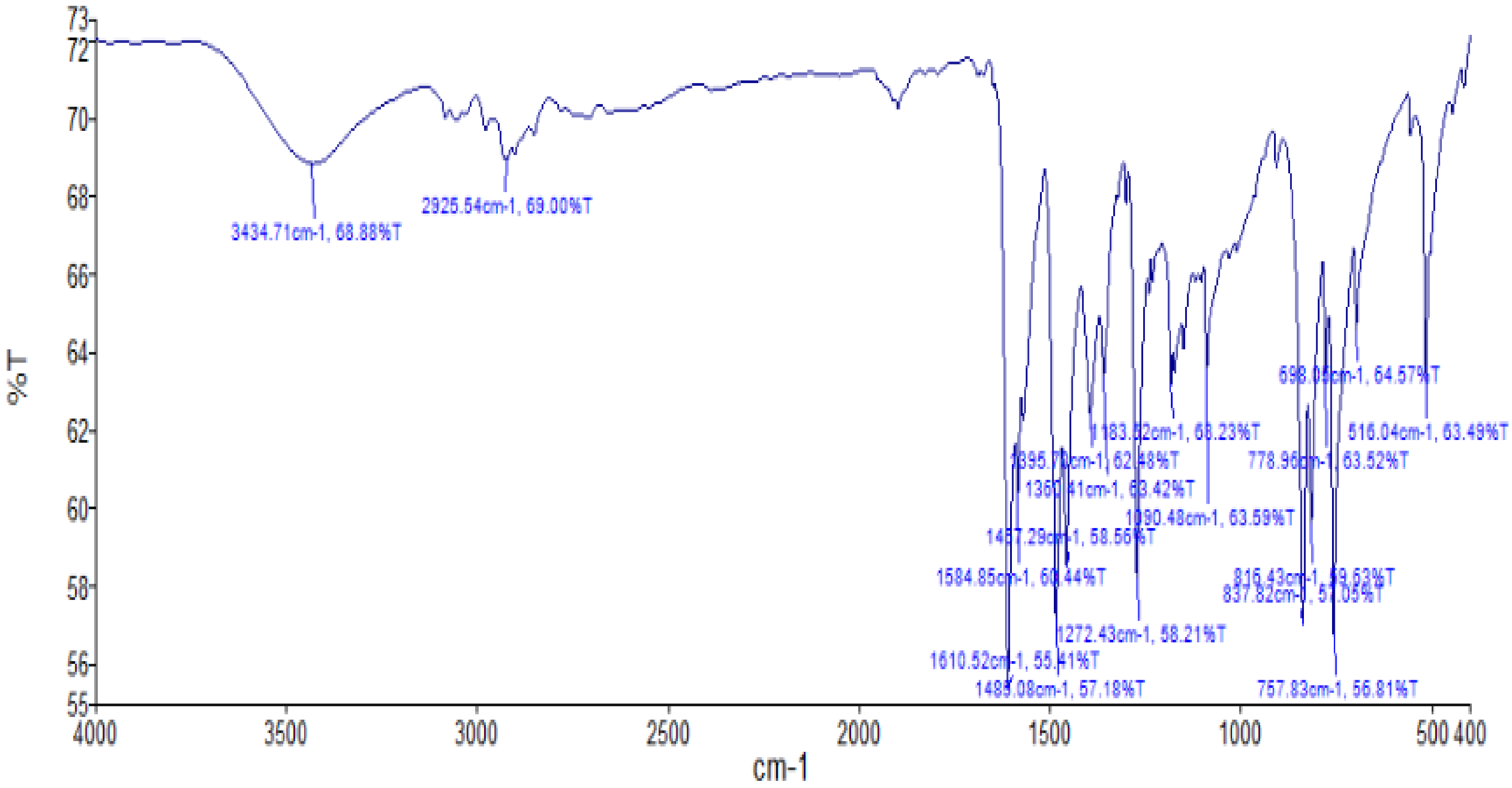
Mass Spectra of compound (3b)

ANNEXURE IX



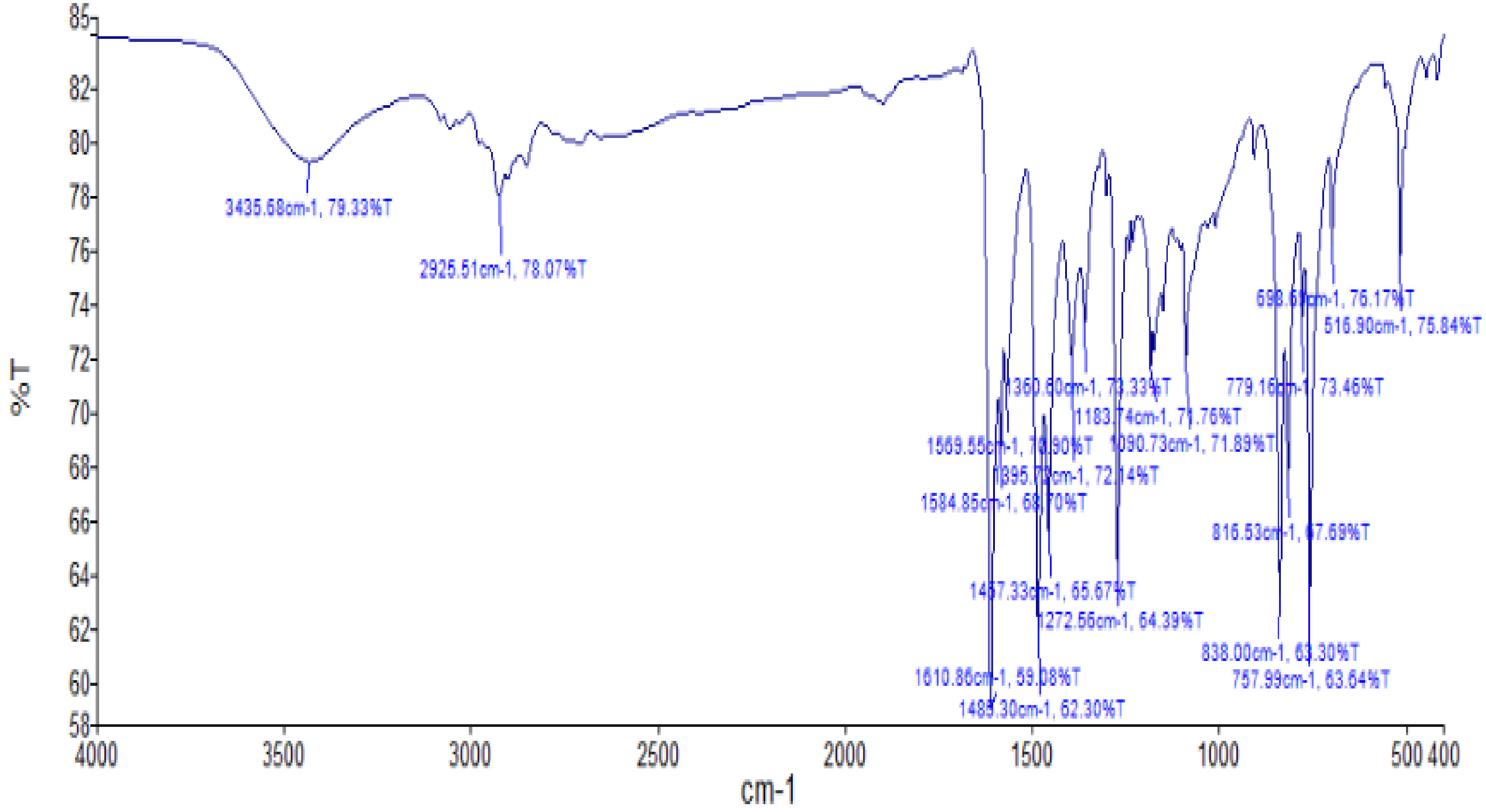
IR Spectrum of compound (4)

ANNEXURE X



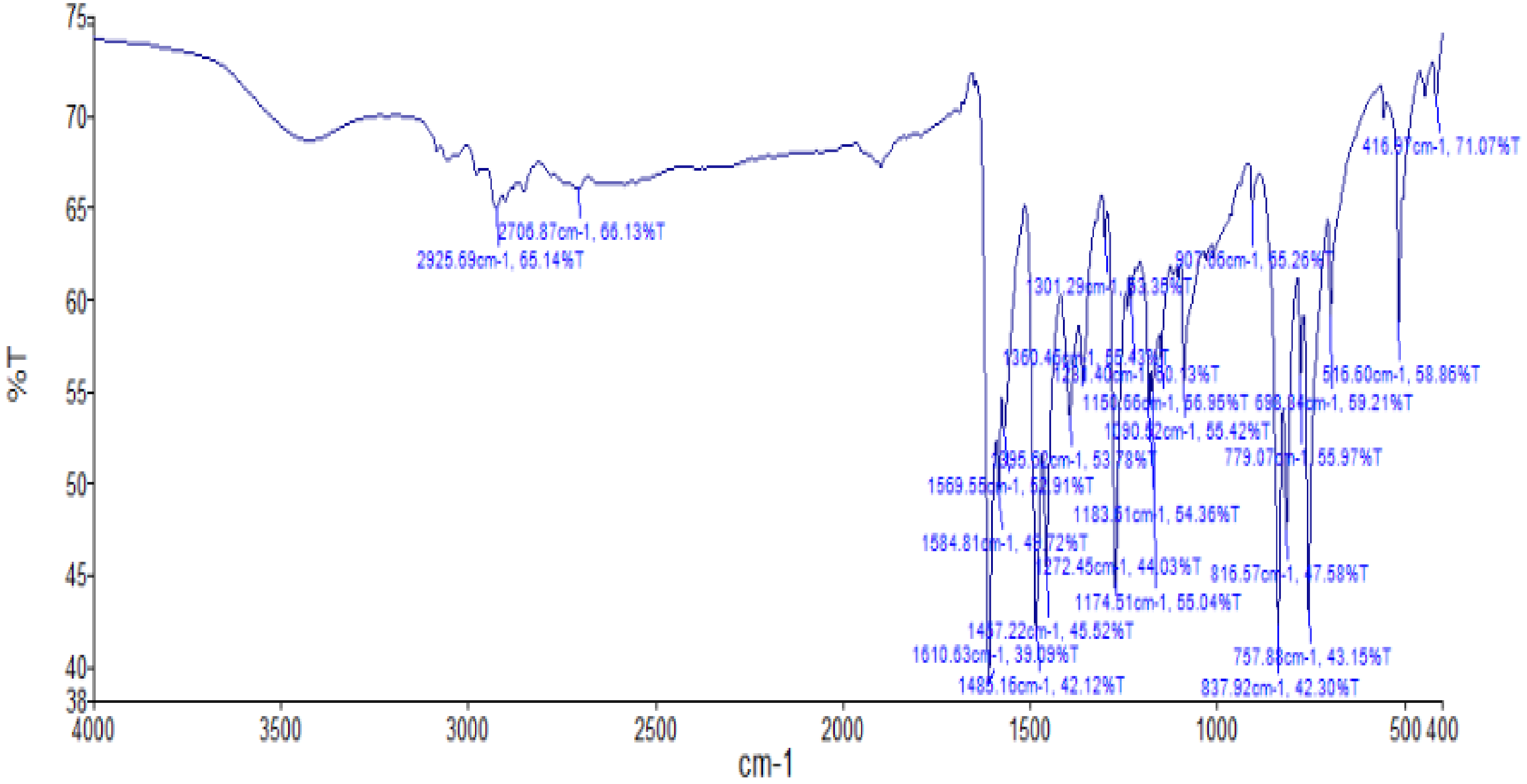
IR Spectrum of compound (5)

ANNEXURE XI



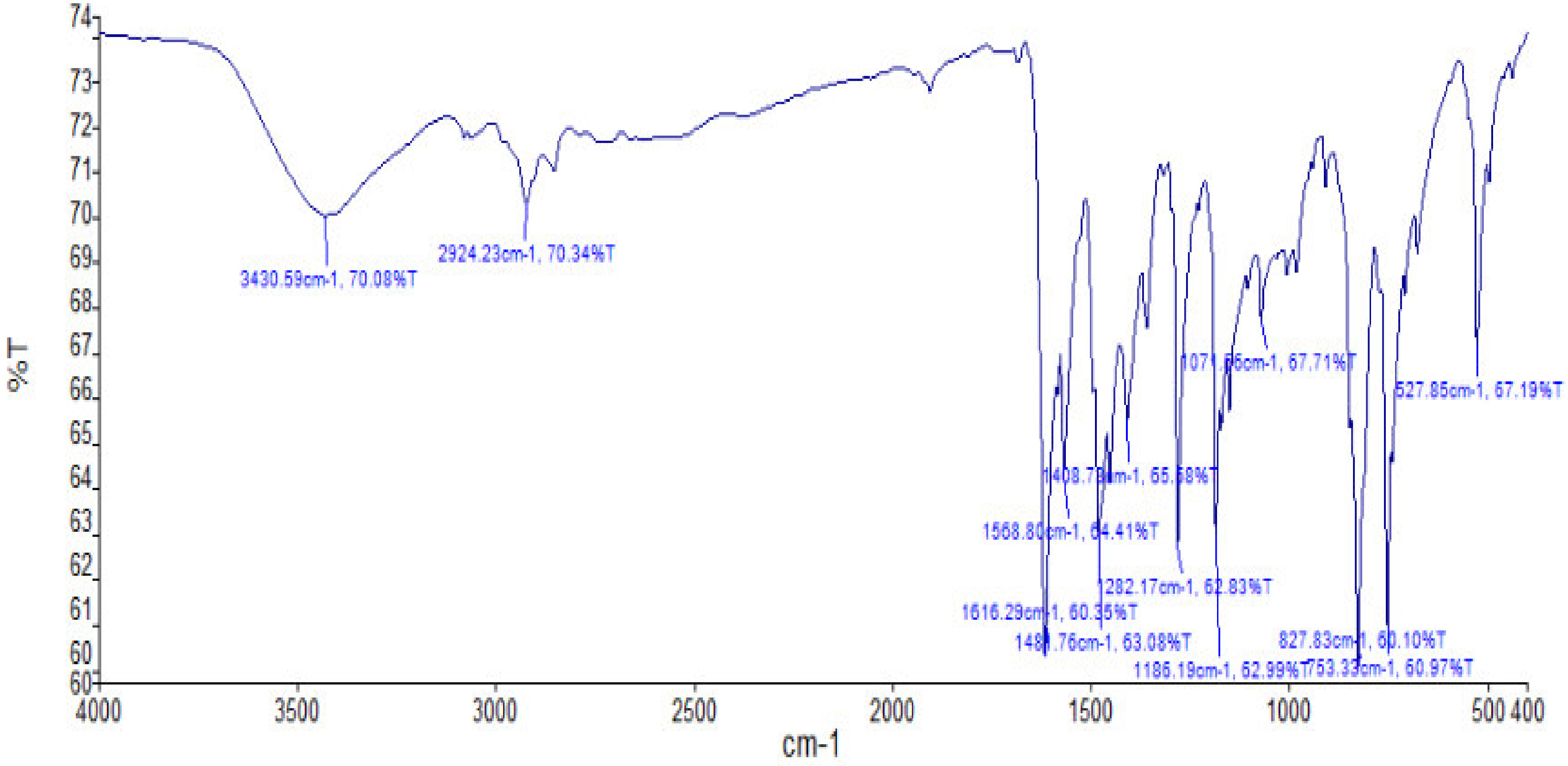
IR Spectrum of compound (6)

ANNEXURE XII



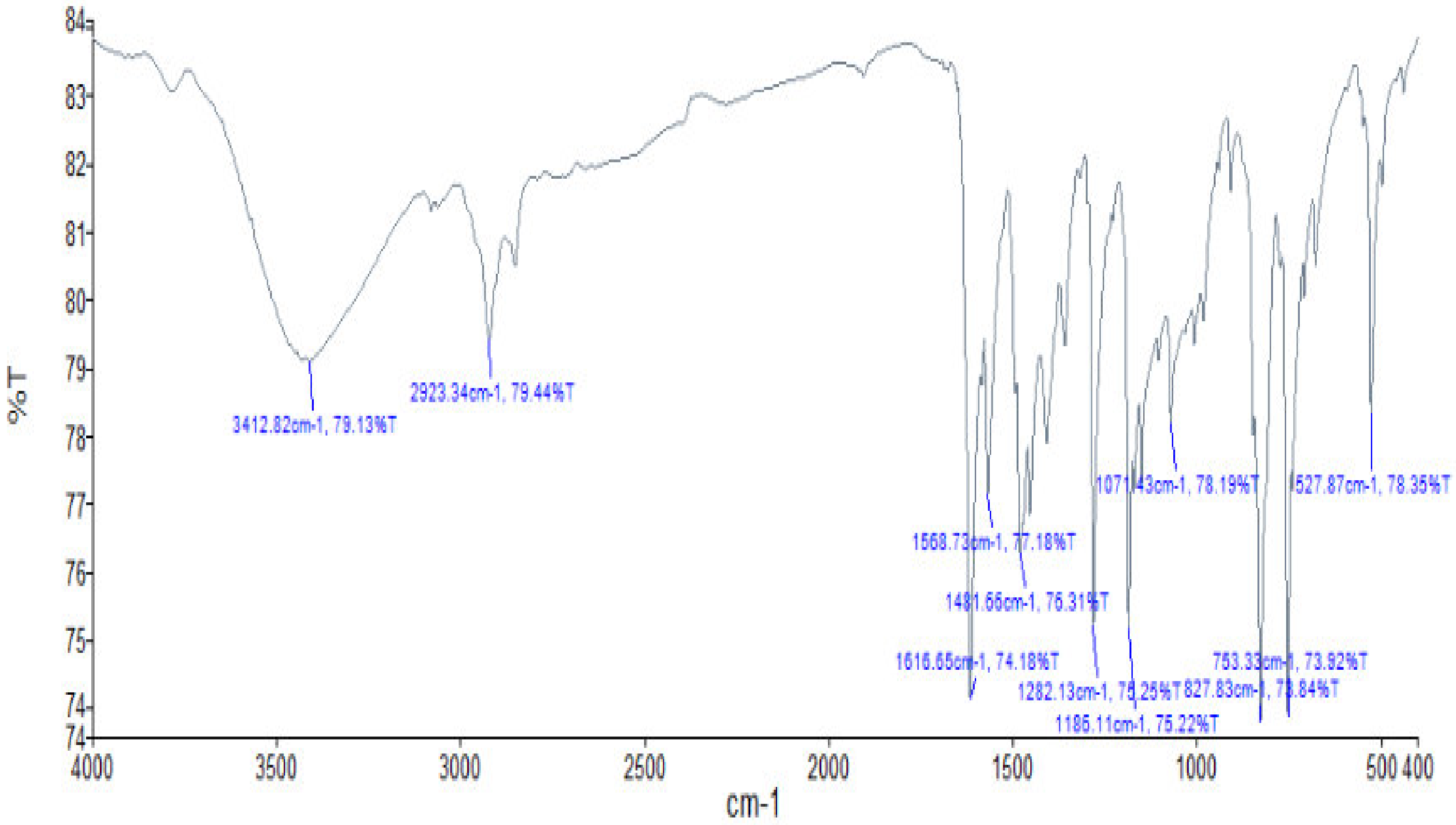
IR Spectrum of compound (7)

ANNEXURE XIII



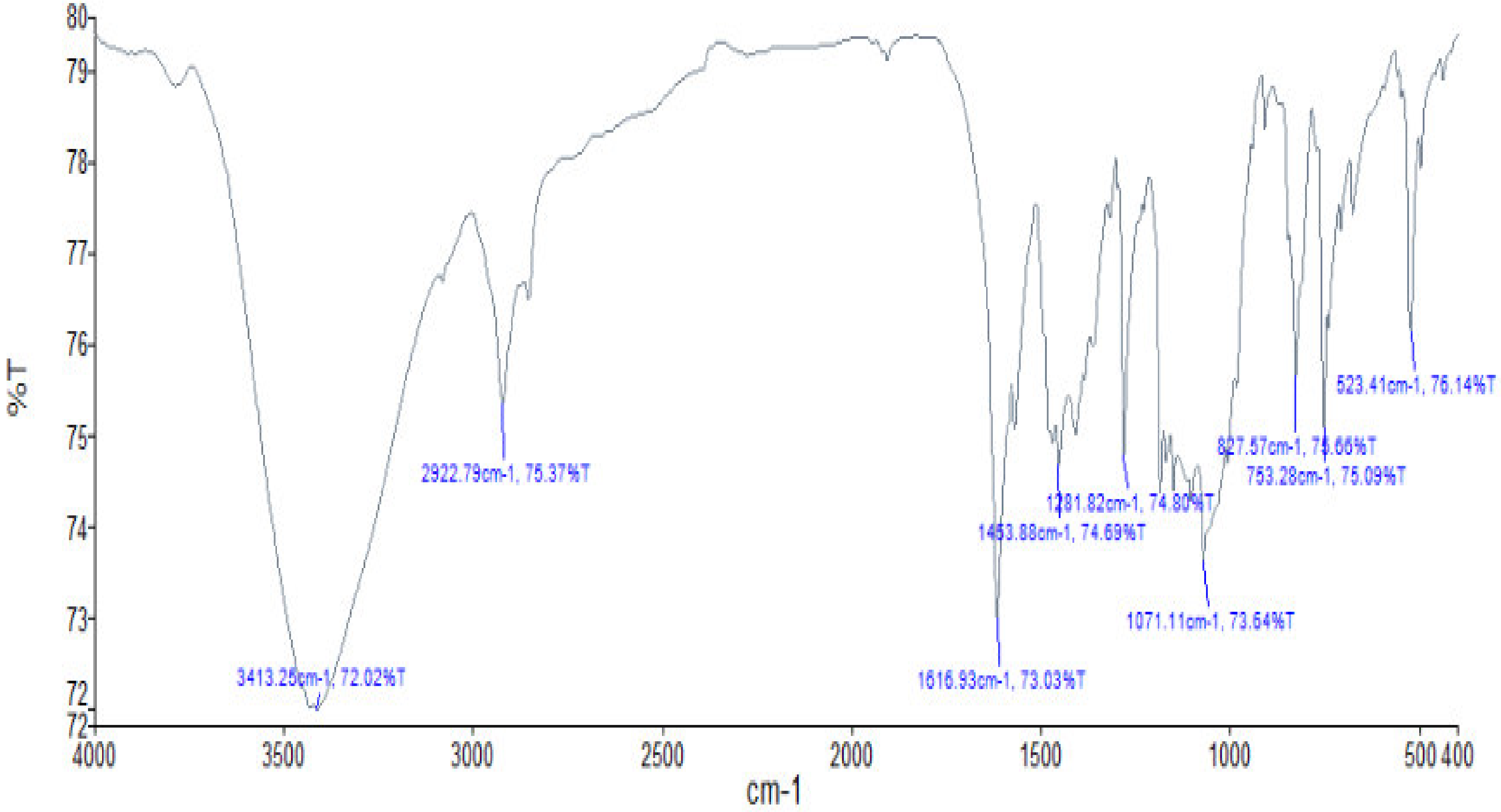
IR Spectrum of compound (8)

ANNEXURE XIV



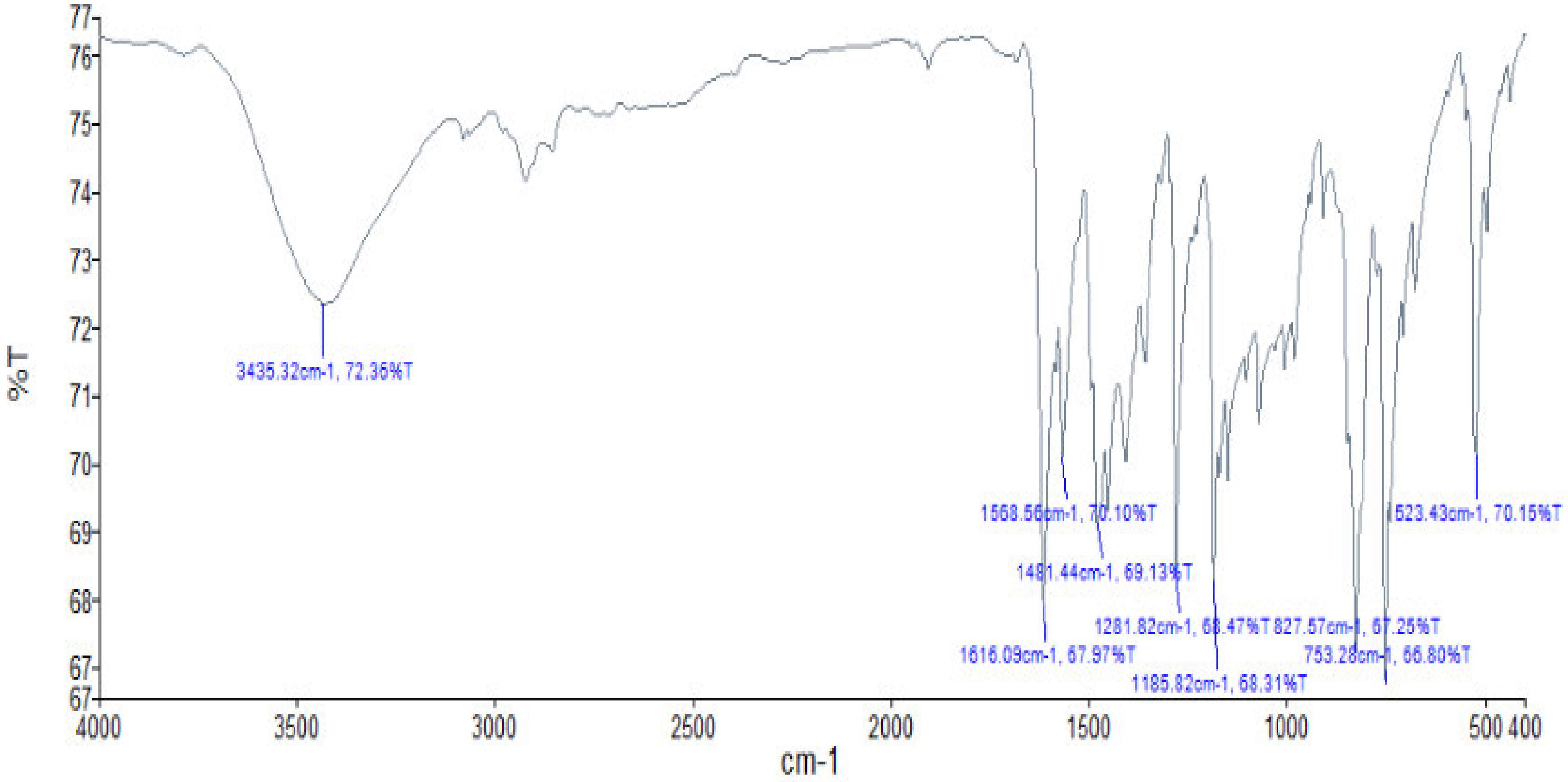
IR Spectrum of compound (9)

ANNEXURE XV



IR Spectrum of compound (10)

ANNEXURE XVI



IR Spectrum of compound (11)

VITA

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