An-Najah National University

Faculty of Graduate Studies

Synthesis and Biological Activity of New Heterocyclic Schiff Bases

By

Ala'a Abed Al-Raheem Janem

Supervisor

Prof. Dr. Mohammed Al-Nuri

Co-supervisor

Prof. Dr. Ismail Warad

This Thesis is submitted in Partial Fulfillment of the Requirements for the Degree of Master of Chemistry, Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.

2016

Synthesis and Biological Activity of New Heterocyclic Schiff Bases

By

Ala'a Abed Al-Raheem Janem

This Thesis was Defended Successfully on 10/11 /2016 and approved by:

Defense Committee Members	Signature
1. Dr. Mohammed Al-Nuri /Supervisor	Mohammed A. Al-Nuri
2. Dr. Ismail Warad /Co- Supervisor	
3. Dr. Orwa Houshia /External Examiner	
4. Dr. Nizar Matar/Internal Examiner	

Dedication

To my parents for helping, taking care and praying for me.

To my Fiance Ameed Amireh for his support, love, and encouragement.

To my sisters Noura , Amira and Nida'a who supported me and shared my worries.

To my brothers for their love.

To my friends for their continuous support.

To all who prayed for me.

To all whom I loved and knew.

Acknowledgments

First, I need to express my deep gratitude to Almighty Allah who gifted me his blessings, and aided me to accomplish my studies for the Master's degree. Thanks to Allah for granting me more than what I deserve, and for Allah's continuous care and generosity.

I would like to thank both of my supervisors Prof. Mohammed Al-Nuri and Prof. Ismail Warad for their support throughout the several months of work of my Master thesis, keeping me going when times were tough, asking insightful questions, and offering invaluable advice.

I am also grateful to Dr. Ahmad Hussein for his continuous support and guidance from day one of my Master's work.

I am also grateful to my external and internal examiners, Dr. Orwa Houshia and Dr. Nizar Matar whose attendance of my defense is appreciated.

I also appreciate the lab technicians at An-Najah National University. In this respect, I especially thank Mr. Nafeth Dwekat. I am also thankful to Ameed Amireh for his help.

Very great help was also provided by Jordan university for the measurements of ^{1}H NMR and ^{13}C NMR data; and thanks are extended to BERC Centre Til Village- Nablus for the study of the biological impact of my studied compounds.

Finally, it is my family, siblings and close friends who deserve my deep gratitude. Thank you for being in my life, what made this achievement and success possible. Lastly and exclusively, my warmest thanks must be extended to my fiance Ameed, for his continuous and unfailing love. With his support and understanding, he underpinned my persistence in my Master study and made the completion of my thesis possible.

الإقرار

انا الموقعه ادناه مقدم الرسالة التي تحمل العنوان:

Synthesis and Biological Activity of New Heterocyclic Schiff Bases

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو بحث علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work and has not been submitted elsewhere for any other degree or qualification.

Student's Name: اسم الطالب: الاء جانم

Signature: التوقيع:

Date: التاريخ: 10/11/2018

List of Contents

List of Figures

List of Tables

List of Equations

List of Schemes

List of Abbreviations

Symbol	Abbreviation
DMSO	Dimethyl Sulfoxide
NMR	Nuclear Magnetic Resonance
PPM	Part per million
Hz	Hertz
S	Singlet
D	Doublet
T	Triplet
E. coli	*Escherichia coli*
S. aureus	*Psedudomonas aeruginosa*
DPPH	1,1-Diphenyl-2-picryl-hydrazyl
TG/DTG	Thermogravimetry/ Differential thermogravimetry

Synthesis and Biological Activity of New Heterocyclic Schiff Bases

By
Ala'a Abed Al-Raheem Janem
Supervisors
Prof. Dr. Mohammed Al-Nuri
Prof. Dr. Ismail Warad

Abstract

A series of novel biologically active hydrazides -hydrazone Schiff base were synthesized from various types of heterocyclic carbonyl compounds and amino groups of heterocyclic hydrazides using ethanol as a solvent.

Such Schiff bases have general formula: $R_1N=CHR_2$. In which R_1 and R_2 are: 2-amino-3-methylpyridine, nicotinic hydrazide, 2-furoic hydrazide, 2-amino-5-bromopyridine, salicoylhydrazides, dipyridyl ketone, pyridin-2-carbaldehyde, furfural, 2-thiophene-carbaldehyde, 5-bromo-2-thiophene-carboxaldehyde, respectively. Such compounds were characterized by various physiochemical techniques such as melting point, elemental analysis, FT- IR, UV-visible, TG/DTG, ^{1}H and ^{13}C NMR spectroscopy.

Chapter One
Introduction

1.1.Definition and History of Schiff Bases

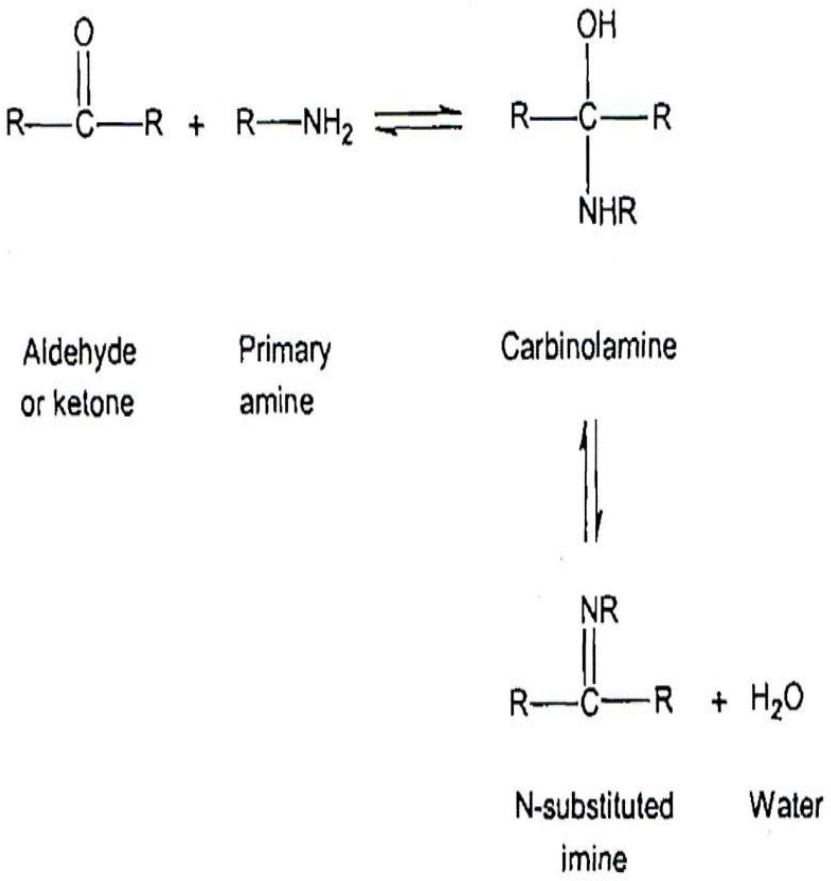
A Schiff base is a nitrogen analog of an aldehyde or ketone in which the C=O group is replaced by C=N-R group(azomethine). It is usually formed by condensation of an aldehyde or ketone with a primary amine and they were first reported by Schiff in 1864 as shown in the following equation:

The common structural feature of these compounds is the azomethine group with a general formula RHC=N-R', where R and R' are alkyl, aryl, cycloalkyl or heterocyclic groups which may be variously substituted. These compounds are also known as anils, imines or azomethines[1].

Schiff bases that contain aryl substituents are substantially more stable and more readily synthesized, while those which contain alkyl substituents are relatively unstable[2]. Schiff bases of aliphatic aldehydes are relatively unstable and readily polymerizable while those of aromatic aldehydes, having effective conjugation, are more stable[3-5].

The formation of a Schiff base from an aldehydes or ketones is a reversible reaction and generally takes place under acid or base catalysis, or upon

heating, as shown in **Scheme 1.1**. The formation of the Schiff base is generally driven to completion by separation of the product or removal of water, or both. Many Schiff bases can be hydrolyzed back to their aldehydes or ketones and amines by aqueous acid or base[6].



Scheme 1.1: Formation of the Schiff base

Several studies showed that the presence of a lone pair of electrons in an sp^2 hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical application and biological importance[7-13], such activities including antibacterial[14-20], antifungal[21-24], antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties. As an example they have shown activities against wide range of organisms

as : *Candida Albicans, Escherichia coli, Staphylococcus aureus, Bacillus licheniformis, Paenibacillus polymyxa, Trychophyton gypseum,* Mycobacteria, *Erysiphe graminis and Plasmopora viticola* [14-25].

Schiff bases have also shown clinical properties ,antiviral, anti-HIV, antiprotozoal and anthelmintic activities [26]. They also exhibit significant anticonvulsant activity, apart from other pharmacological properties [27].

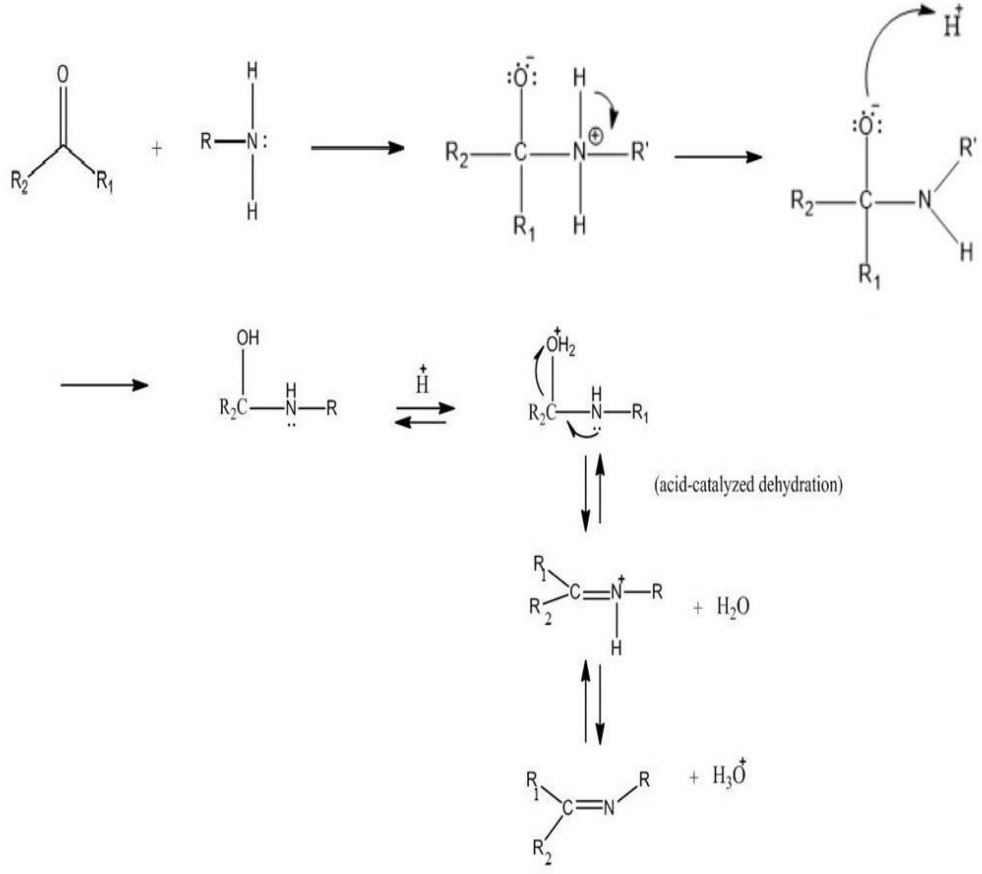
Schiff bases derived from 4-dimethylaminobenzaldehyde,o-phenylenediamine have also shown antibacterial activity, since they have been used as antibodies and anti-inflammatory agents [28-32].

Schiff bases appear to be an important intermediates in a number of enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate. One of the most important types of catalytic mechanism is the biochemical process which involves the condensation of a primary amine in an enzyme usually that of a lysine residue, with a carbonyl group of the substrate to form an imine, or Schiff base[33].

Stereochemical investigation, carried out with the aid of molecular model, showed that Schiff base formed between methylglyoxal and the amino group of the lysine side chains of proteins can bent back in such a way towards the nitrogen atom of peptide groups that a charge transfer can occur between these groups and oxygen atoms of the Schiff bases[34].

1.2 Mechanism of Schiff Bases Formation

The formation of Schiff base is a good example of nucleophilic addition reaction of nucleophilic amine to the carbonyl group. In the first part of the mechanism, the amine reacts with the aldehyde or ketone to give an unstable addition compound called carbinolamine. The carbinolamine loses water by either acid or base catalyzed pathways. Since the carbinolamine is an alcohol, it undergoes acid catalyzed dehydration[35]. **(Scheme 1.2)**

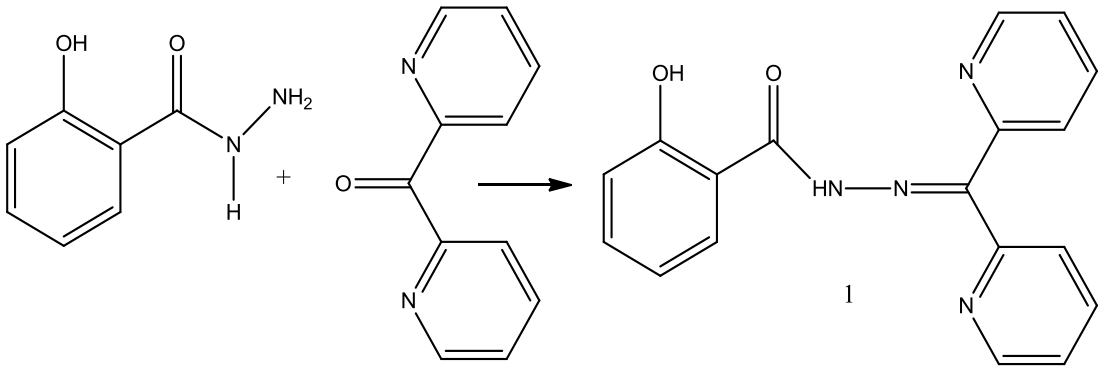


Scheme 1.2: Mechanism of formation of Schiff bases

Typically, the dehydration of the carbinolamine is the rate-determining step of Schiff base formation and that is why the reaction is catalyzed by acids. Yet the acid concentration cannot be too high because amines are

basic compounds. If the amine is protonated and becomes non-nucleophilic, equilibrium is pulled to the left and carbinolamine formation cannot occur. Therefore, many Schiff bases synthesis are best carried out at mildly acidic conditions.

The dehydration of carbinolamine is also catalyzed by base. This reaction is somewhat analogous to the E2 elimination of alkyl halides except that it is not a concerted reaction. It proceeds in two steps through an anionic intermediate, the Schiff base formation is really a sequence of two types of reactions, i.e. **addition** followed by **elimination**. In our present work ,we have synthesized new heterocyclic Schiff bases by utilizing hydrazides that contain the amino group that acts as the nucleophile which attacks the carbonyl group of the heterocyclic carbonyl compounds to produce heterocyclic Schiff bases. The following equation is an example of our present work (**Scheme 1.3**).



Salicoyl hydrazide Di(pyridin-2-yl)methanone *N*'-(di(pyridin-2-yl)methylidene)-2-hydroxybenzohydrazide

Scheme 1.3: (Synthesis of N'- (di (pyridine-2-yl) methylidene) -2-hydroxybenzoylhydrazide).

1.3.Biological Activities

1.3.1Biological Activity of Some Modified Compound

Biological activity refers to substances having or producing an effect on the living tissue or its ability to effect a change in a biological process.

The relation between the molecular entity and the biological activity can be tested by answering the following questions: (1) What is it? (2) What does it do? and (3) How much of it is present? These questions can express the activity of the compound. The importance of biological processes refers to the description of functional relationships between biological activities and the chemical substances that express them [36].

1.3.2 Anti-microbial (antibacterial)

Microbes are tiny organisms seen by a microscope. These microbes are found in air, soil, rock, plants, bodies and water. Microbes are known to replicate and spread rapidly. Microbial organisms include bacteria, viruses, fungi, and protozoa. Some microbes cause disease and are called parasites. However, many others exist in the body as normal flora without causing harm and may be beneficial[37].

Antimicrobial drugs are synthesized to inhibit the microbe without any side effects on the patients[38]. Antibiotics are one of the most important weapons we have in the fight against bacterial infections, and the

manufacture of these antibiotics has a strong relationship with the nature of life associated with human health[39].

1.3.3 Anti-microbial (antifungal activities)

An antifungal medicine is a drug that works selectively to eliminate fungal pathogens from a host with minimal toxicity to the host [40].Unlike bacterial disease, fungal diseases are more difficult to treat. Topical and oral treatments are long term and partially successful in controlling the fungus.

Fungal infections of the skin are the most abundant and widespread group of all mycoses. Skin mycoses affect more than 20–25% of the world's population, which makes them one of the most frequent forms of infections. [41].

The presentations of tinea infections range and its causative species are shown in the **Table 1.1**[42].

1.3.4 Anti-oxidants

Anti-oxidant "free radical scavengers" are substances that may prevent or delay some types of cell damage by reacting with and blocking the activity of free radicals and preventing them from causing the damage of scavengers so as to prevent/delay different diseased states. These free radicals are considered as highly reactive species that have an odd number of electrons, which gives them high potentials to cause damage to cells

called cellular pathologies. Some of these damages may lead to cancer. In the biological system, oxygen gives rise to a large number of free radicals and other reactive species collectively known as ‘reactive oxygen species’

(ROS).‘Reactive nitrogen species’ (RNS) are another group of reactive species that play a dual role as both deleterious and beneficial species [43, 44, 45].

Antioxidants are very important organic compounds especially in designing new novel drugs. Two types of free radicals exist. The first type is synthesized naturally by the body. The second type is introduced to our bodies through external sources. Sources of radicals are tobacco smoke, exposure to the sun, and other pollution forms of the body. This makes endogenous antioxidants, which are used to neutralize free radicals. However, the body also needs external sources of antioxidants called (exogenous) sources or dietary antioxidants like fruits and vegetables [46]. The high potential of free radicals gives them the high reactivity which harms the cells. They are created when an atom or a molecule either gains or loses an electron (a small negatively charged particle found in atoms)[48].

As the concentrations of free radicals increase, their hazard on the body increases and causes the damage to all major components of cells, including proteins, DNA, and cell membranes. Many of these mutagens and carcinogens may act through the generation of oxygen radicals, as a

result of the damage of DNA. Such conditions are suitable environments for the establishment and progression of cancer [49, 50].

Plants convert the solar energy into chemical energy so there's a hazard due to the excess energy and due to fear of oxidative damage of the plant cell. Nonetheless, the presence of antioxidant in plants will prevent the oxidative damage. Many of such compounds that protect plant cells are also found in human and protect human cells [51].

1.4 Aim of the study

The main objectives of this study are the following :

1- Synthesis of several heterocyclic Schiff bases using various types of carbonyl compounds .

2- Characterization of the synthesized compounds by elemental analysis, IR, NMR &UV spectroscopy.

3- Evaluate the biological activities of the synthesized Schiff bases including (antimicrobial, antifungal, antibacterial activities).

Table 1.1: Tinea Infections Range and its Causative Species

Tinea Infections- Type	Common Causative Species
Tinea corporis (arms, legs and trunk)	*Microsporum canis*
	Trichophyton rubrum M. Canis
Tinea cruris (gorin)	*T. rubrum*
Tineapedis (feet)	*Trichophyton mentagrophytes* *T. rubrum*
Tinea manuum(hand)	*T. rubrum*
Tinea unguium (finger,nails,and toe nails)	*T. rubrum*
Tinea unguium (finger,nails,and toe nails)	*Trichophyton mentagrophytes*

Chapter Two

Experimental

All chemicals were purchased from Sigma-Aldrich Chemical Company and used without further purification unless otherwise specified. Ethyl acetate, diethyl ether, ethanol, cyclohexane, methanol and dichloromethane were purchased from Chemical Science Company (CS company).

All prepared Schiff bases were characterized by IR , ^{1}H NMR , ^{13}C NMR and UV- visible spectroscopy. Nuclear magnetic resonance spectra were recorded on Bruker 500MHz-Avance III at the Chemistry Department at the University of Jordan.

Infrared spectroscopy were recorded on Fourier transform infrared spectrophotometer (Nicolet Is5 –Id3).

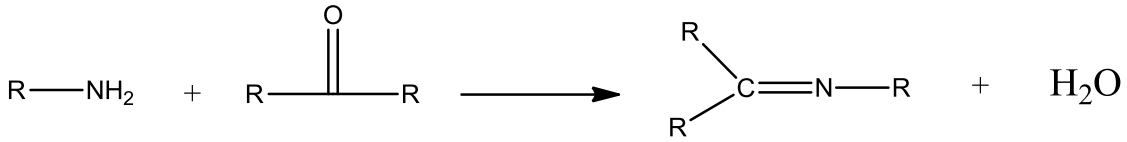
All ^{13}C NMR and ^{1}H NMR were reported in δ units, parts per million (ppm) downfield from tetramethylsilane (TMS). TLC analysis was performed on silica gel plates pre-coated with Merck kieselgel 60 F254 (purchased from Aldrich Chemical Company) and visualization was done using UV lamp. Sample purifications were performed by crystallization. Melting points were measured using Stuart melting point apparatus ,R00102618.

The antibacterial activity of the synthesized compounds was determined against the following microorganisms: *Staphylococcus aureus* (ATCC 25923), *Salmonella, Klebsiella pneumonia* (ATCC 13883), *Proteus*

vulgaris (ATCC 13315), and *Pseudomonas aeruginosa* (ATCC 27853). On the other hand, the antifungal activity test was done against the following dermatophytes: *Trichophyton rubrum* (CBS 392.58), Trichophyton mentagophytes *(CBS 106.67 and* Microsporum canis (CBS 132.88). *All the isolates were purchased from* Biodiversity & Environmental Research Center *BERC /Til Village*-Nablus.

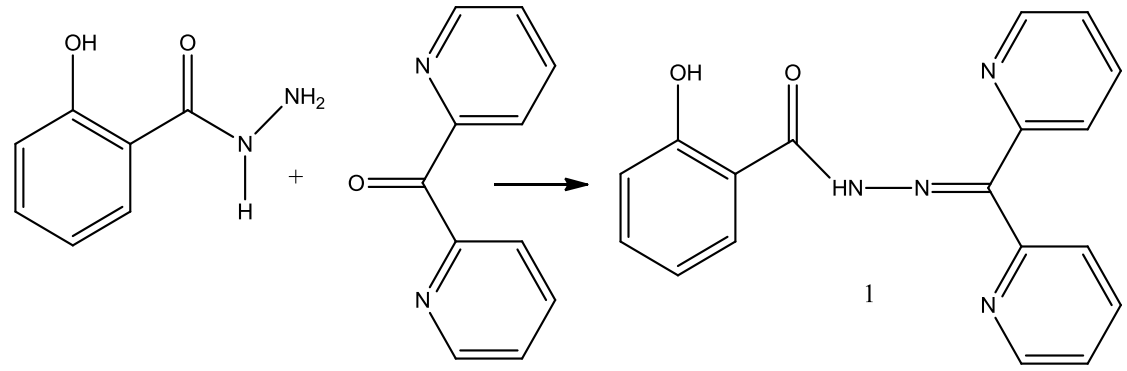
2.1 General procedure for synthesis of Schiff Base Compounds

The compounds were prepared by the Hugo method, in which the amine and aldehyde or ketone are mixed in round bottomed flask(RBF) and dissolved in absolute ethanol according to the following general equation:



Primary amine Aldehyde or ketone Schiff base

2.1.1 : Synthesis of N'-(di(pyridin-2-yl)methylene)-2-hydroxybenzoylhydrazide 1



Salicoyl hydrazide Di(pyridin-2-yl)methanone *N*'-(di(pyridin-2-yl)methylidene)-2-hydroxybenzohydrazide

Equation 2.1: Synthesis of Compound 1

A mixture of salicoylhydrazide (0.323 g, 2.00 mmol) and dipyridyl ketone (0.368 g, 2.00mmol) in 50 mL absolute ethanol containing few drops of concentrated sulphuric acid were placed in 100 mL RBF. The reaction mixture was refluxed for 4 hours. The reaction was monitored by TLC and then cooled to room temperature after completion. The solid product was filtered, washed with diethyl ether to remove sulphuric acid, dried and recrystallized from ethanol to afford 0.532 g ,(83.6%) of a white powder, m.p.(142-147°C).

IR: 3202.27; 2574.37; 1656.46; 1601.37; 1515.48; 1482.58; 1447.35; 1378.10; 1266.20; 118.54; 127.55; 1047.54 cm^{-1}.

^{1}H NMR ppm: δ = 8.880 (1H, d, *J* =5.057 Hz); 8.298 (1H, t, *J* =7.049 Hz); 7.944 (1H, d, *J* =4.870 Hz); 7.599 (1H, d, *J* =4.106 Hz); 7.402 (1H, t, *J* =3.248 Hz); 7.180 (1H, t, *J* =1.701 Hz); 6.948 (1H, d, *J* =2.926 Hz) ppm.

^{13}C NMR ppm:δ= 117.334, 118.014, 120.296, 125.528, 125.774, 126.435, 131.710, 134.579, 142.344, 146.455, 149.940, 156.857, 162.993 ppm.

Molecular ion [M^{+}] *m/z* = 318.1 (M. Wt = 318.3 theoretical). (Calcd. for $C_{18}H_{14}N_4O_2$:C, 67.91; H, 4.43; N, 17.60. Found: C, 67.75; H, 4.21; N, 17.35).

The UV-visible Electronic Absorption Spectroscopy

The electronic absorption of the starting materials and prepared compound in EtOH was monitored before and after refluxing by UV- Visible, as seen in **Fig.1.** Before refluxing , a) salicoyl hydrazide which revealed two electron transition maxima at λ_{max} = 265 and 300 nm, b) di(pyridin-2-yl)methanone with four electron transition maxima at λ_{max} = 208, 223, 243 and 273 nm, after refluxed, c) only two new electron transfer maxima at λ_{max} = 273 and 330 nm were observed due to formation of N'-(di(pyridin-2-yl)methylidene)-2-hydroxybenzohydrazide. All electron absorbance in both starting and product material were resonated n- π* or π- π* electron transition.

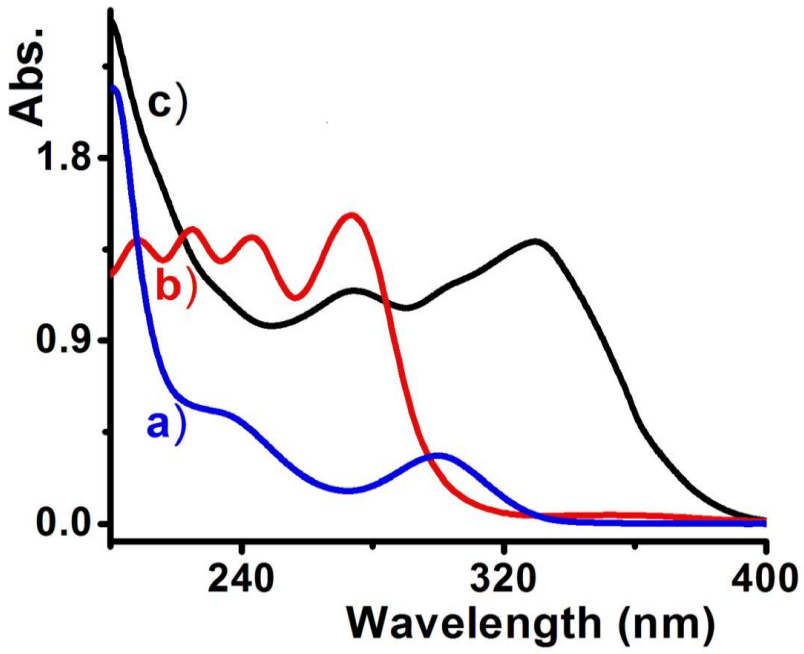


Fig.1. UV–Vis spectra of a) salicoyl hydrazide b) di(pyridin-2-yl)methanone and c) N'-(di(pyridin-2-yl)methylidene)-2-hydroxybenzohydrazidein EtOH at RT.

The Geometrical Structure of the Desired Hydrazide

The molecular structure geometry of the desired compound on ground-state was first optimized in gaseous state without symmetry constraints at B3LYP level. The optimized geometry is illustrated in **Fig.2**

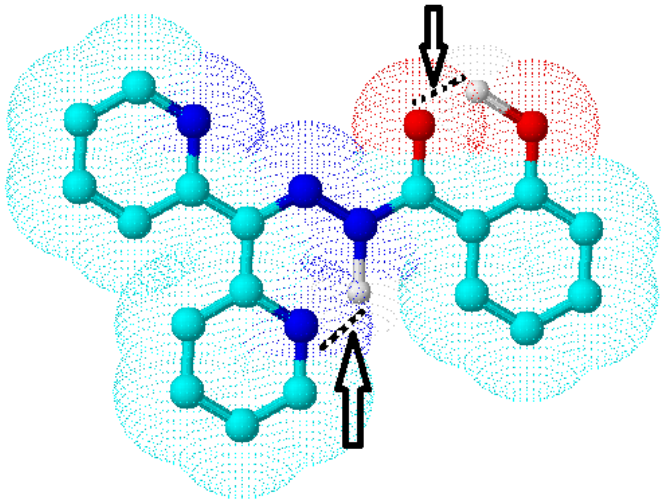


Fig. 2. Optimized geometrical structure of N'-(di(pyridin-2-yl)methylene)-2-hydroxybenzohydrazide.

The optimized structure revealed two important points: two intramolecular hydrogen-bonds per molecule were detected in the structure O-H….O with bond length = 2.2 A and N-H….Npy with bond length = 2.4 A, as labeled in **Fig. 2,** such H-bonds stabilized the compound in two semi-hexacyclic heteroatomic rings and effected the physical properties, phenyl occupied semi-perpendicular plane to the two pyridine ring planes and this is due to minimized the internal repulsion.

The frontier molecular orbital's HOMO–LUMO energies and their corresponding density of state of the desired product are shown in **Fig. 3.** The HOMO–LUMO gap in vacuum is 0.390 au. DFT 6-31++g(d,p).

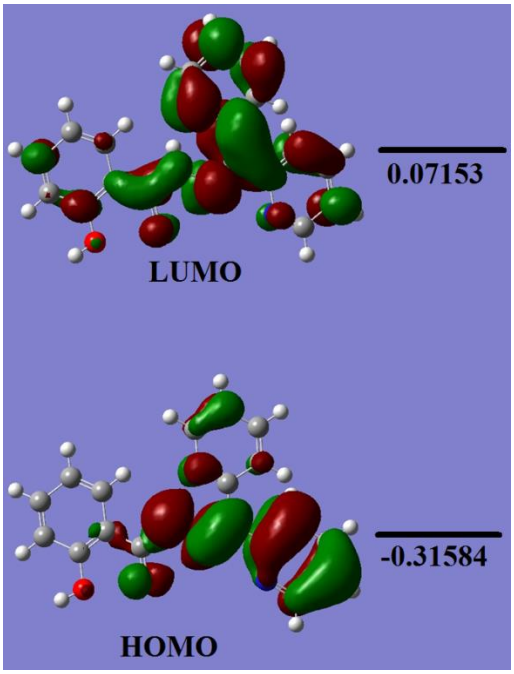
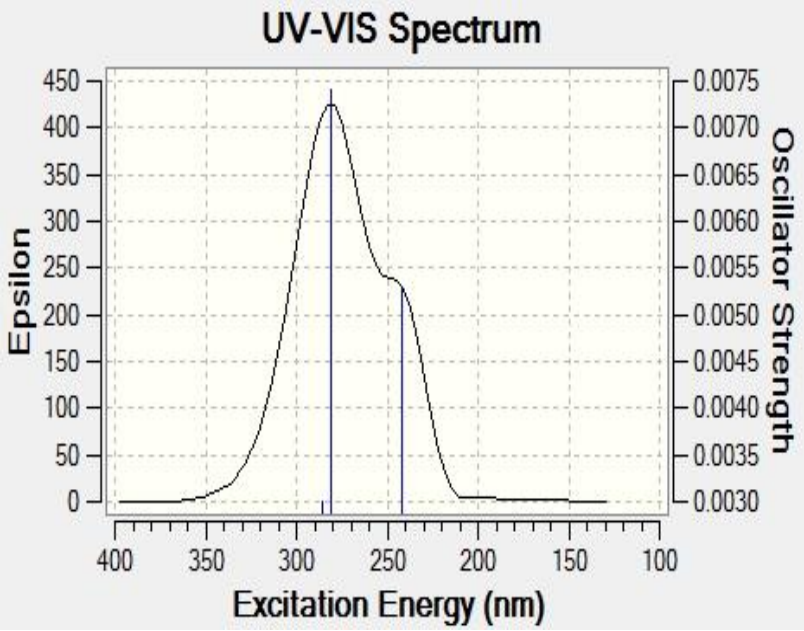


Fig. 3. HOMO and LUMO Plots of the Prepared Hydrazide.

TDSCF-DFT Electronic Absorption Spectra

The time dependence electronic absorption spectra of the desired hydrazide in gaseous state were performed using TDSCF-DFT (B3LYP and HF)/at different level of calculations. The results are reported in **Fig. 4**. It is observed for the molecule at each level that the absorption in the visible region is much weaker than that in the UV region. The maximum value of the oscillator strength is reached for 0.035at a wavelength of around 398 nm using B3LYP 3-31 level (as in Fig. 5a) and 0.045 at the same wave length using B3LYP 6-31++g(d,p) level (as in Fig. 5b). The maximum

value of the oscillator strength is reached for 0.007at a wavelength of around 280 nm using HF 3-31 level (as in Fig. 5c) and 0.45 at wave length around 250 nm using HF 6-31++g(d,p) level (as in Fig. 5d). Experimental measurements of electronic absorption are performed in water and revealed two maxima at λ_{max} = 273 and 330 nm. The TD-DFT B3LYPcalculations have an appreciable red-shift, while TD-DFT HF calculation revealed blue-shift compared by experimental result in water. The discrepancy between experimental and TD-DFT theoretical may result due to two reasons: solvent effects, i.e. polar solvent. could affect the electronic structure and the geometry through the expected interaction between solute and solvent molecules. The smaller HOMO-LUMO gap of compound induced smaller excited energies.



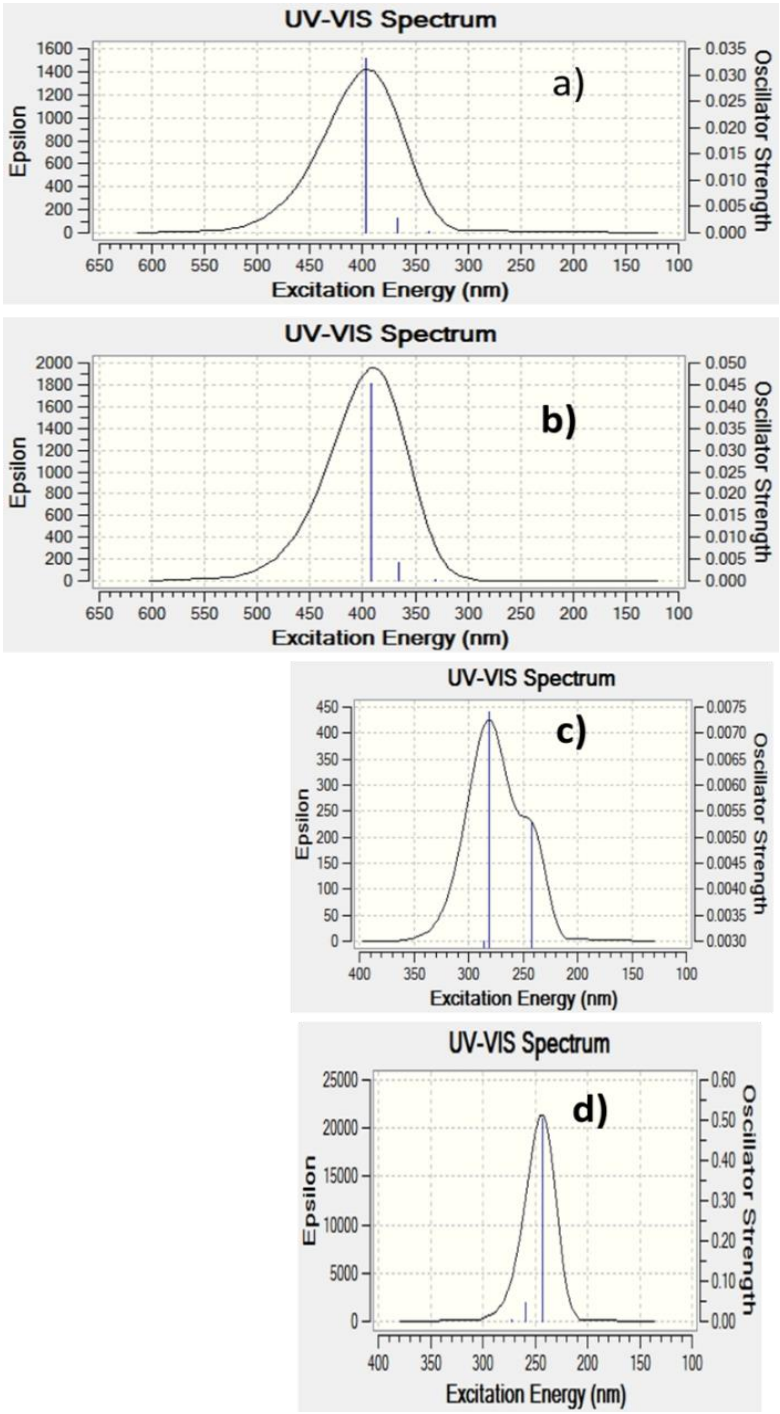


Fig. 4.TD-DFT UV/Vis electronic absorption spectra of the titled compound at different levels of calculations a)B3LYP 3-31, b) B3LYP 6-31++g(d,p), c)HF 3-31 and d) HF 6-31++g(d,p) .

TG/DTG thermal analysis

The thermal properties TG/DTG of the N'-(di(pyridin-2-yl)methylene)-2-hydroxybenzohydrazide was investigated under an open atmosphere in the range of 0–700 °C and heating rate of 10 °C/min. **Fig.5** showed simple decomposition process with one broad step typical decomposition, started from 160 °C and ended at 280 °C with weight loss 99%.

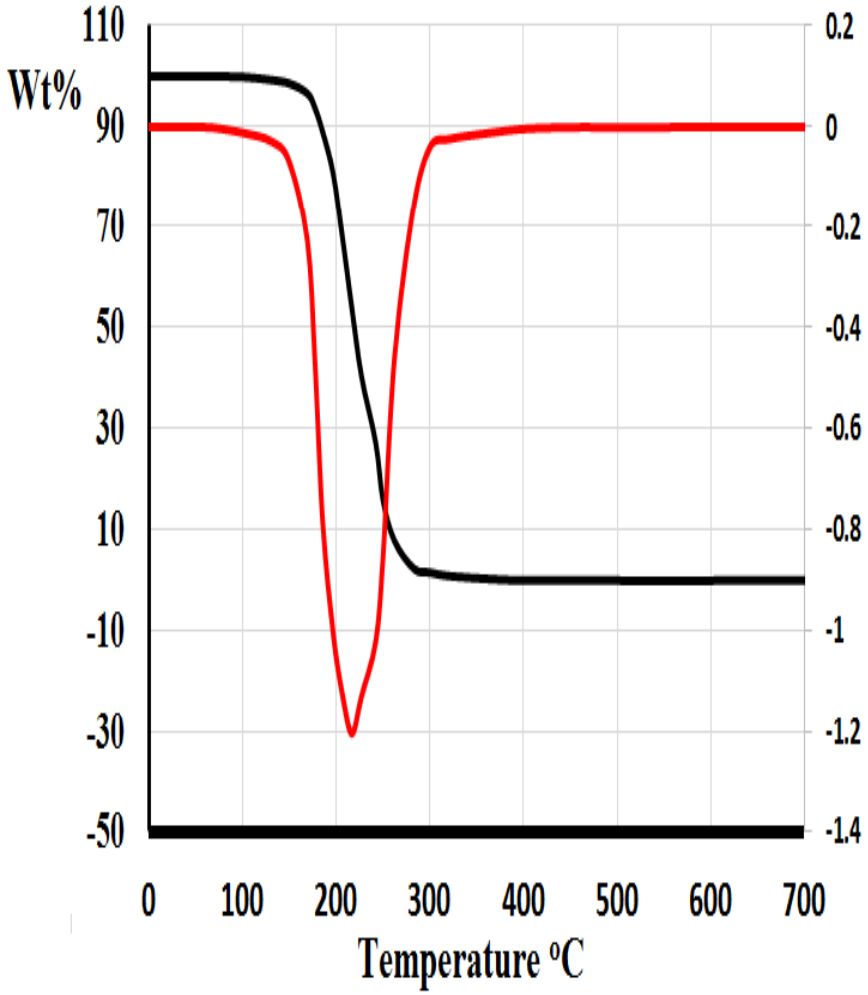
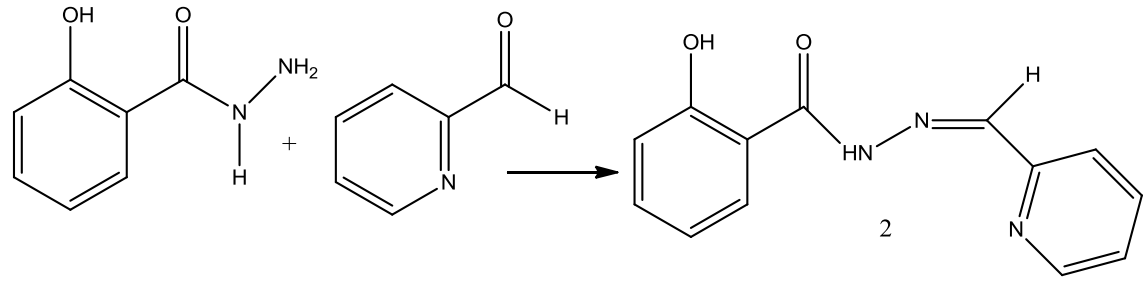


Fig. 5. TG/DTG thermal curve of the desired compound at heating rate of 10 °C/min

2.1.2 Synthesis of 2-hydroxy-N-(pyridine-ylmethylene) benzohydrazid



Salicoyl hydrazide Pyridine-2-carbaldehyde (*Z*)-2-hydroxy-*N*'-(pyridin-2-ylmethylidene)benzohydrazide

Equation 2.2: Synthesis of Compound 2

A mixture of salicoylhydrazide (0.300 g, 2.00 mmol) and pyridine-2-carbaldehyde (0.368 g 3.400 mmol) in 50 mL absolute ethanol were placed in 100 mL RBF under acidic conditions. The reaction mixture was refluxed for 9 hours. The reaction was monitored by TLC and then cooled to room temperature after completion. The solid product was filtered, washed with diethyl ether to remove sulphuric acid, dried and recrystallized from ethanol to afford 0.276 g, (58%) of bright brown solid, m.p.(259-265°C).

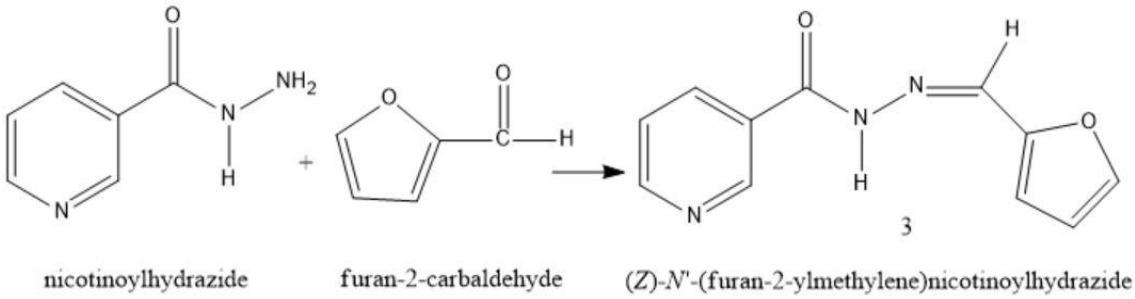
IR: 3235.87; 2544.41; 2353.23; 1612.45; 1519.67; 1481.89; 1290.81; 1147.79 cm^{-1}.

^{1}H NMR ppm: δ = 8.725 (1H, d, *J* =6.090 Hz); 8.168 (1H, s); 7.911 (1H, d, *J* =7.668 Hz); 7.815(1H, s, *J* =811.041 Hz); 7.734 (1H, t, *J* =12.984 Hz); 7.435 (1H, t, *J* =10.439 Hz); 7.251 (1H, t, *J* =8.07 Hz); 7.201 (1H, t, *J* =5.128 Hz); 6.995 (1H, d, *J* =7.964 Hz) ppm.

^{13}C NMR ppm: δ 117.180, 117.635, 119.622, 121.327, 126.369, 129.597, 134.502, 142.624, 143.881, 146.283, 149.891, 158.784, 165.366 ppm.

UV :λ_{max} = 302, 487 nm.

2.1.3 Synthesis of (Z)-N'-(furan-2-ylmethylene)nicotinoylhydrazide 3



Equation 2.3: Synthesis of Compound 3

A mixture of furfural (0.122 g 2.00mmol) and nicotinoylhydrazide (0.274 g, 2.00mmol) in 50 mL absolute ethanol containing a few drops of concentrated sulphuric acid were placed in 100 mL RBF. The reaction mixture was refluxed for 10 hours. The reaction was monitored by TLC and then cooled to room temperature after completion. The solid product was cooled to room temperature. The separated Schiff base was filtered, washed with diethyl ether to remove sulphuric acid, dried and recrystallized from ethanol to afford 0.562 g, (65%) of white crystals,m.p.(193-196°C).

IR: 3219.52; 3070.58; 1647.98; 1613.83; 1559.79; 1538.59; 1471.33; 141.28; 1332.22; 1291.83; 1146.47; 963.28; 936.37; 897.29; 783.68; 748.32; 731.22; 701.32; 616.35; 589.50; 518.05; 504.06 cm^{-1}.

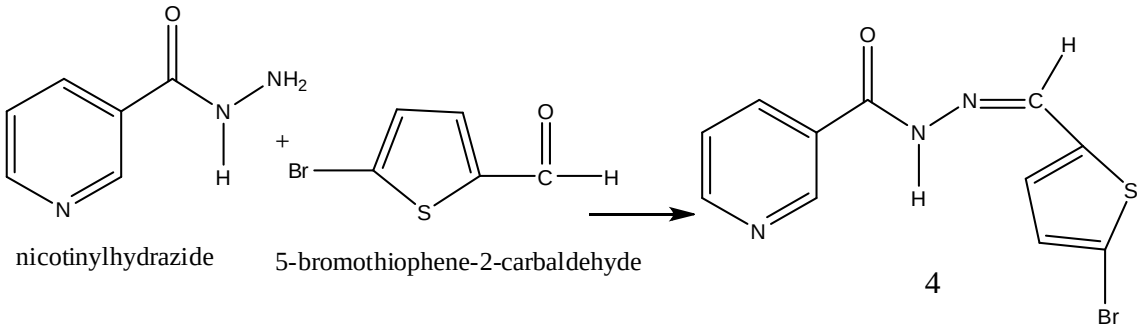
^{1}H NMR ppm:δ = 9.017 (1H, s, *J* =5.509Hz); 8.729 (1H, d, *J* =4.610Hz); 8.297 (1H, s, *J* =15.968Hz); 8.211 (1H, d, *J* =7.152Hz); 7.831 (1H, t, *J*

=3.437 Hz); 7.525 (1H, t, *J* =8.061 Hz); 6.930 (1H, s, *J* =4.330Hz); 6.161 (H, t, *J* =10.439Hz); ppm.

^{13}C NMR ppm: δ= 112.724, 114.289, 124.079, 129.079, 134.866, 135.875, 145.572, 148.002, 149.433, 149.722, 162.071ppm.

UV :λ_{max} = 250, 340 nm.

2.1.4 Synthesis of N'- ((5-bromothiophen-2yl) methylene) nicotinoylhydrazide 4



Scheme 2.4: Synthesis of Compound 4

A mixture of 5-bromothiophene-2-carbaldehyde (0.100 g, 0.5mmol) and nicotinoylhydrazide (0.0700 g, 0.5mmol) in 50 mL absolute ethanol containing few drops of concentrated sulphuric acid were placed in 100 mL RBF. The reaction mixture was refluxed for 11 hours. The reaction was monitored by TLC and then cooled to room temperature after completion. The solid product was filtered, washed with diethyl ether to remove sulphuric acid, dried and recrystallized from ethanol to afford 0.313 g, (66%) as a bright brown crystals, m.p.(216-219°C).

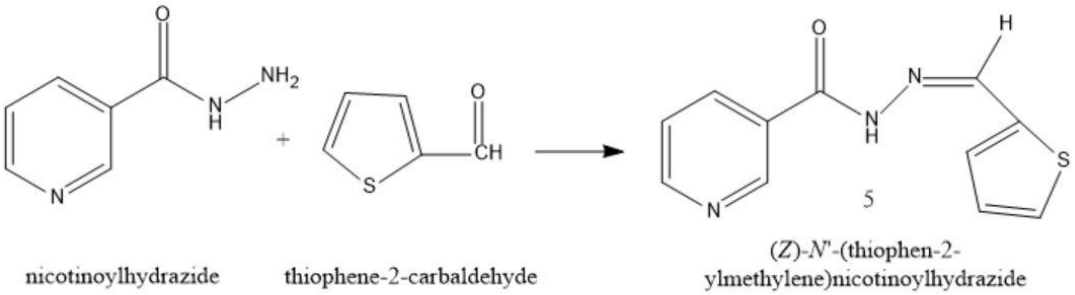
IR: 3225.76; 3065.31; 1638.16; 1586.43; 1555.14; 1473.43; 1427.59; 1412.52; 1344.99; 1290.69; 1242.16; 1209.37; 1153.49; 1067.06; 1050.93; 1035.66 cm^{-1}.

^{1}H NMR ppm:δ= 8.998 (1H,s, *J* =5.428Hz); 8.720 (1H,d, *J* =4.690Hz); 8.546 (1H, s, *J* = 7.398Hz) 8.194(1H, d, *J* =6.408Hz); 7.320 (1H, d, *J* =4.512Hz); 7.530 (1H, t, *J* =4.253Hz); 7.255(1H, d, *J* =3.447Hz)ppm.

^{13}C NMR ppm: δ = 115.535, 124.090, 129.529, 131.839, 132.321, 135.881, 141.265, 143.182, 149.00, 152.809, 162.091ppm.

UV : λ_{max} = 329, 446 nm.

2.1.5 Synthesis of N'-(thiophen-2-ylmethylene)nicotinoylhydrazide 5



Equation 2.5: Synthesis of Compound 5

A mixture of 2-thiophene-carbaldehyde (0.100 g, 0.9 mmol) and nicotinoylhydrazide (0.122 g, 0.9mmol) in 50 mL absolute ethanol in 100 mL RBF without adding any acid, was refluxed for 11 hours. The reaction was monitored by TLC and then cooled to room temperature after completion. The light brown solid was filtered, washed with diethyl ether

to remove sulphuric acid, dried and recrystallized from ethanol to afford 0.205 g, (82.9%),m.p.(221-224°C).

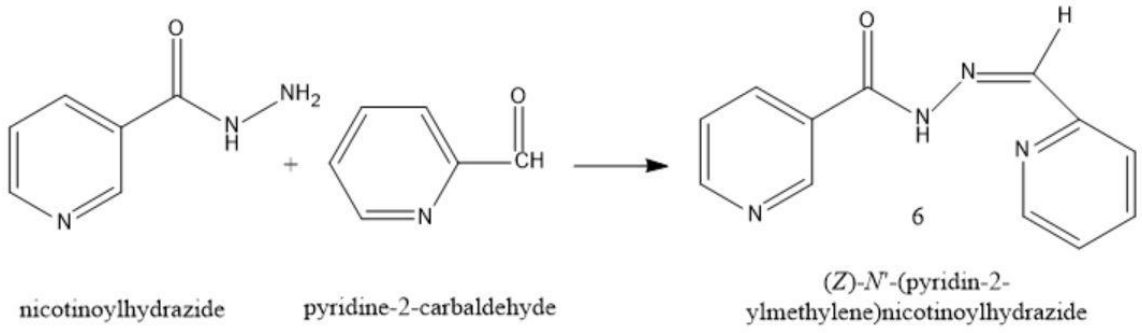
IR: 3229.36; 2963.20; 2836.88; 1672.27; 1643.87; 1593.25; 1557.38; 1481.53; 1416.52; 1363.35; 1315.06; 1282.47; 1092.73; 1025.31 cm^{-1}.

^{1}H NMR ppm: δ= 8.998 (1H, s); 8.729 (1H,d, *J* =4.846Hz); 8.546(1H, s, *J* =3.850Hz); 8.178 (1H, d, *J* =7.280Hz); 7.568 (1H, t, *J* =4.485 Hz); 7.530(1H, d,*J* =4.176 Hz); 7.328(1H, t, *J* =4.171 Hz); 7.240(1H, t, *J* =3.954)ppm.

^{13}C NMR ppm: δ= 124.691, 129.528, 130.008, 131.697, 131.881, 132.318, 135.831, 143.284, 149.639, 153.350, 162.146.

UV :λ_{max} = 321, 755 nm

2.1.6 Synthesis of (Z)-N'-(pyridin-2-ylmethylene) nicotinoylhydrazide 6



Scheme 2.6: Synthesis of Compound 6

A mixture of pyridine-2-carbaldehyde (0.107 g, 0.1mmol) and nicotinoylhydrazide (0.137 g, 0.1mmol) in 50 mL absolute ethanol were

placed in 100 mL RBF without adding any acid. The reaction mixture was refluxed for 8 hours. The reaction was monitored by TLC and then cooled to room temperature after completion. The solid product was filtered, washed with diethyl ether, dried and recrystallized from ethanol to afford 0.29 g, (67%) as bright yellow crystals, m.p.(118-122°C).

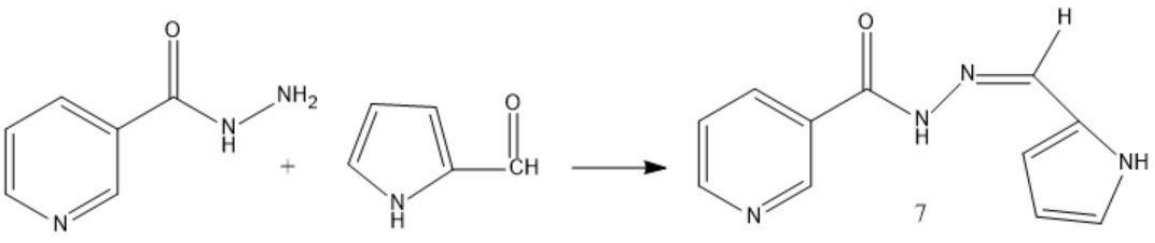
IR: 3216.45; 1634.10; 1600.20; 1556.60; 1528.53; 1438.32; 1415.04; 1288.15; 1144.35; 1093.16; 1058.70; 1025.73 cm^{-1}.

^{1}H NMR ppm:δ = 9.025 (1H, s,*J* =7.177Hz); 8.706 (1H, d, *J* =5.414Hz); 8.255 (1H, d, *J* =3.679Hz); 8.215 (1H, d, *J* =5.762Hz); 7.955 (1H, t, *J*=8.098Hz); 7.514 (1H, t, *J* =4.176Hz); 6.899 (1H, s, *J* =7.119Hz); 6.479 (1H, t, *J* =7.864Hz); 6.114 (1H, d, *J* = 4.883Hz)ppm.

^{13}C NMR ppm: δ = 118.820, 122.868, 124.175, 127.322, 132.301, 135.869, 142.964, 145.450, 148.837, 148.778, 152.560, 161.646 ppm.

UV : λ_{max} =332, 575 nm.

2.2.7 Synthesis of N'-((1H-pyrrol-2-yl)methylene)nicotinoylhydrazide



nicotinoylhydrazide 1*H*-pyrrole-2-carbaldehyde (*Z*)-*N*'-((1*H*-pyrrol-2-yl)methylene)nicotinoylhydrazide

Scheme 2.7: Synthesis of Compound 7

A mixture of nicotinoylhydrazide (0.19 g, 0.1mmol) and pyrrole-2-carbaldehyde(0.274 g, 0.3 mmol) in 50 mL absolute ethanol was placed in 100 mL RBF without adding any acid. The reaction mixture was refluxed for 14 hours. The reaction was monitored by TLC and then cooled to room temperature after completion. The solid product was filtered, washed with diethyl ether, dried and recrystallized from ethanol to afford 0.29 g,(49%) as bright yellow crystals, m.p.(120-122C°).

IR: 3330.85; 2973.98; 2889.75; 1637.22; 1602.69; 1525.53; 1415.59; 1274.04; 1087.99; 1046.47 cm^{-1}.

^{1}H NMR ppm:δ = 9.037 (1H, s, *J* =5.179Hz); 8.705 (1H, d , *J* =4.724 Hz); 8.215 (1H, d, *J* =5.580Hz); 7.515 (1H, t, *J* =4.330 Hz); 7.418 (1H, s, *J* =3.945 Hz); 6.906 (1H, d, *J* =7.339 Hz); 6.481 (1H, t, *J* =8.036 Hz); 6.119 (1H, d, *J* = 4.909 Hz)ppm.

^{13}C NMR ppm: δ 109.758, 114.082, 123.259, 124.002, 130.243, 131.035, 132.281, 135.794, 141.973, 149.141, 152.348, 162.011 ppm.

UV : λ_{max}=512, 581 nm.

Chapter Three

Results and Discussion

3.1 Physical Data of the New Schiff Bases

All the physical data of new Schiff bases compounds were listed in **Table 3.1.**

Table3.1:Physical and Elemental Analysis of New Synthesized Schiff bases.

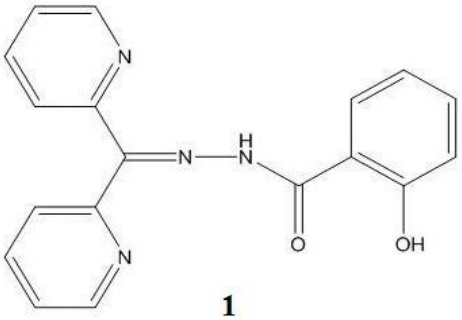
Compound	Molecular Formula	Color	Yield %	M.P.	M.wt. g/mol
1	$C_{18}H_{14}N_4O_2$	White powder	83.6	142-147	318
2	$C_{13}H_{11}N_3O_2$	Bright brown	58%	259-265	227
3	$C_{11}H_9N_3O_2$	White crystals	65%	193-196	215
4	$C_{11}H_8BrN_3OS$	Bright brown crystals	66%	216-219	294
5	$C_{11}H_9N_3OS$	Light brown solid	82.9%	221-224	231
6	$C_{12}H_{10}N_4O$	Bright yellow crystals	67%	118-122	218
7	$C_{11}H_{10}N_4O$	Bright yellow crystals	49%	120-122	206

3.2.Identification of New Synthetic Schiff Bases Compounds:

The structures of products were determined by their melting point, elemental analysis, FT-IR, UV-visible, ^{1}H and ^{13}C NMR spectral data.

3.2.1 Synthesis of compound (1)

I. [N'-(di(pyridin-2-yl)methylidene)-2-hydroxybenzoylhydrazide] was prepared by condensation of salicoyl hydrazide (0.323 g, 2.00 mmol) and dipyridyl ketone (0.368 g, 2.00 mmol) in 50 mL absolute ethanol in (83.6%) yield, the structure was determined by IR, ^{1}H and ^{13}C NMR spectroscopy.



1

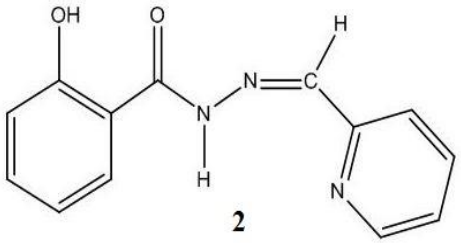
Compound (1).

II. Light yellow solid product was collected, and recrystalized several time with ethanol, normal hexane, ether and distilled water, where the product still in solid form.

III. The synthesized Schiff base is stable in air and completely soluble in chlorinated solvent, DMSO and DMF and partially soluble in ROH. The product is insoluble in non-polar like *n*-hexane and polar solvent like water.

3.2.2 Synthesis of Compound (2)

I. [2-hydroxy-N-(pyridine-ylmethylidene)benzohydrazid] was prepared by condensation of salicoyl hydrazide (0.300 g, 2.00 mmol) and pyridine-2- carbaldehyde (0.368 g 3.400 mmol) in 50 mL absolute ethanol in (58%) yield, the structure was determined by IR, ^{1}H and ^{13}C NMR spectroscopy.



2

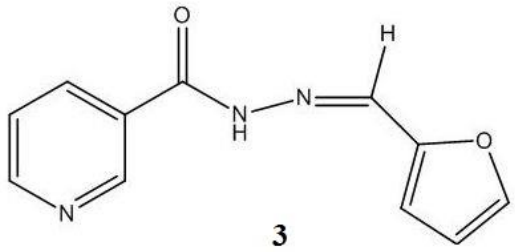
Compound (2).

II. The bright brown solid product was collected, and purified by washing it with ether and set aside to give pure solid compound

III. The synthesized Schiff base is stable in air and soluble in organic solvents such as methanol, ethanol, and dimethyl sulfoxide and insoluble in dichloromethane and n-hexane.

3.2.3 Synthesis of Compound (3)

I. [(Z)-N'-(furan-2-ylmethylene)nicotinoylhydrazide] was prepared by condensation of furfural (0.122 g 2.00 mmol) and nicotinoylhydrazide (0.274 g, 2.00 mmol) in 50 mL absolute ethanol in (65%) yield, the structure was determined by IR, ^{1}H and ^{13}C NMR spectroscopy.



3

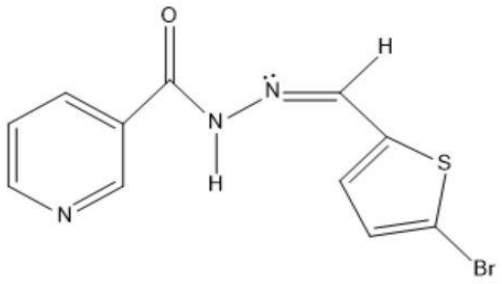
Compound (3).

II. The product was collected and purified by washing it with distilled water, and n-hexane, a small quantity of CH_2Cl_2 was added to the product and set aside at room temperature to give pure solid compound,then recrystallized from ethanol (99%) to produce white crystalline product.

III. The synthesized Schiff base is stable in air and soluble in organic solvents such as methanol, ethanol, and dimethyl sulfoxide and insoluble in dichloromethane and n-hexane.

3.2.4 Synthesis of Compound (4)

I. [N'-((5-bromothiophen-2yl)methylene)nicotinoylhydrazide] was prepared by condensation of 5-bromothiophene-2-carbaldehyde (0.100 g, 0.5mmol) and nicotinoylhydrazide (0.0700 g, 0.5mmol) in small amount of absolute ethanol in (66%) yield. The structure was determined by IR, ^{1}H and ^{13}C NMR spectroscopy.



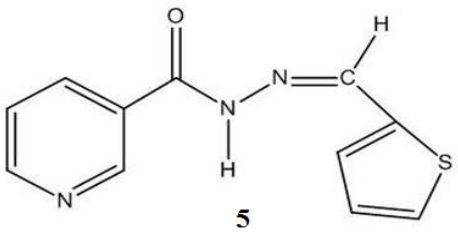
Compound (4).

II. The product was collected and purified by washing it with distilled water, and n-hexane, then 5 mL of CH_2Cl_2 was added to the product and set aside at room temperature to produce pure solid compound, then recrystalized from ethanol (99%) to produce bright brown crystals.

III. The synthesized Schiff base is stable in air and soluble in organic solvents such as methanol, ethanol, and dimethyl sulfoxide and insoluble in dichloromethane and n-hexane.

3.2.5 Synthesis of Compound (5)

I. [N'-(thiophen-2-ylmethylene)nicotinoylhydrazide] was prepared by condensation of 2-thiophene-carbaldehyde (0.100 g, 0.9 mmol) and nicotinoylhydrazide (0.122 g, 0.9mmol) in 50 mL absolute ethanol in (82.9%) yield. The structure was determined by IR, ^{1}H and ^{13}C NMR spectroscopy.



5

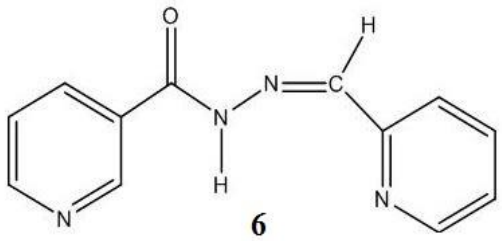
Compound (5).

II. The light brown solid product was collected, and recrystallized several times with ethanol, normal hexane, ether and distilled water, where the product was still in solid form.

III. The synthesized Schiff base is stable in air and soluble in organic solvents such as methanol, ethanol, and dimethyl sulfoxide and insoluble in dichloromethane and n-hexane.

3.2.6 Synthesis of Compound (6)

I. [(Z)-N'-(pyridin-2-ylmethylene)nicotinoylhydrazide] was prepared by condensation of pyridine-2-carbaldehyde (0.107 g, 0.1 mmol) and nicotinoylhydrazide (0.137 g, 0.1mmol) in 50 mL absolute ethanol in (67%) yield, the structure was determined by IR, ^{1}H and ^{13}C NMR spectroscopy.



6

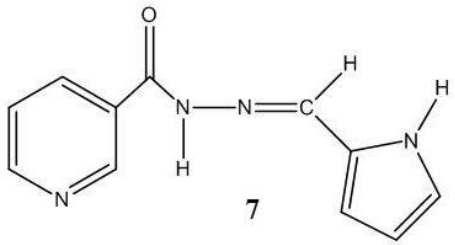
Compound (6).

II. The product was collected and purified by washing it with distilled water, and n-hexane, then 5 mL of CH_2Cl_2 was added to the product and set aside at room temperature to produce pure solid compound, then recrystallized from ethanol (99%) to produce bright yellow crystals.

III. The synthesized Schiff base is stable in air and soluble in organic solvents such as methanol, ethanol, and dimethyl sulfoxide and insoluble in dichloromethane and n-hexane.

3.2.7 Synthesis of Compound (7)

I. [N'-((1H-pyrrol-2-yl)methylene)nicotinoylhydrazide] was prepared by condensation of nicotinoylhydrazide (0.19 g, 0.1 mmol) and pyrrole-2-carbaldehyde (0.274 g, 0.3 mmol) in 50 mL absolute ethanol in (49%) yield, the structure was determined by IR, ^{1}H and ^{13}C NMR spectroscopy.



7

Compound (7).

II. The prepared compound was collected and purified by washing it with distilled water, and n-hexane, then 5 mL of CH_2Cl_2 was added to the product and set aside at room temperature to produce pure solid compound, then recrystallized from ethanol (99%) to produce bright yellow crystals.

III. The synthesized Schiff base is stable in air and soluble in organic solvents such as methanol, ethanol, and dimethyl sulfoxide and insoluble in dichloromethane and n-hexane.

3.3 Spectroscopic Analysis

3.3.1 IR Spectra Investigations

The IR spectra of Schiff bases displayed more or less strong bands in the 4,000 - 400 cm^{-1} range.

The IR spectra of the corresponding Schiff bases have been examined in comparison with the spectra of the starting materials (1° amine, and carbonyl compound).

The spectra of the desired compound in particular show three main sets of characteristic absorptions, ν C=N, ν N-H, and ν C-H aromatic.

The IR spectra of the synthesized compounds were recorded by using FT-IR Fourier Transform Infrared Spectrophotometer (Necolet Is5 - Id3) at room temperature.

3.3.2 The General Observation about Schiff Bases IR Spectra :

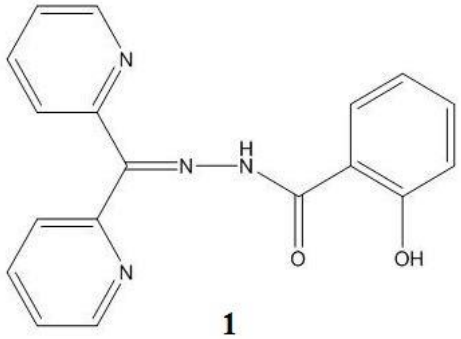
* The vibration of carbonyl group (ν C=O) in the spectra of aldehyde compound shifted to less frequency which means the formation of azomethine group (C=N).

* The vibration of N-H in the spectra of 1° amine appears in the spectra of Schiff bases.

* The vibration of C=C of aromatic system shifted to less frequency in the Schiff bases spectra.

* The vibration of C-H shifted to less frequency in the Schiff bases spectra.

3.3.2.1 IR Spectrum of Compound 1



1

♦ In the spectrum of dipyridyl ketone the major band appears at 1678.09 cm^{-1} that is related to vibration of C=O, (this low value due to the conjugation of C=O with aromatic rings, (while normal ketone appears at 1715 cm^{-1}), and there is a small peak as an overtone at 3414 cm^{-1} ; a frequency of about twice of ν C=O vibration.

• (C-H) vibration of aromatic rings appears at 3052.14 cm^{-1}.

• (C=C) vibration of the ring appears at 1577.83 cm^{-1}.

♦ In the spectrum of salicoyl hydrazide, the major peak appears at 3317.12 cm^{-1}, which is related to (N-H) bond vibration, and at 1581.52 cm^{-1} for (C=C) vibration.

• 1652.54 cm^{-1} is related to (C=O).

• 3258.65 cm^{-1} is related to (O-H).

♦ In the spectrum of Schiff base **1**, a major peak observed at 1656.46 cm^{-1} which is related to azomethine group (C=N), and disappearance of (N-H) peak of amine.

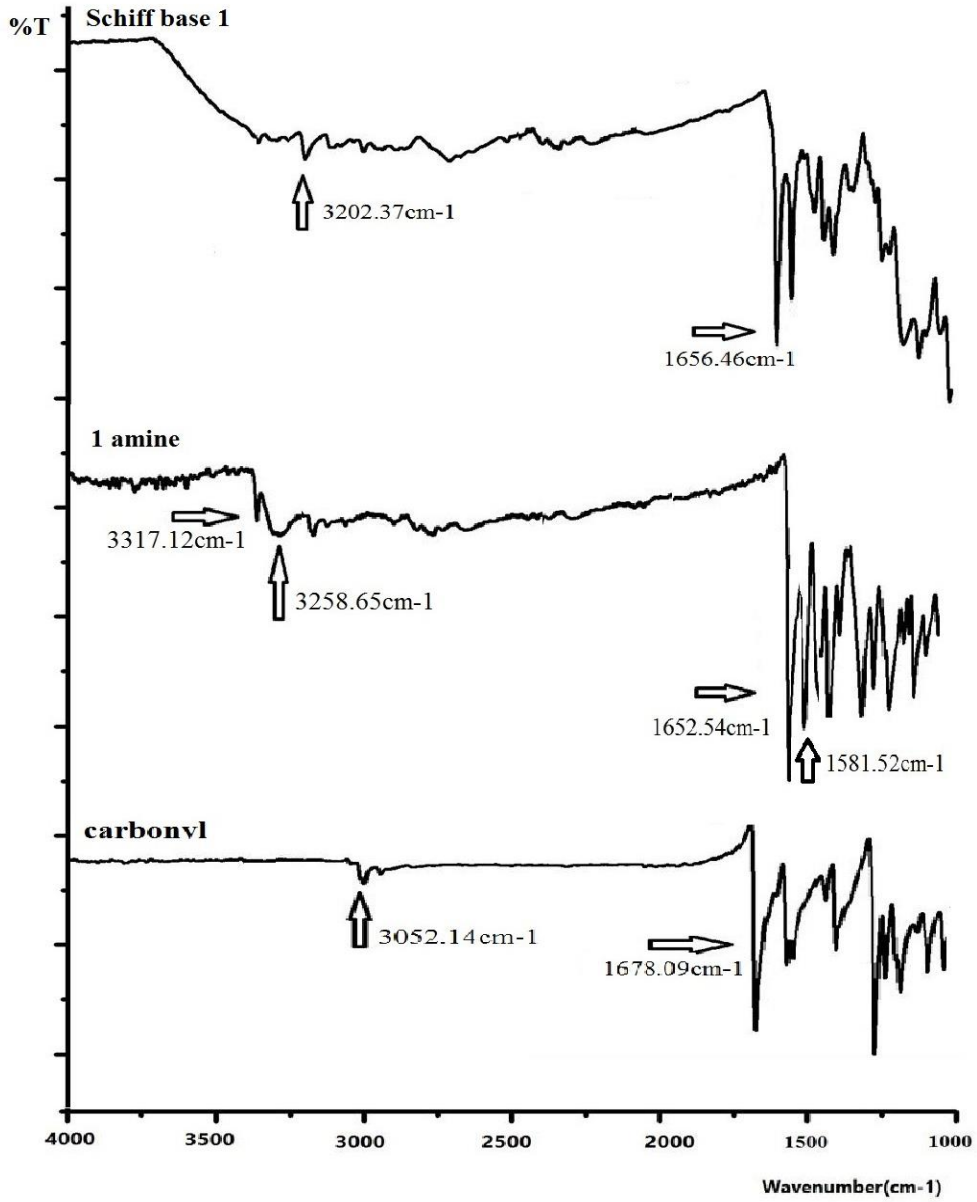
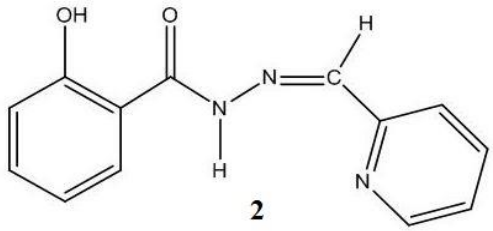


Fig.3.1 IR spectra (4000-1000 cm^{-1}) of: 1 amine = (salicoyl hydrazide), carbonyl = (di (pyridin-2-yl) methanone) and Schiff base 1 = (N'- (di (pyridin-2-yl) methylidene)-2-hydroxybenzohydrazide).

3.3.2.2 IR Spectrum of 2



2

♦ In the spectrum of salicoyl hydrazide, the major peak appears at 3317.12 cm^{-1}, which is related to (N-H) bond vibration, and at 1581.52 cm^{-1} for (C=C) vibration.

• 1652.54 cm^{-1} is related to (C=O).

• 3258.65 cm^{-1} is related to (O-H).

♦ In the spectrum of pyridine-2-carbaldehyde the major peak of carbonyl vibration observed at 1707.44 cm^{-1}.

• The (C-H) vibration of aromatic ring appears at 3055.59 cm^{-1}.

• 2821.00 cm^{-1} indicates aldehyde (C-H) vibration.

• The (C=C) vibration of ring appears at 1583.57 cm^{-1}.

♦ In the spectrum of Schiff base **2**, a major peak observed at 1612.45 cm^{-1} which is related to azomethine group (C=N), and there is disappearance of (N-H) peak of amine.

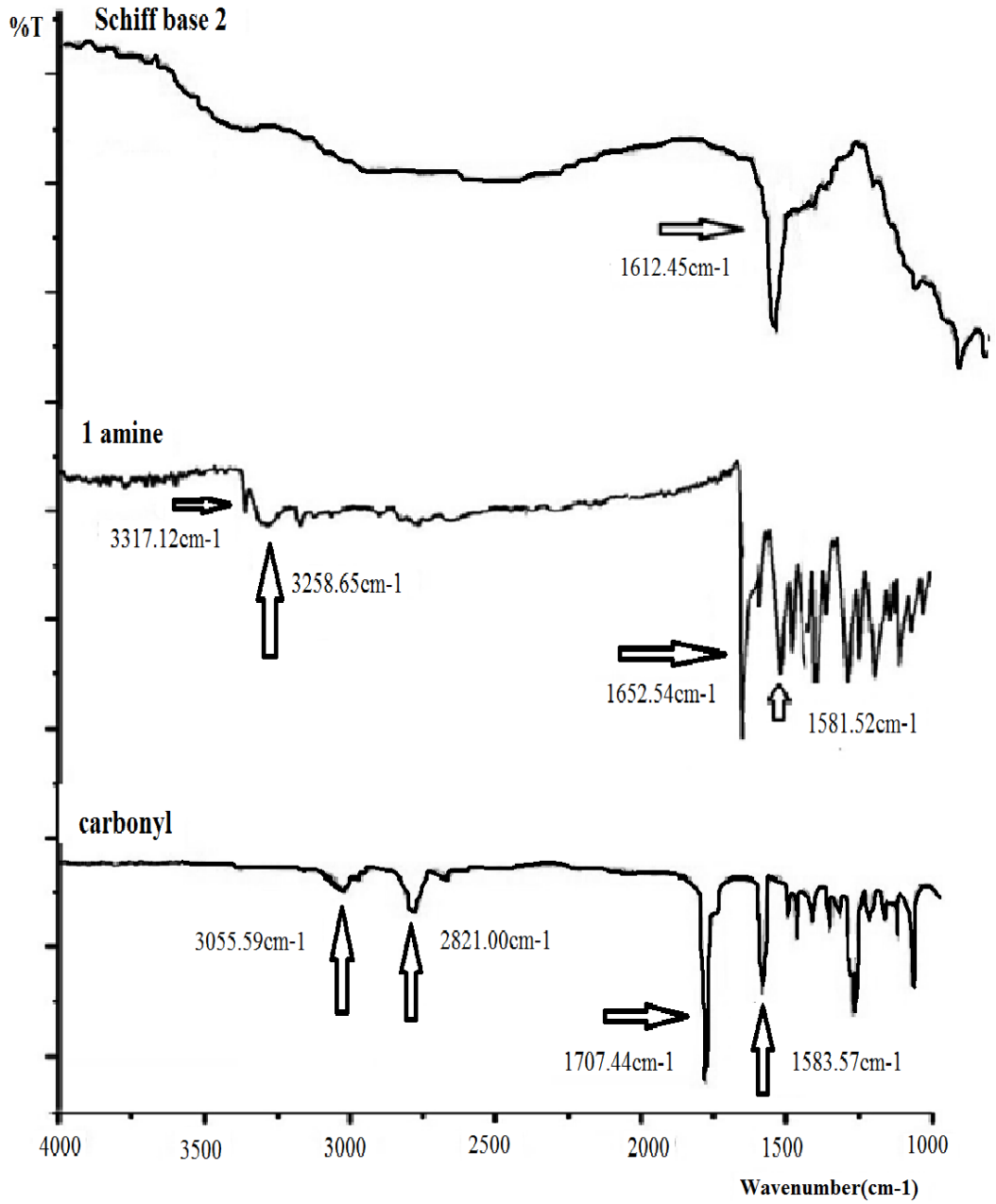
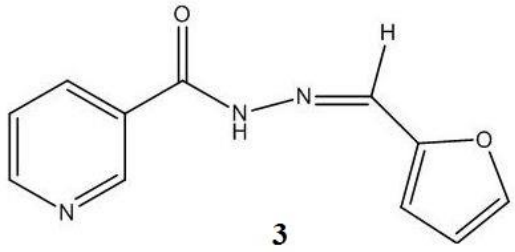


Figure 3.2 IR spectra (4000-1000 cm^{-1}) of: 1 amine = (salicoyl hydrazide), carbonyl = (pyridine-2-carbaldehyde) and Schiff base 2 = (2-hydroxy-N-(pyridine-ylmethylidene)benzohydrazide).

3.3.2.3 IR Spectrum of 3



3

♦ In the spectrum of furfural, the major peak appears at 1658.34 cm^{-1}, which is related to (C=O) bond vibration, and at 1563.93 cm^{-1} for (C=C) vibration.

♦ In the spectrum of nicotinoylhydrazide, the major peak of carbonyl vibration observed at 1664.97 cm^{-1}, while (N-H) vibration appears at 3321.11 cm^{-1}.

• The (C-H) vibration of pyridine ring appears at 3007.18 cm^{-1}.

• The (C=C) vibration of ring appears at 1558.56 cm^{-1}.

♦ In the spectrum of Schiff base **3**, the major peak observed at 1647.98 cm^{-1} which is related to azomethine group (C=N), and there is disappearance of (N-H) peak of amine.

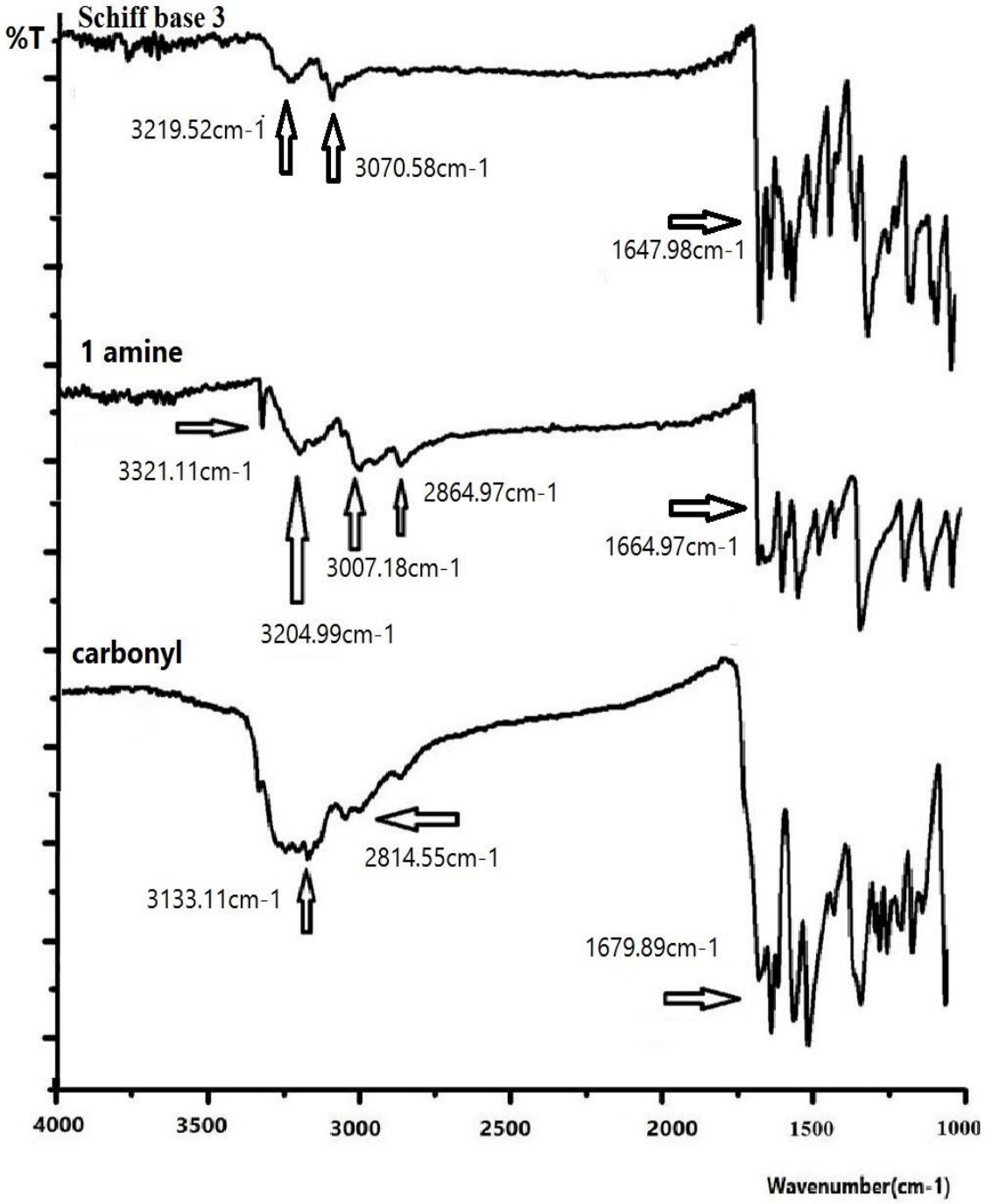
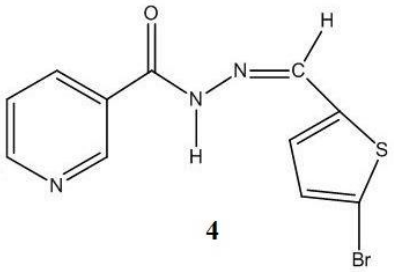


Figure 3.3 IR spectra (4000-1000 cm^{-1}) of: 1 amine = (nicotinoylhydrazide), carbonyl = (furfural) and Schiff base 3 = ((Z)-N'-(furan-2-ylmethylene)nicotinoylhydrazide).

3.3.2.4 IR Spectrum of 4



4

♦ In the spectrum of 5-Bromo-2-thiophenecarbaldehyde, the major peak must appear at 1710 cm^{-1}, which is related to (C=O) bond, due to the conjugation with aromatic ring the frequency decreases to 1658.8 cm^{-1}.

• The aromatic (C-H) vibration observed at 3091.99 cm^{-1}.

• Aldehyde (C-H) vibration appears at 2830.57 cm^{-1}, 2777.22 cm^{-1}.

•1521.06 cm^{-1} is related to ring C=C vibration.

♦ In the spectrum of nicotinoylhydrazide, the major peak of carbonyl vibration observed at 1664.97 cm^{-1}, while (N-H) vibration appears at 3321.11 cm^{-1}.

• The (C-H) vibration of pyridine ring appears at 3007.18 cm^{-1}.

• The (C=C) vibration of ring appears at 1558.56 cm^{-1}.

♦ In the spectrum of Schiff base **4**, the major peak observed at 1538.16 cm^{-1} which is related to azomethine group (C=N), and disappearance of (N-H) peak of amine.

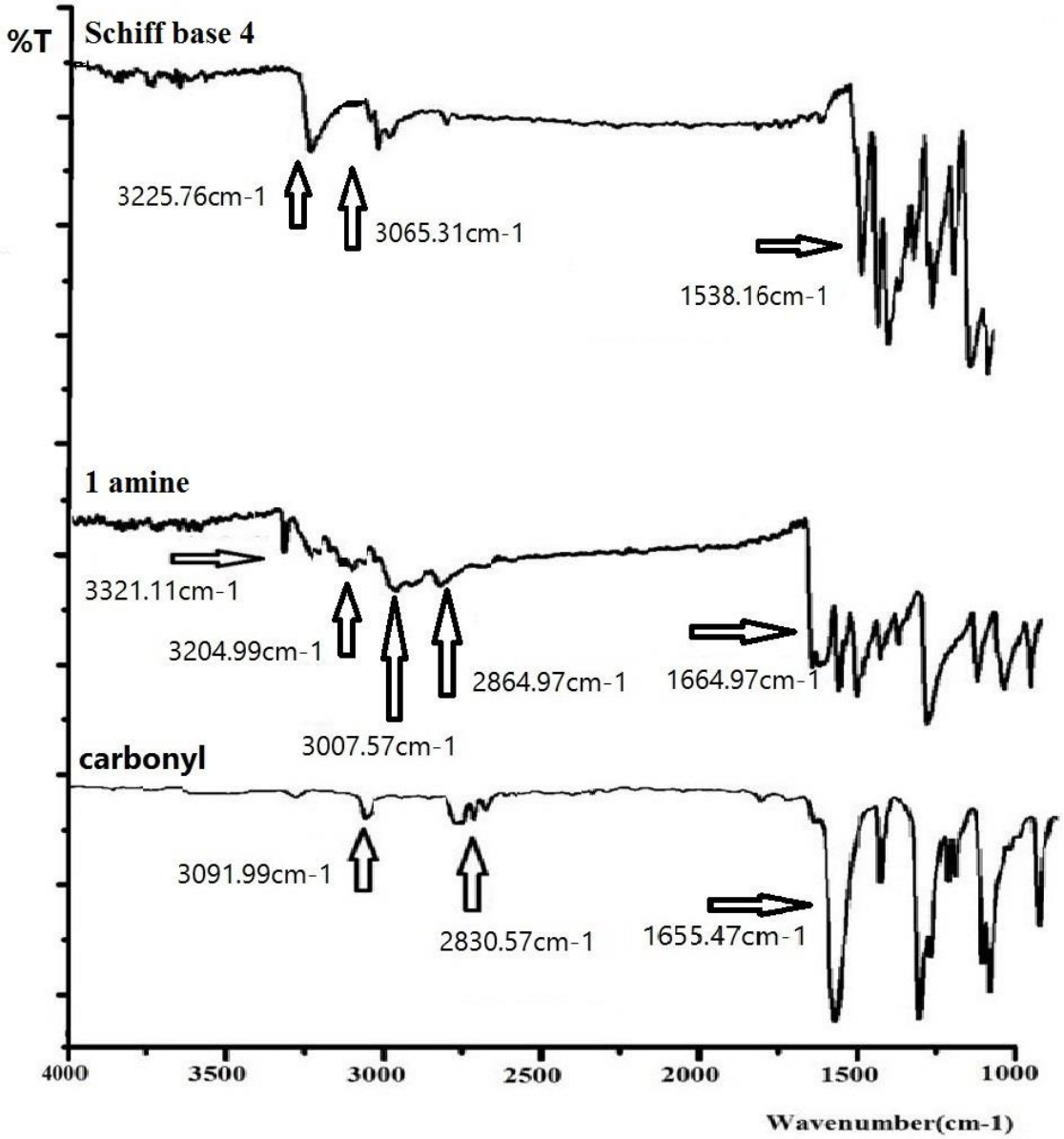
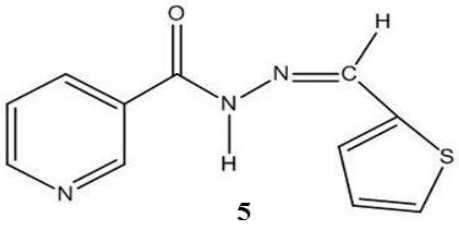


Figure 3.4 IR spectra (4000-1000 cm^{-1}) of: 1 amine = (nicotinoylhydrazide), carbonyl = (5-bromothiophene-2-carbaldehyde) and Schiff base 4 = (N'-((5-bromothiophen-2yl)methylidene)nicotinoylhydrazide).

3.3.2.5 IR Spectrum of 5



5

♦ In the spectrum of 2-thiophenecarbaldehyde, the major peak must appear at 1710 cm^{-1}, which is related to (C=O) bond, due to the conjugation with aromatic ring the frequency decreases to 1657.69 cm^{-1}.

• The aromatic (C-H) vibration observed at 3092.62 cm^{-1}.

• Aldehyde (C-H) vibration appears at 2823.50 cm^{-1}.

•1515.48 cm^{-1} is related to ring C=C vibration.

♦ In the spectrum of nicotinoylhydrazide, the major peak of carbonyl vibration observed at 1664.97 cm^{-1}, while (N-H) vibration appears at 3321.11 cm^{-1}.

• The (C-H) vibration of pyridine ring appears at 3007.18 cm^{-1}.

• The (C=C) vibration of ring appears at 1558.56 cm^{-1}.

♦ In the spectrum of Schiff base **5**, the major peak observed at 1672.27 cm^{-1} which is related to azomethine group (C=N), and there is disappearance of (N-H) peak of amine.

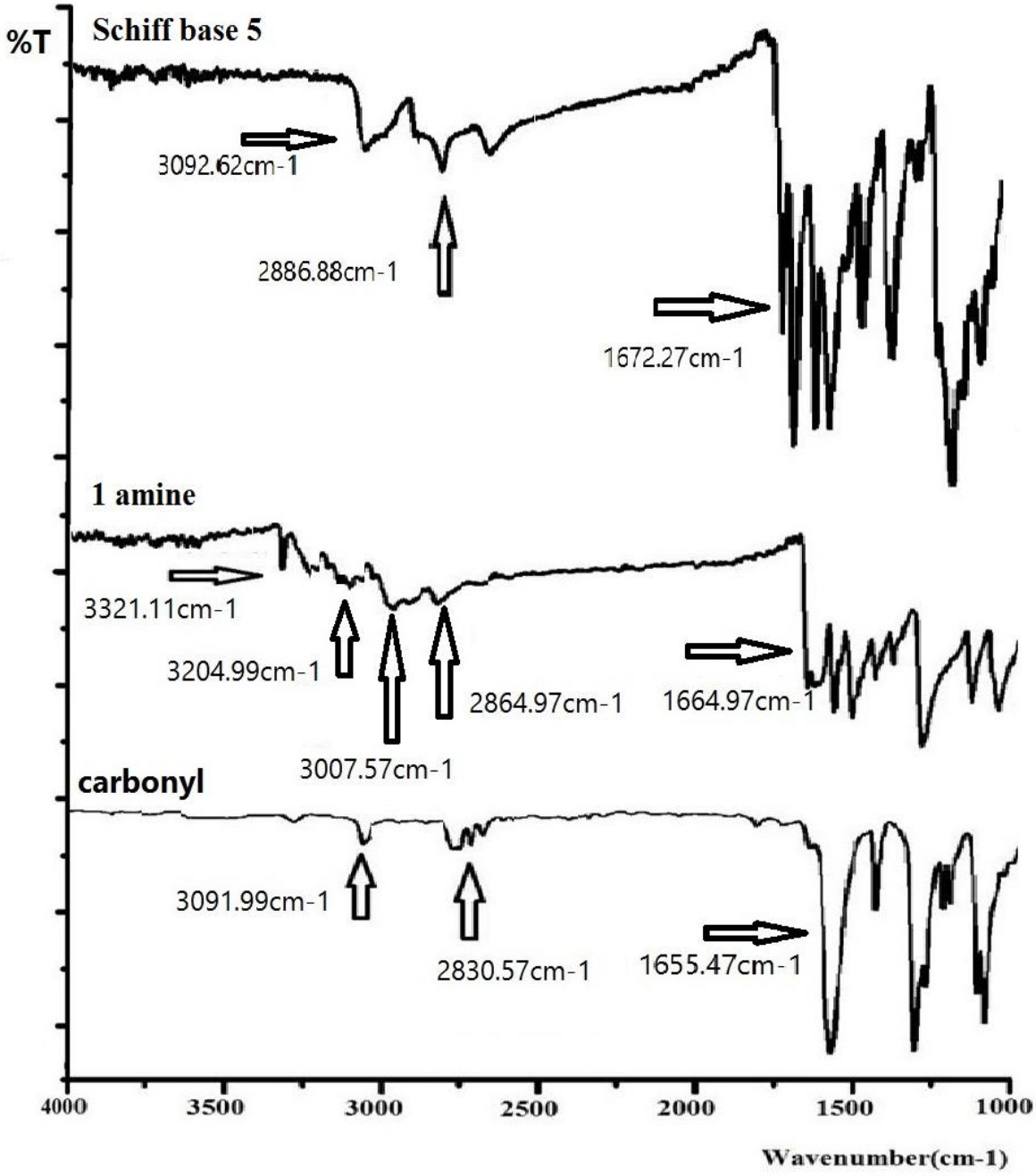
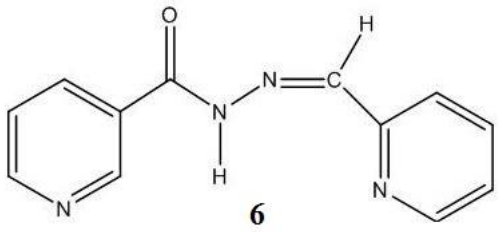


Figure 3.5 IR spectra (4000-1000 cm^{-1}) of: 1 amine = (nicotinoylhydrazide), carbonyl = (2-thiophene-carbaldehyde) and Schiff base 5 = (N'-(thiophen-2-ylmethylene)nicotinohydrazide).

3.3.2.6 IR Spectrum of 6



6

♦ In the spectrum of pyridine-2-carbaldehyde, the major peak of carbonyl vibration is observed at 1707.44 cm^{-1}.

• The (C-H) vibration of aromatic ring appears at 3055.59 cm^{-1}.

• 2821.00 cm^{-1} is related to aldehyde (C-H) vibration.

• The (C=C) vibration of ring appears at 1583.57 cm^{-1}.

♦ In the spectrum of nicotinoylhydrazide, the major peak of carbonyl vibration observed at 1664.97 cm^{-1}, while (N-H) vibration appears at 3321.11 cm^{-1}.

• The (C-H) vibration of pyridine ring appears at 3007.18 cm^{-1}.

• The (C=C) vibration of ring appears at 1558.56 cm^{-1}.

♦ In the spectrum of Schiff base **6**, the major peak is observed at 1634.10 cm^{-1} which is related to azomethine group (C=N), and there is disappearance of (N-H) peak of amine.

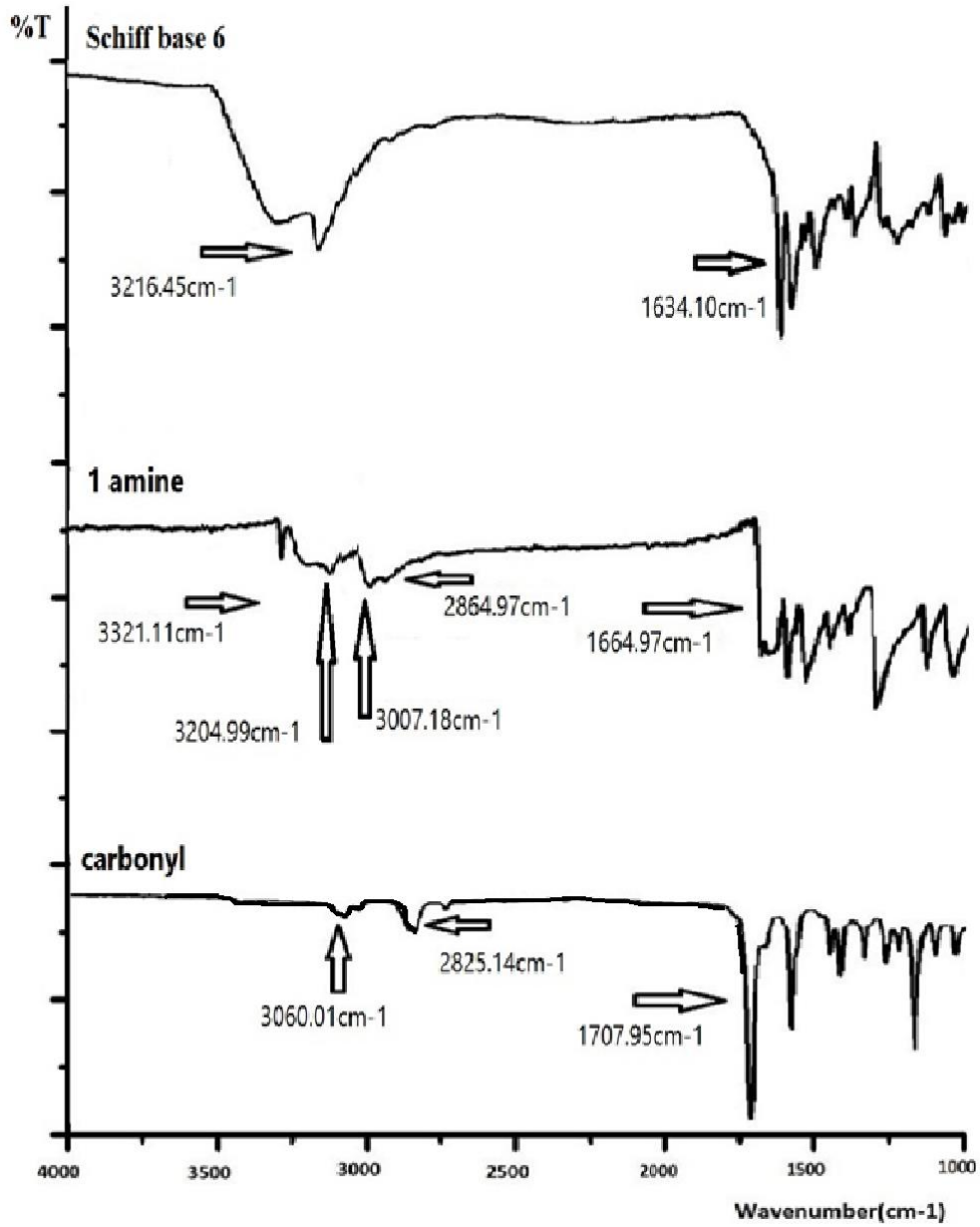
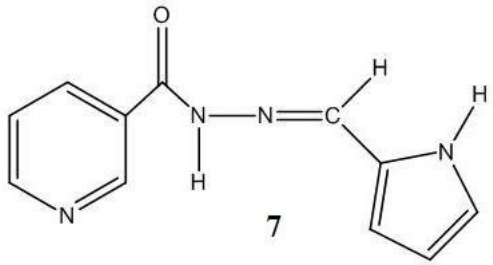


Figure 3.6 IR spectra (4000-1000 cm^{-1}) of: 1 amine = (nicotinoylhydrazide), carbonyl = (pyridine-2-carbaldehyde) and Schiff base 6 = ((Z)-N'-(pyridin-2-ylmethylene)nicotinoylhydrazide).

3.3.2.7 IR Spectrum of 7



7

♦ In the spectrum of pyrrole-2-carbaldehyde, the major peak of carbonyl vibration is observed at 1715.65 cm^{-1}.

• (C-H) vibration of aldehyde appears at 2825.07 cm-1 and 2730.15cm-1

♦ In the spectrum of nicotinoylhydrazide, the major peak of carbonyl vibration is observed at 1664.97 cm^{-1}, while (N-H) vibration appears at 3321.11 cm^{-1}.

• The (C-H) vibration of pyridine ring appears at 3007.18 cm^{-1}.

• The (C=C) vibration of ring appears at 1558.56 cm^{-1}.

♦ In the spectrum of Schiff base **7**, the major peak is observed at 1647.98 cm^{-1} which is for azomethine group (C=N), and there is disappearance of (N-H) peak of amine.

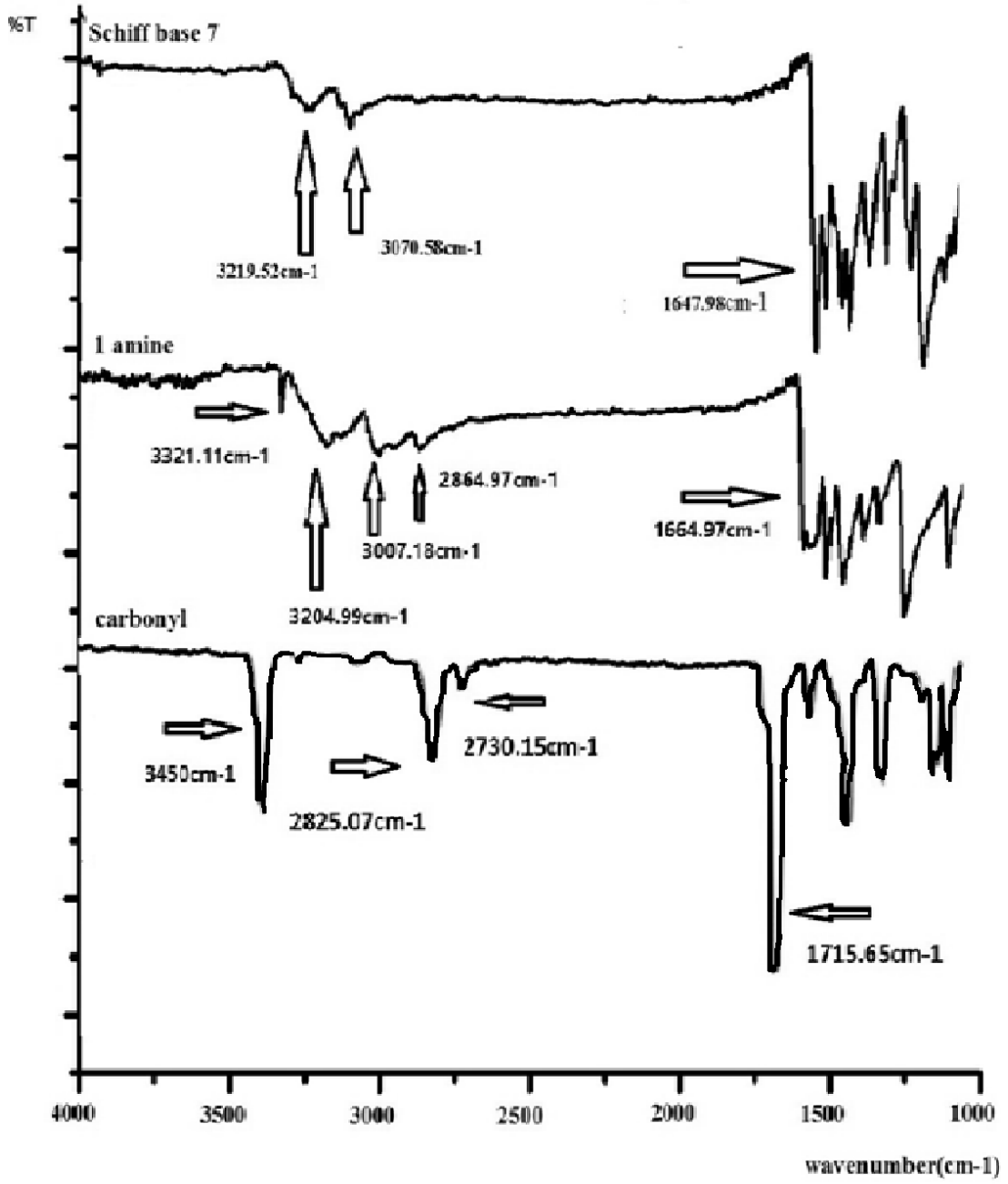


Figure 3.7 IR spectra (4000-1000 cm^{-1}) of: 1 amine = (nicotinoylhydrazide), carbonyl = (pyrrole-2-carbaldehyde) and Schiff base 7 = (N'-((1H-pyrrol-2-yl)methylene)nicotinoylhydrazide).

3.4 ^{1}H-NMR Investigations of Schiff's Bases

The ^{1}H-NMR spectra of Schiff bases were recorded at room temperature by using DMSO as a solvent.

In general, the spectrum is divided for two regions ; 7-8 ppm for aromatic and 8-12 ppm for aldehyde protons.

3.4.1 ^{1}H-NMR Spectra of 1

The ^{1}H-NMR spectrum of Schiff base **1** is shown in **Fig 3.4.1.** The signals in 6.9480-8.8805 ppm were related to the aromatic protons, in which (H_a ,d = 6.948), (H_b ,t = 7.402), (H_c ,t = 7.180), (H_d ,d = 7.944), (H_e ,d = 7.599), (H_f ,t = 8.298), (H_g ,d = 8.880) ppm.

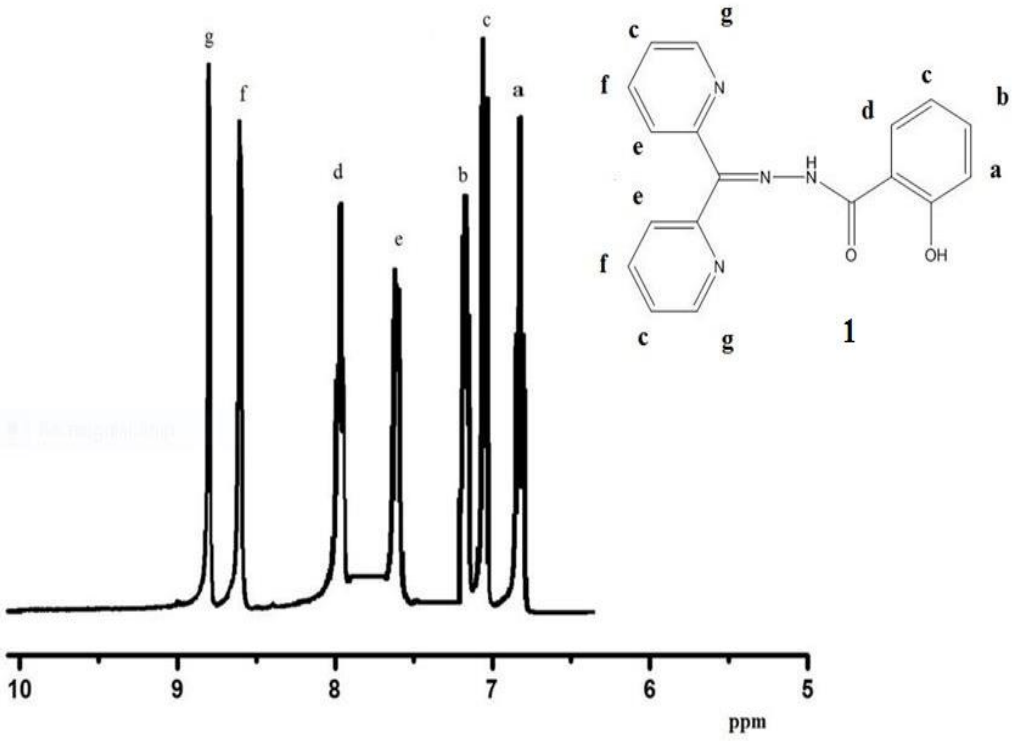


Figure 3.4.1 : ^{1}H-NMR Spectrum of 1

3.4.2 ^{1}H-NMR Spectra of 2

The ^{1}H-NMR spectrum of Schiff base **2** is shown in **Figure 3.4.2.** The signals in 6.995-8.725 ppm were related to the aromatic protons, in which

(H_a ,d = 7.911), (H_b ,t = 7.734), (H_c ,t = 7.201), (H_d ,d = 8.725), (H_e ,s = 8.168), (H_f ,s = 7.815), (H_g ,t = 7.251), (H_h ,t = 7.435), (H_i ,d = 6.995) ppm.

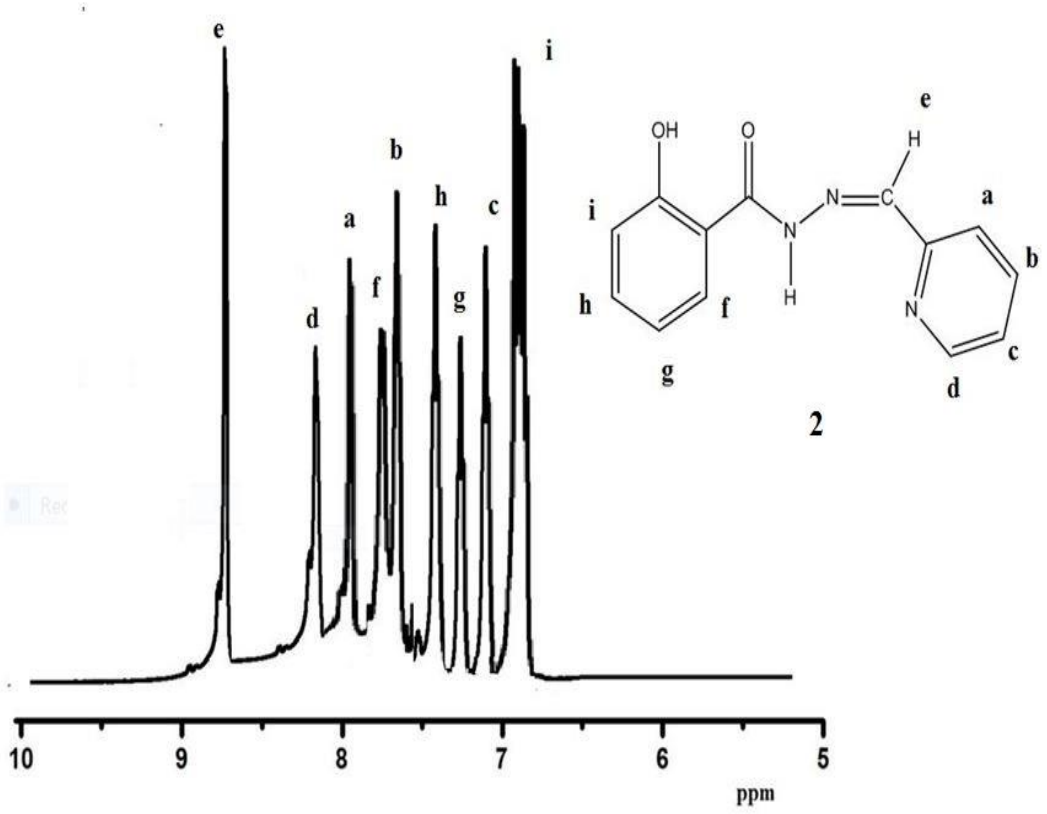


Figure 3.4.2 : ^{1}H-NMR Spectrum of 2

3.4.3 ^{1}H-NMR Spectra of 3

The ^{1}H-NMR spectrum of Schiff base **3** is shown in **Figure 3.4.3.** The signals in 6.614-9.017 ppm were related to the aromatic protons, in which

(H_a ,d = 6.614), (H_b ,t = 7.831), (H_c ,d = 6.930), (H_d ,s =8.297), (H_e ,s = 9.017), (H_f ,d = 8.729), (H_g ,t = 7.525), (H_h ,d = 8.211) ppm.

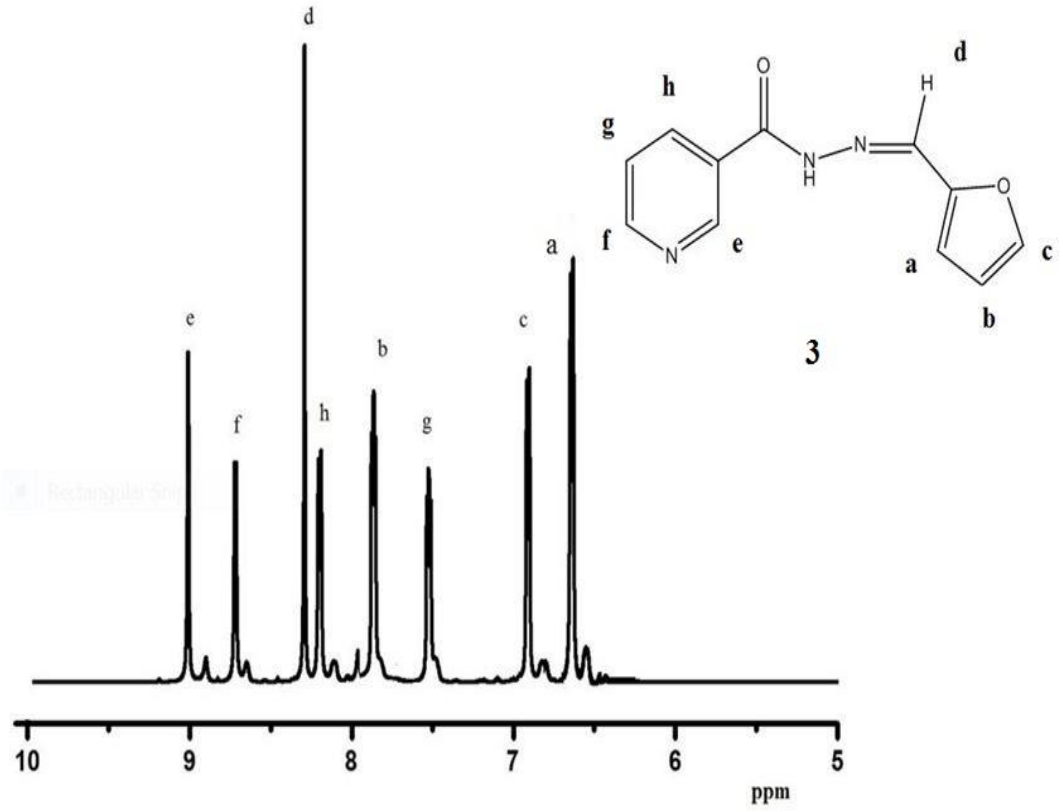


Figure 3.4.3 : ^{1}H-NMR Spectrum of 3

3.4.3 ^{1}H-NMR Spectra of 4

The ^{1}H-NMR spectrum of Schiff base **4** is shown in **Figure 3.4.4.** The signals in 7.255-8.998 ppm were related to the aromatic protons, in which (H_a ,d = 7.255), (H_b ,d = 7.320), (H_c ,s = 8.546), (H_d ,s = 8.998), (H_e ,d = 8.720), (H_f ,t = 7.530), (H_g ,d = 8.194) ppm.

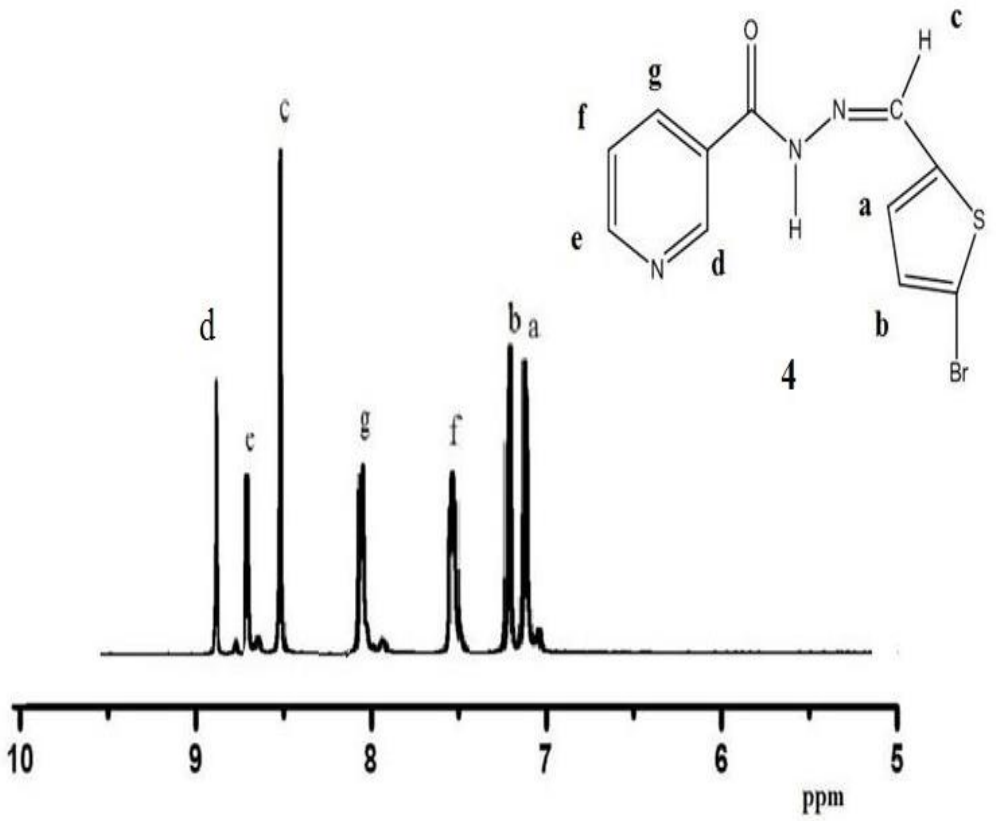


Figure 3.4.4 : [1]H-NMR Spectrum of 4

3.4.4 [1]H-NMR Spectra of 5

The [1]H-NMR spectrum of Schiff base **5** is shown in **Figure 3.4.5.** The signals in 7.240-8.998 ppm were related to the aromatic protons, in which (H_a ,d = 7.328), (H_b ,t = 7.240), (H_c ,d = 7.530), (H_d ,s = 8.546), (H_e ,s = 8.998), (H_f ,d = 8.178), (H_g ,t = 7.568), (H_h ,d = 8.729) ppm.

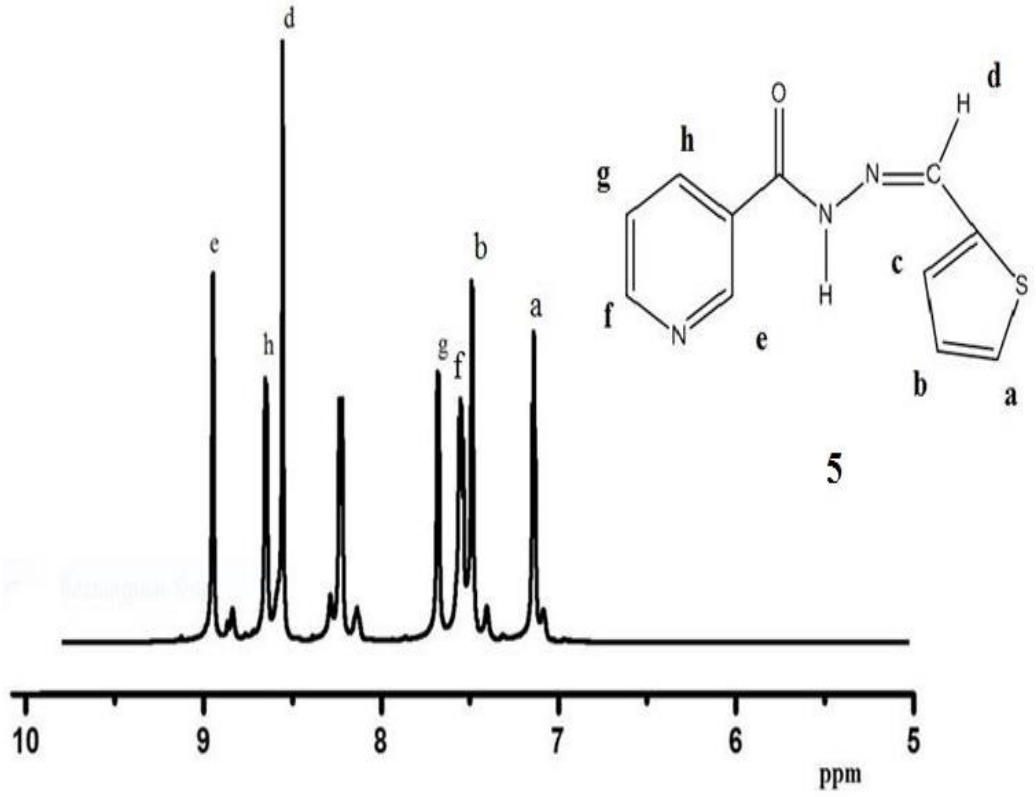


Figure 3.4.5 : [1]H-NMR Spectrum of 5

3.4.6 [1]H-NMR Spectra of 6

The [1]H-NMR spectrum of Schiff base **6** is shown in **Figure 3.4.6.** The signals in 6.114-9.025 ppm were related to the aromatic protons, in which (H_a ,d = 8.706), (H_b ,t = 6.479), (H_c ,t = 7.514), (H_d ,d = 6.114), (H_e ,s = 6.899), (H_f ,s = 9.025), (H_g ,d = 8.215), (H_h ,t = 7.955), (H_i ,d = 8.255) ppm.

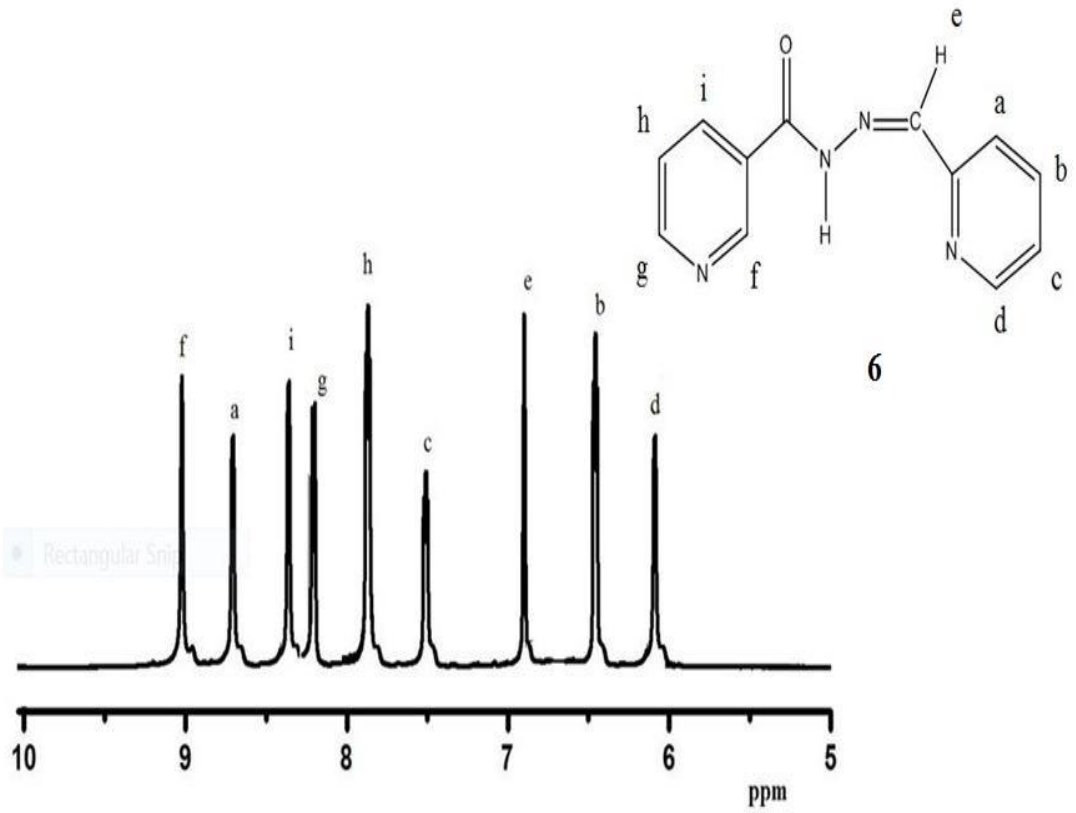


Figure 3.4.6 : [1]H-NMR Spectrum of 6

3.4.7 [1]H-NMR Spectra of 7

The [1]H-NMR spectrum of Schiff base **7** is shown in **Figure 3.4.7.** The signals in 6.119-9.037 ppm were related to the aromatic protons, in which (H_a ,d = 6.119), (H_b ,t = 6.481), (H_c ,d = 6.906), (H_d ,s = 7.418), (H_e ,s = 9.037), (H_f ,d = 8.705), (H_g ,t = 7.515), (H_h ,d = 8.215) ppm.

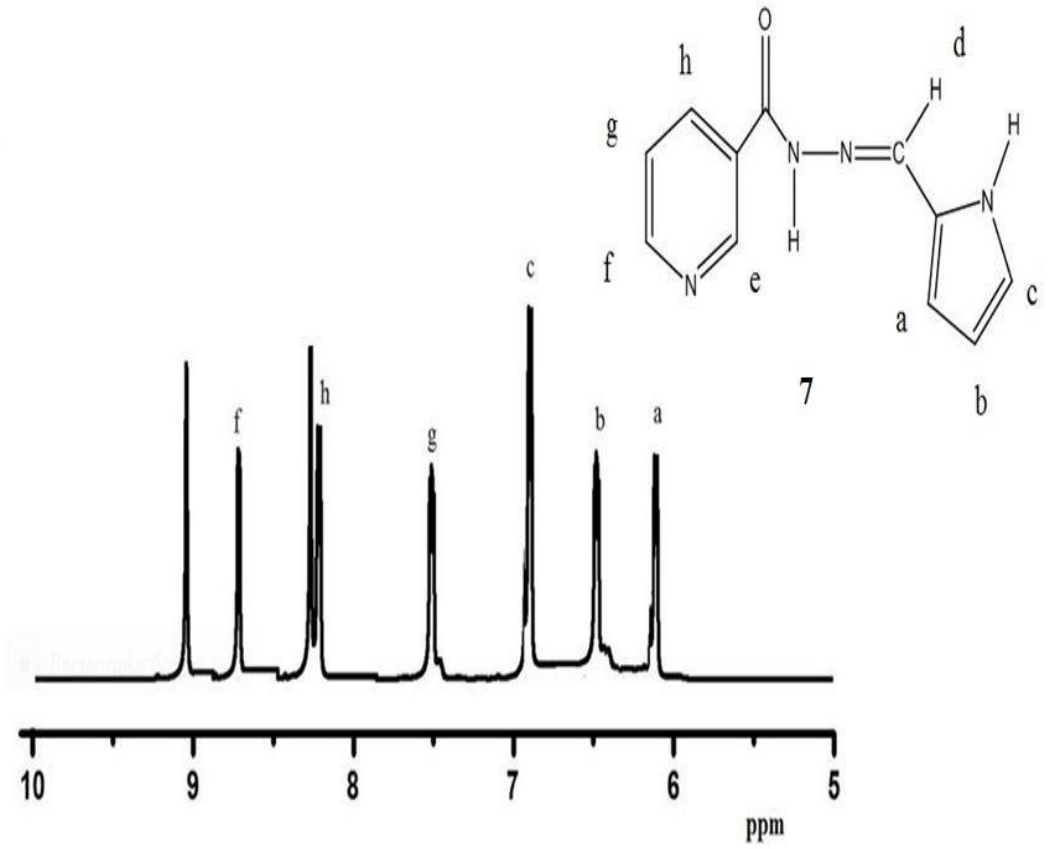


Figure 3.4.7 : ^{1}H-NMR Spectrum of 7

3.5 ^{13}C-NMR Investigation of Schiff's Bases

The ^{13}C-NMR spectra of the designed Schiff bases 1-8 have been recorded in DMSO solvent at room temperature. The spectral data confirms the ^{1}H-NMR spectral results. In all ^{13}C-NMR, the number of signals corresponds to the number of magnetically non-equivalent carbon atoms in the Schiff bases. In ^{13}C-NMR spectra, the state of hybridization is the dominating factor in determining the chemical shift of a carbon atom sp^3 -hybrid carbon atoms absorb up field while sp^2 carbon atoms absorbs at lower field strength i.e. sp^3>sp>sp^2.

3.5.1 ^{13}C-NMR Spectrum of 1

The signal of carbon (C_8) atom of azomethine group (C=N) appeared at 149.940 ppm. The signal of carbon atoms of aromatic ring (C_1-C_6) appeared in (117.334-156.575) ppm range, while the signal at 162.993 ppm is related to the carbon (C_7) of carbonyl group (C=O).

The signals of carbon atoms of pyridine rings appeared in (120.296-149.940) ppm range, in which the signals at 146.455, 142.344 ppm, 125.774 ppm, 134.579 ppm and 120.296 ppm are related to (C_9, C_{10}, C_{11}, C_{12}, C_{13}) respectively, as shown in **Figure 3.5.1**.

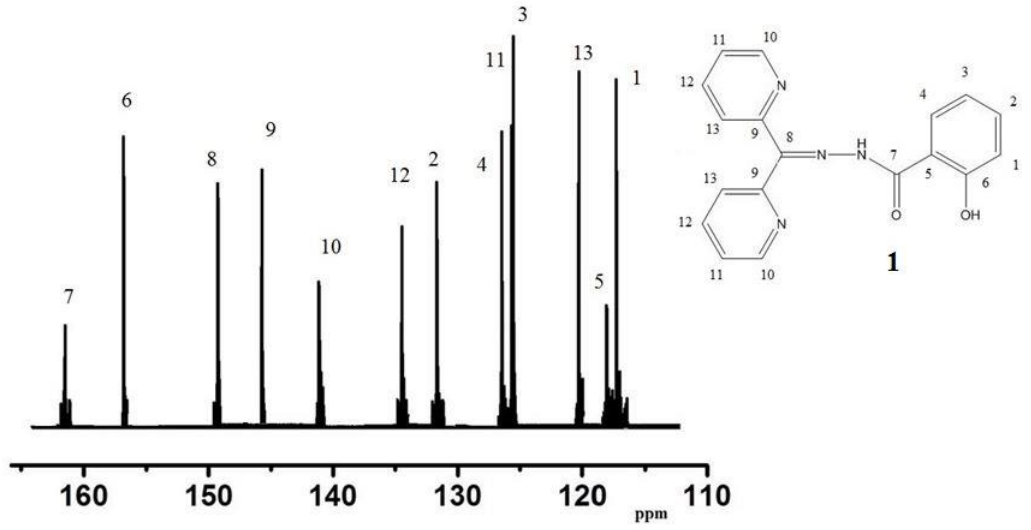


Figure 3.5.1 : ^{13}C-NMR Spectrum of 1

3.5.2 ^{13}C-NMR Spectrum of 2

The signal of (C_6) carbon atom of azomethine group (C=N) appeared at 143.881ppm. The signal of carbon atoms (C_1-C_5) of aromatic ring appeared

in (117.180 - 149.891) ppm range. while the signal at 165.366 ppm is related to the carbon of carbonyl group (C_7).

The signals of carbon atoms of pyridine rings appeared in (117.635-158.784) ppm range, in which the signals at 117.635 ppm, 158.784 ppm, 117.180 ppm, 134.502 ppm, 121.327 ppm, and 129.597 ppm are related to (C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}) respectively, as shown in **Figure 3.5.2**.

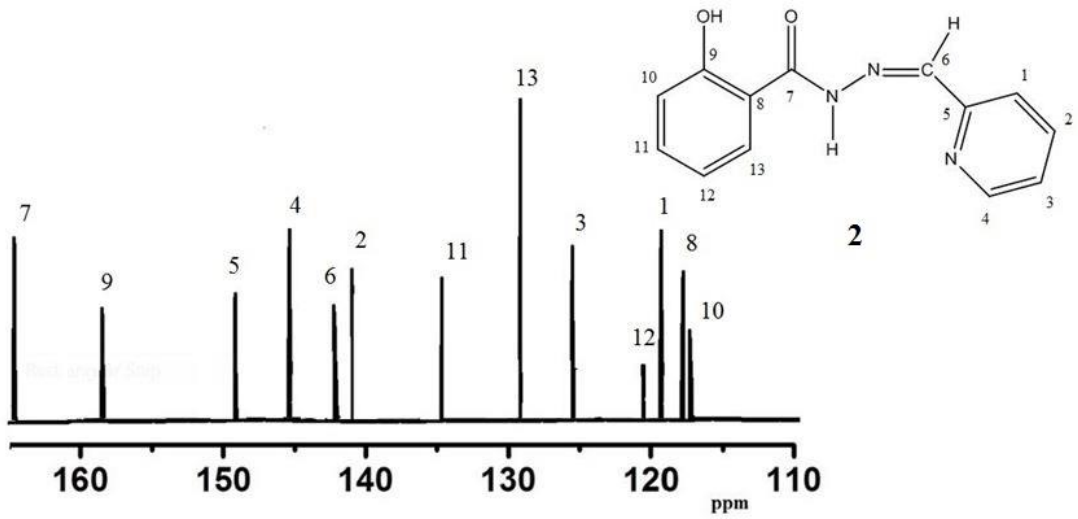


Figure 3.5.2 : ^{13}C-NMR Spectrum of 2

3.5.3 ^{13}C-NMR Spectrum of 3

The signals of (C_5) carbon atom of azomethine group (C=N) appeared at 134.866 ppm. The signal of carbon atoms of aromatic ring (C_1-C_4) appeared in (112.724- 149.433) ppm range, while the signal at 162.071 ppm is related to the carbon of carbonyl group (C_6).

The signals of carbon atoms of pyridine rings appeared in (124.079-149.722) ppm range, in which the signals at 129.079 ppm, 148.002 ppm,

149.722 ppm, 124.079 ppm, 135.875ppm are related to (C_7, C_8, C_9, C_{10}, C_{11}) respectively, as shown in **Figure 3.5.3**.

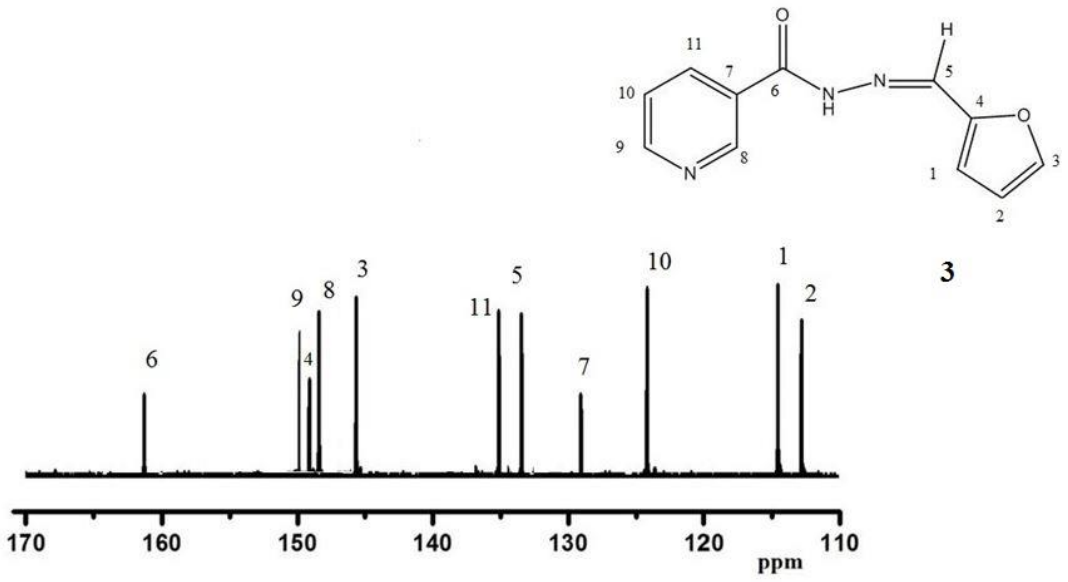


Figure 3.5.3 : ^{13}C-NMR Spectrum of 3

3.5.4 ^{13}C-NMR Spectrum of 4

The signal of carbon atom (C_5) of azomethine group (C=N) appeared at 135.881 ppm. The signals of carbon atoms of aromatic ring (C_1-C_4) appeared in (115.535 - 143.182) ppm, while the signal at 162.091 ppm is related to the carbon of carbonyl group (C_6).

The signals of carbon atoms of pyridine ring appeared in (124.095-152.809) ppm range, in which the signals at 132.321 ppm, 149.00 ppm, 152.809 ppm, 124.095 ppm, 141.265 ppm are related to (C_7, C_8, C_9, C_{10}, C_{11}) respectively, as shown in **Figure 3.5.4**.

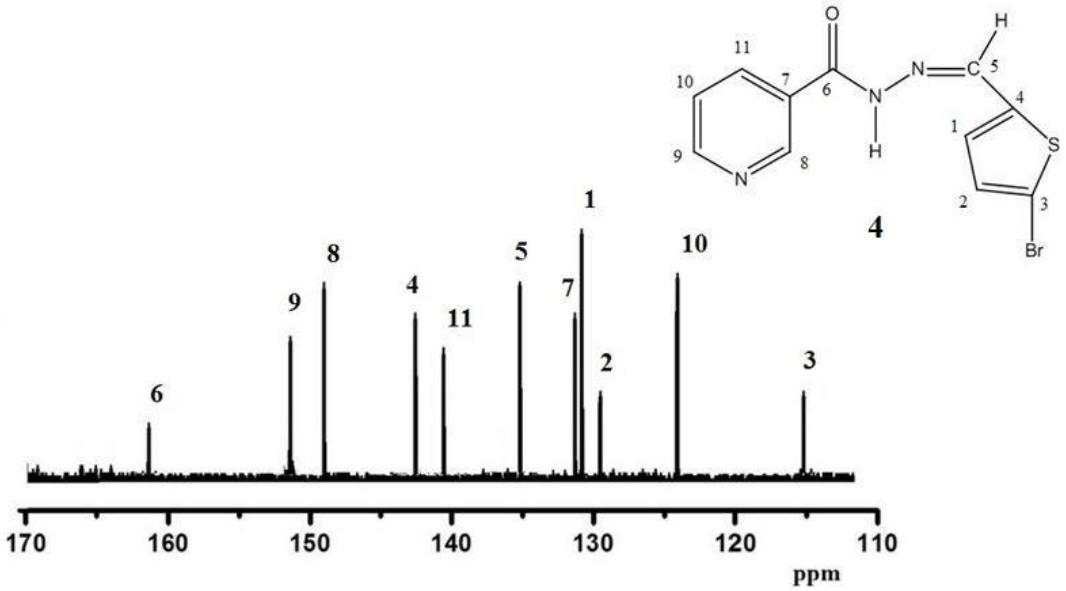


Figure 3.5.4 : ^{13}C-NMR Spectrum of 4

3.5.5 ^{13}C-NMR Spectrum of 5

The signal of carbon atom (C_5) of azomethine group (C=N) appeared at 132.318 ppm. The signals of carbon atoms of aromatic ring (C_1-C_4) appeared in (129.528- 143.284) ppm range, while the signal at 162.146 ppm is related to the carbon of carbonyl group (C_6).

The signals of carbon atoms of pyridine ring appeared in (124.691-153.350) ppm range, in which the signals at 131.697 ppm, 149.639 ppm, 153.350 ppm, 124.691 ppm, 135.831 ppm are related to (C_7, C_8, C_9, C_{10}, C_{11}) respectively, as shown in **Figure 3.5.5**.

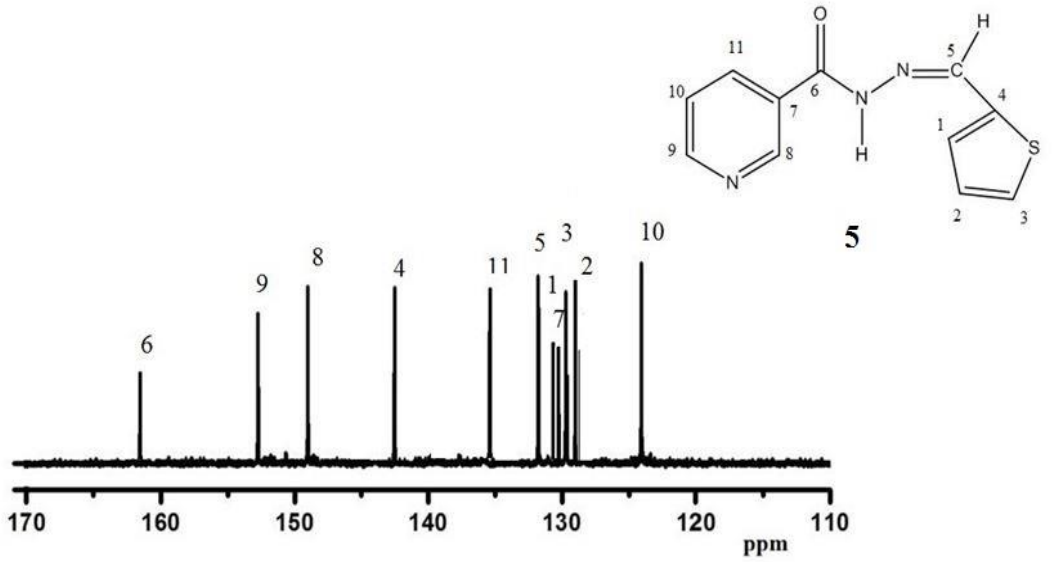


Figure 3.5.5 : ^{13}C-NMR Spectrum of 5

3.5.6 ^{13}C-NMR Spectrum of 6

The signal of carbon atom (C_6) of azomethine group (C=N) appeared at 145.450 ppm. The signals of carbon atoms of pyridine ring (C_1-C_5) appeared in (118.820- 152.560) ppm range, while the signal at 161.646 ppm indicate the carbon of carbonyl group (C_7).

The signals of carbon atoms of pyridine ring appeared in (124.175-148.837) ppm range, in which the signals at 124.175 ppm, 142.964 ppm, 148.837 ppm, 127.322 ppm, 132.301 ppm are related to (C_8, C_9, C_{10}, C_{11}, C_{12}) respectively, as shown on **Figure 3.5.6**.

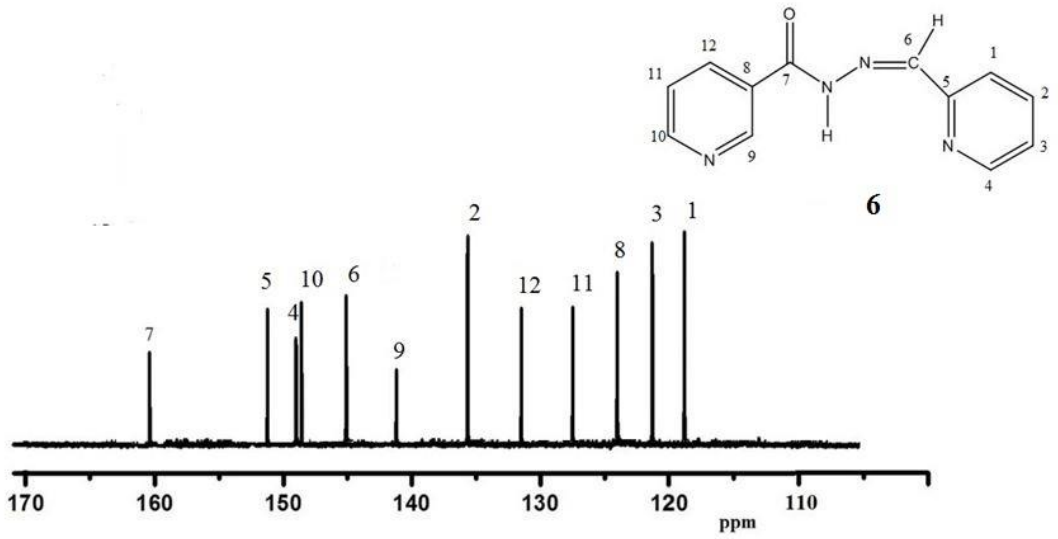


Figure 3.5.6 : ^{13}C-NMR Spectrum of 6

3.5.7 ^{13}C-NMR Spectrum of 7

The signal of carbon atom (C_5) of azomethine group (C=N) appeared at 131.035 ppm. The signals of carbon atoms of pyridine ring (C_1-C_4) appeared in (109.758- 141.973) ppm range, while the signal at 162.011 ppm is related to the carbon of carbonyl group (C_6).

The carbon atoms of pyridine ring appeared in (124.002-152.348) ppm range, in which the signals at 130.243ppm, 149.141 ppm, 152.348 ppm, 124.002 ppm, 132.281 ppm are related to (C_7, C_8, C_9, C_{10}, C_{11}) respectively, as shown in **Figure 3.5.7**.

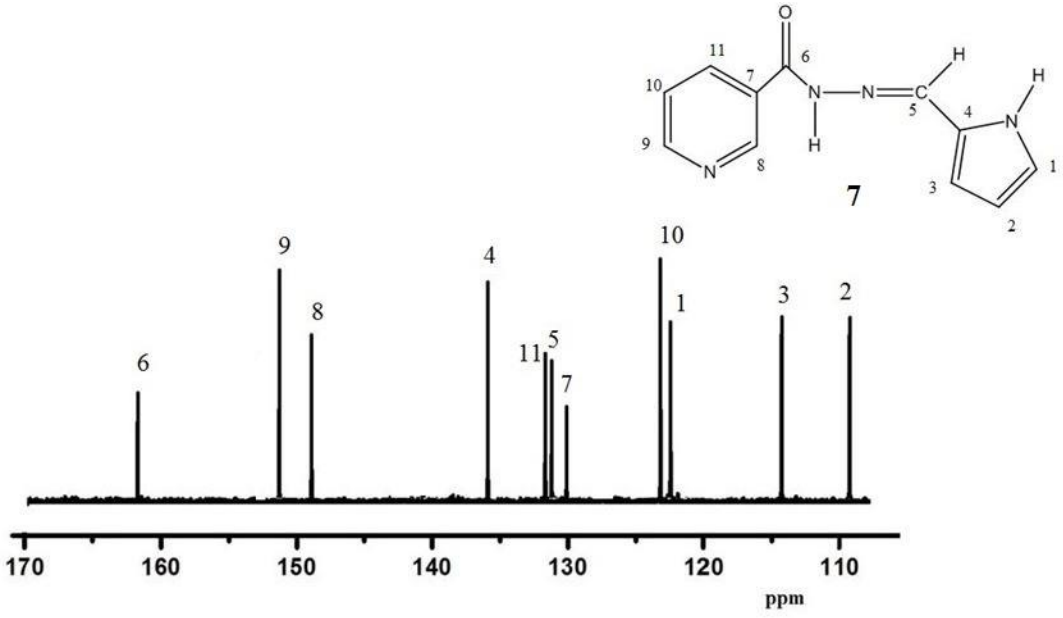


Figure 3.5.7 : ^{13}C-NMR Spectrum of 7

Chapter Four

Conclusion

Seven of Schiff' bases were synthesized and characterized of heterocyclic hydrazides and the selected carbonyl compounds. Such bases were identified and determined by various spectral analysis (IR, ^{1}H-NMR,^{13}C-NMR). Some Schiff bases were tested for their biological activities. It has been found that some of our synthesized Schiff bases have shown promising biological activities. in this project, seven Schiff bases were synthesized and characterized by various analytical and spectral techniques.

♦ Schiff base **1**

[N'-(di(pyridin-2-yl)methylene)-2-hydroxybenzoylhydrazide] formed readily in 83.6% yield. The structure was identified by IR, ^{1}H-NMR,^{13}C-NMR spectroscopy.

♦ Schiff base **2**

[2-hydroxy-N-(pyridine-ylmethylene)benzohydrazide] formed readily in 58% yield. The structure was identified by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy.

♦ Schiff base **3**

[(Z)-N'-(furan-2-ylmethylene)nicotinoylhydrazide] formed readily in 65% yield. The structure was identified by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy.

♦ Schiff base **4**

[N'-((5-bromothiophen-2yl)methylene)nicotinoylhydrazide] was synthesized in 66% yield. And the structure was identified by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy.

♦ Schiff base **5**

[N'-(thiophen-2-ylmethylene)nicotinoylhydrazide] formed readily in 82.9% yield. And the structure was identified by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy.

♦ Schiff base **6**

[(Z)-N'-(pyridin-2-ylmethylene)nicotinoylhydrazide] was synthesized in 67% yield. The structure was identified by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy.

♦ Schiff base **7**

[N'-((1H-pyrrol-2-yl)methylene)nicotinoylhydrazide] was in 49% yield. The structure was identified by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy.

Chapter Five

Biological activities

5.1 General procedure of anti-fungal test for Schiff bases

5.1.1 Preparation of samples for testing

Each compound (50 mg) was dissolved in 10 mL of mixed solution (7 ml ethanol and 3 ml ethyl acetate), and the solution was sterilized using membrane filtration (0.45 μm millipore filters) for all of the following tests.

5.1.2 Antifungal testing

All Schiff bases compounds were tested at different concentrations (**Table 5.1.)** for their antifungal activities against the test pathogens by a modified "poisoned food" technique. Different amounts of each compound were incorporated in pre-sterilized SDA medium to prepare a series of concentrations of the compound (0.6, 1.2, 2.4μg/ml). A mycelial agar disk of 5 mm diameter was cut out of 12 days old culture of the test fungus and inoculated on to the freshly prepared agar plates. In controls, sterile distilled water was used in place of the tested sample. Four replicate plates were used for each treatment (concentration).The inoculated plates were incubated in

the dark at 24˚C and the observations were recorded after 10 days. Percentage of mycelial inhibition was calculated using the following formula:

$$\% \text{ mycelial inhibition} = \left(\frac{dc-ds}{dc}\right) \text{x}100\%$$

where,

dc: colony diameter of the control

ds: colony diameter of the sample

As already introduced in the previous section, the seven samples are listed in **Table 5.1**. The three mentioned fungi underwent the seven different tests for the efficiency of Schiff bases compounds, namely *Trichophyton mentagrophytes* **Table 5.2**, Trychophyton rubrum **Table 5.3**,and *Microsporum canis* **Table 5.4**

Table 5.1 Schiff Bases Compounds

Sample Number	Name of Compound
I	N'-(di(pyridin-2-yl)methylene)-2-hydroxybenzoylhydrazide
II	2-hydroxy-N-(pyridine-ylmethylidene)benzohydrazid
III	(Z)-N'-(furan-2-ylmethylene)nicotinoylhydrazide
IV	N'-((5-bromothiophen-2yl)methylene)nicotinoylhydrazide
V	N'-(thiophen-2-ylmethylene)nicotinoylhydrazide
VI	(Z)-N'-(pyridin-2-ylmethylene)nicotinoylhydrazide
VII	N'-((1H-pyrrol-2-yl)methylene)nicotinoylhydrazide

Table 5.2 Diameter zone (mm) of *T. mentagrophytes* against three different concentration (c_1,c_2 and c_3).

Control	Diameter zone (mm) = 45,44,41,42,43					
Compound	**C1=0.6ug/ml**	**Mean**	**C2=1.2ug/ml**	**Mean**	**C3=2.4ug/ml**	**Mean**
I	15,14,15,16	15	no growth	no growth	no growth	no growth
II	31,32,33,31	31.75	15,16,14,15	15	9,10,8,10	9.25
III	30,31,21,20	25.5	21,22,21,20	21	7,6,7,7	6.75
IV	22,23,23,22	22.5	18,20,19,22	19.75	15,14,15,13	14.25
V	11,12,13,14	12.5	10,11,10,12	10.75	6,7,6,7	6.5
VI	2526,25,23	24.75	22,18,20,19	19.75	6,7,6,7	6.5
VII	30,31,32,29	30.5	25,26,25,23	24.75	15,14,15,13	14.25

Table 5.3 Diameter zone (mm) of *T. rubrum* CBS 392.58 against three different concentration (c_1,c_2 and c_3)

Control	Diameter zone (mm) = 45,44,41,42,43					
Compound	**C1=0.6ug/ml**	**Mean**	**C2=1.2ug/ml**	**Mean**	**C3=2.4ug/ml**	**Mean**
I	7,8,7,6	7	no growth	no growth	no growth	no growth
II	21,22,21,20	21	15,16,14,15	15	7,8,8,8	7.75
III	15,14,15,13	14.25	7,8,8,8	7.75	no growth	no growth
IV	11,12,13,14	12.5	10,9,11,10	10	45,46,47,45	5.5
V	6,7,6,7	6.5	no growth	no growth	no growth	no growth
VI	45,46,47,45	5.5	no growth	no growth	no growth	no growth
VII	11,12,13,14	12.5	10,11,10,7	10.75	6,7,6,7	6.5

Table 5.4 Diameter zone (mm) of *M. canis* CBS 132.88 against three different concentration (c_1,c_2 and c_3)

Control	Diameter zone (mm) = 45,44,41,42,43					
Compound	**C1=0.6ug/ml**	**Mean**	**C2=1.2ug/ml**	**Mean**	**C3=2.4ug/ml**	**Mean**
I	30,31,21,20	25.5	0,0,11,11	5.5	0,0,11,11	5.5
II	37,36,38,37	37	18,20,19,22	19.75	7,8,7,6	7
III	30,31,32,29	30.5	7,8,8,8	7.75	7,8,8,8	7.75
IV	25,26,24,25	25	10,11,10,12	10.75	8,9,8,7	26
V	32,33,31,30	31,5	15,16,14,15	15	0,0,11,11	5.5
VI	21,22,21,20	21	15,16,14,15	15	7,8,8,8	7.75
VII	37,36,38,37	37	15,14,15,13	14.25	7,8,8,8	7.75

5.2 General procedure of anti-oxidant test for Schiff bases compounds.

The hydrogen atom or electron donation abilities of the corresponding compounds were measured from the bleaching of the purple-colored methanolic solution of DPPH (1,1-Diphenyl-2-picryl-hydrazyl). This spectrophotometric assay uses the stable radical DPPH as a reagent.

One mL of various concentrations of the compounds (10,20,30,40,50 ug/ml) in ethanol was added to 4 mL of 0.004% methanol solution of DPPH (OD= 1.1128). Gallic acid (0.25mg/ml) used as standard. After 30 minutes of an

incubation period at room temperature, the absorbance was read against a blank at 517 nm. The percent Inhibition I (%) of free radical by DPPH was calculated as follows:

$$I(\%)=((A_{blank}-A_{sample})/A_{blank})x\ 100\% \qquad \textbf{Equation (1)}$$

Compounds concentrations providing 50% inhibition (IC_{50}) were calculated from the plot of inhibition (%) against compound concentration. The control is Galic acid as Shown in **Table 5.2.1**

Table 5.2.1 Absorbance for Blank at Different Concentrations

Concentration ug/ml		Abs	% inhibition
C1	12.5	0.87	20.79
C2	25	0.679	38.18
C3	37.5	0.579	47.28
C4	50	0.411	62.58
C5	62.5	0.385	64.94

Table 5.2.2 Absorbance for the Samples at Different Concentrations

Sample Number	Sample Name	Absorbance (Mean)	Concentration ug/ml	% inhibition
I	N'-(di(pyridin-2-yl)methylene)-2-hydroxybenzoylhydrazide	1.0579	10	3.68
		1.0851	20	1.21
		0.9888	30	9.97
		1.0277	40	6.43
		1.0399	50	5.32
II	2-hydroxy-N-(pyridine-ylmethylidene)benzohydrazide	1.0804	10	1.63
		1.0414	20	5.18
		1.056	30	3.86
		1.0472	40	4.66
		1.0563	50	3.83
III	(Z)-N'-(furan-2-ylmethylene)nicotinoylhydrazide	1.0619	10	3.32
		1.031	20	6.13
		1.0034	30	8.64
		0.9901	40	9.85
		1.0216	50	6.99
IV	N'-((5-bromothiophen-2yl)methylene)nicotinoylhydrazide	1.0452	10	3.323
		1.0385	20	6.13
		1.0357	30	8.64
		1.0319	40	9.85
		1.0378	50	6.99
V	N'-(thiophen-2-ylmethylene)nicotinoylhydrazide	1.0347	10	4.84
		1.0301	20	5.45
		0.9665	30	5.70
		0.9751	40	6.05
		1.0016	50	5.51
VI	(Z)-N'-(pyridin-2-ylmethylene)nicotinoylhydrazide	1.0438	10	5.79
		1.0438	20	6.21
		1.0333	30	12.21

		1.0472	40	11.22
		1.0472	50	8.81
VII	N'-((1H-pyrrol-2-yl)methylene)nicotinoylhydrazide	1.0624	10	4.97
		1.0443	20	4.97
		1.0491	30	5.92
		1.0362	40	4.66
		1.0481	50	5.13

5.3 General Procedure of Anti-bacterial Test for Schiff Bases Compounds

5.3.1 Antibacterial activity

The antibacterial activity of the synthesized compounds was determined against the following microorganisms: *Escherichia coli Staphylococcus aureus* (ATCC 25923), *Salmonella, Klebsiella, pneumonia* (ATCC 13883), *Proteus vulgaris* (ATCC 13315), and *Pseudomonas aeruginosa* (ATCC 27853), All the isolates were purchased from BERC /Til Village. Solutions of each synthetic compound (5.0 mg/mL) in ethanol were sterilized by filtration through a 0.45 mm membrane filter. Antibacterial tests were then carried out by disc diffusion method.

Compounds were investigated by the disc diffusion using 6 mm filter discs prepared from Whatman paper 3. Bacteria were cultured overnight at 28°C in LB medium and then adjusted with sterile saline to a concentration of 1.0x105 CFU mL^{-1}. The suspension was swapped on the top of Muller–Hinton agar plates (20 mL agar/1 plate).

Discs were flooded with the 10ul top of Muller–Hinton agar plates

compounds (5.0 mg mL^{-1}) and placed on the inoculated agar. (4 discs per agar plate). After 24 hrs of incubation at 37°C for bacteria, the diameter of the growth inhibition zones was measured. Gentamycin was as a positive

control and 10µL was applied to the discs from stock solution (1 mg mL^{-1}).

All tests were done in duplicate. (Sokovic et al., 2008).

Chapter Six

Results and Discussion

6.1 Antifungal test

Our compounds were tested for their antifungal activities against *T. mentagrophytes, T. rubrum* , *M. canis*, . **Tables 6.1, 6.2 and 6.3** , respectively.

The seven tested compounds showed results as shown below. N'-(di(pyridin-2-yl)methylene)-2-hydroxybenzoylhydrazide showed complete inhibition against *T. mentagrophet* at 1.2 µg/ml and at 2.4 µg/ml, while the other compounds showed excellent significant activity. (Z) - N'- (pyridin-2- ylmethylene) nicotinoyl hydrazide, N'- (thiophen -2- ylmethylene) nicotinoyl hydrazide and N'-(di(pyridin-2-yl)methylene)-2-hydroxybenzoylhydrazide shows complete inhibition at 1.2 and 2.4 µg/mla gainst *T. rubrum.* On the other hand the seven compounds showed different inhibition at 0.2, 1.6 and 2.4 µg/ml against *M. canis.* as shown in **Figure : 6.4**, **6.5** and **6.6**

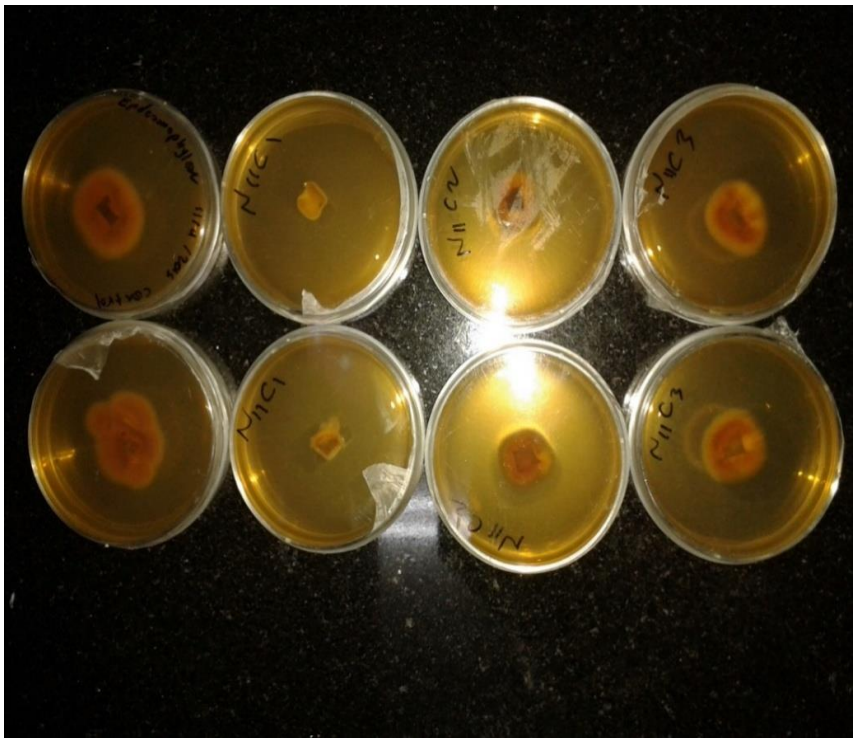


Figure 1: Anti-fungal Testing of Compound Number 5 against *T. mentagrophytes*

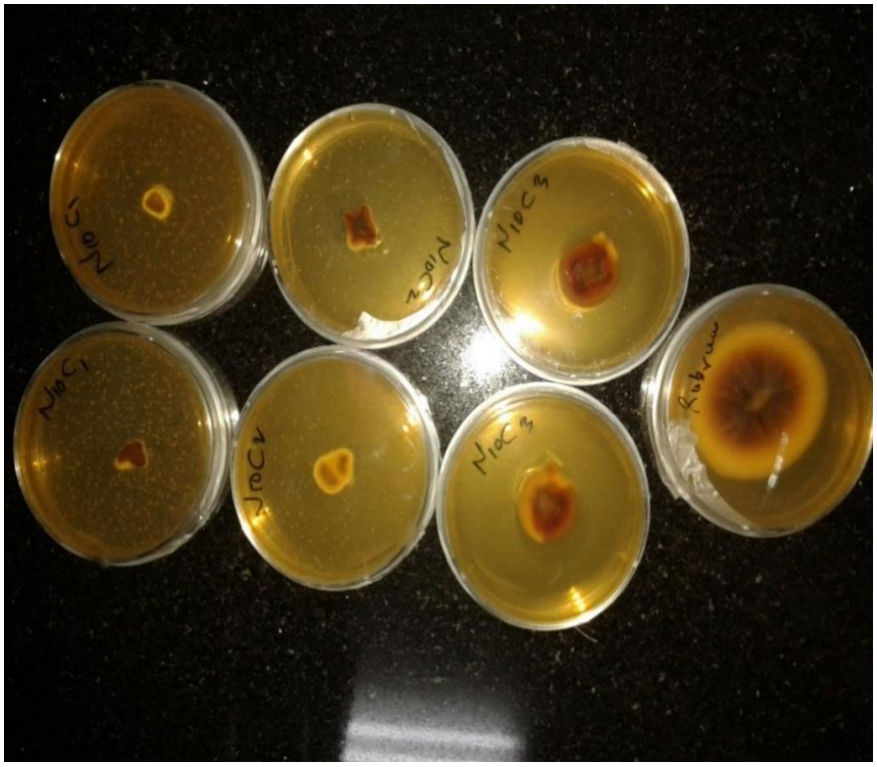


Figure 2: Anti-fungal Testing of Compound Number 3 against *T. rubrum*

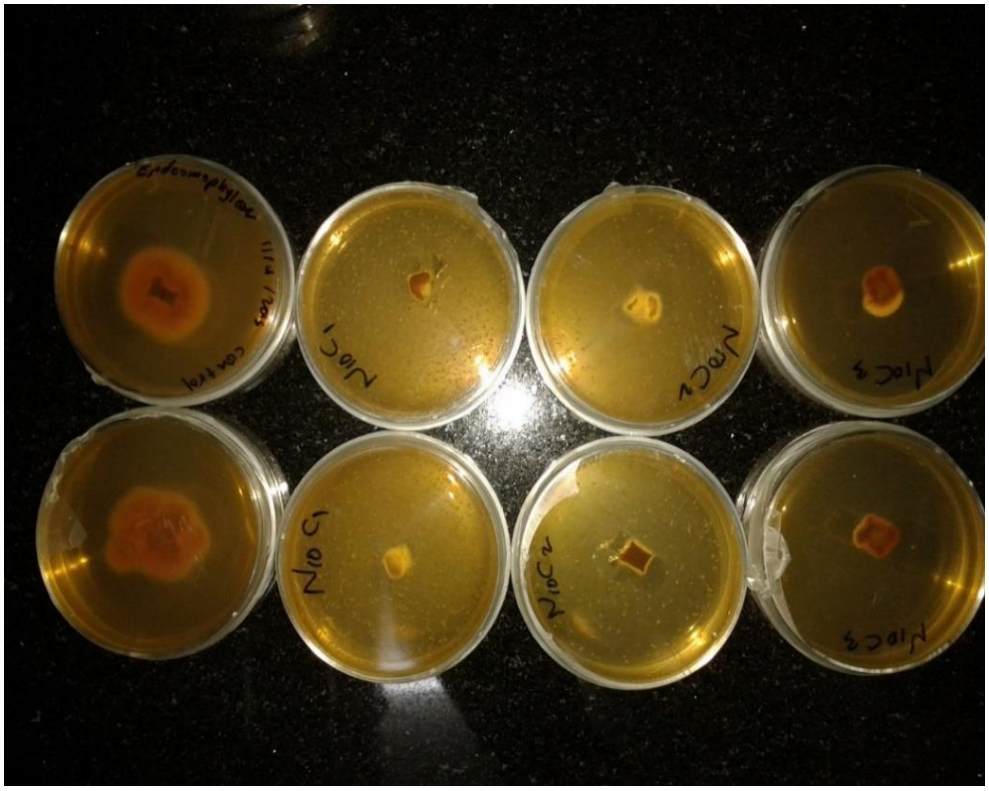


Figure 3: Anti-fungal Testing of Compound Number 7 against *M. canis*

Table 6.1 Anti-fungal activity of Schiff bases compounds against *T. mentagrophyt* at three concentrations

			T. mentagrophyt		
Nx	**no.**	**Compound**	**0.6** µg/ml	**1.2** µg/ml	**2.4** µg/ml
N1	1	N'-(di(pyridin-2-yl)methylene)-2-hydroxybenzoylhydrazide	65	100	100
N2	2	2-hydroxy-N-(pyridine-ylmethylidene)benzohydrazide	26.2	67.2	78.5
N3	3	(Z)-N'-(furan-2-ylmethylene)nicotinoylhydrazide	44.3	51.2	84.3
N4	4	N'-((5-bromothiophen-2yl)methylene)nicotinoylhydrazide	47.7	56.8	68.9
N5	5	N'-(thiophen-2-ylmethylene)nicotinoylhydrazide	63.2	75	84.9
N6	6	(Z)-N'-(pyridin-2-ylmethylene)nicotinoylhydrazide	42.4	54.1	84.9
N7	7	N'-((1H-pyrrol-2-yl)methylene)nicotinoylhydrazide	33.3	42.4	66.9

Table 6.2 Anti-fungal activity of Schiff bases against *T. rubrum* at three concentrations

			T. rubrum		
Nx	**No.**	**Compound**	**0.6** µg/ml	**1.6** µg/ml	**2.4** µg/ml
N1	1	N'-(di(pyridin-2-yl)methylene)-2-hydroxybenzoylhydrazide	79.4	100	100
N2	2	2-hydroxy-N-(pyridine-ylmethylene)benzohydrazide	51.2	67.2	83.1
N3	3	(Z)-N'-(furan-2-ylmethylene)nicotinoylhydrazide	66.9	78.5	100
N4	4	N'-((5-bromothiophen-2yl)methylene)nicotinoylhydrazide	63.2	70.6	88
N5	5	N'-(thiophen-2-ylmethylene)nicotinoylhydrazide	84.9	100	100
N6	6	(Z)-N'-(pyridin-2-ylmethylene)nicotinoylhydrazide	88	100	100
N7	7	N'-((1H-pyrrol-2-yl)methylene)nicotinoylhydrazide	63.2	75	89.9

Table 6.3 Anti-fungal activity of Schiff bases against *M. canis at three concentration*

			M. canis		
Nx	**no.**	**Compound**	**0.6**µg/ml	**1.2** µg/ml	**2.4** µg/ml
N1	1	N'-(di(pyridin-2-yl)methylene)-2-hydroxybenzoylhydrazide	44.3	88	88
N2	2	2-hydroxy-N-(pyridine-ylmethylidene)benzohydrazide	19.1	56.8	79.4
N3	3	(Z)-N'-(furan-2-ylmethylene)nicotinoylhydrazide	33.3	83.1	83.1
N4	4	N'-((5-bromothiophen-2yl)methylene)nicotinoylhydrazide	45.4	75	84.1
N5	5	N'-(thiophen-2-ylmethylene)nicotinoylhydrazide	31.1	67.2	88
N6	6	(Z)-N'-(pyridin-2-ylmethylene)nicotinoylhydrazide	51.2	67	83
N7	7	N'-((1H-pyrrol-2-yl)methylene)nicotinoylhydrazide	19.1	66.9	83.1

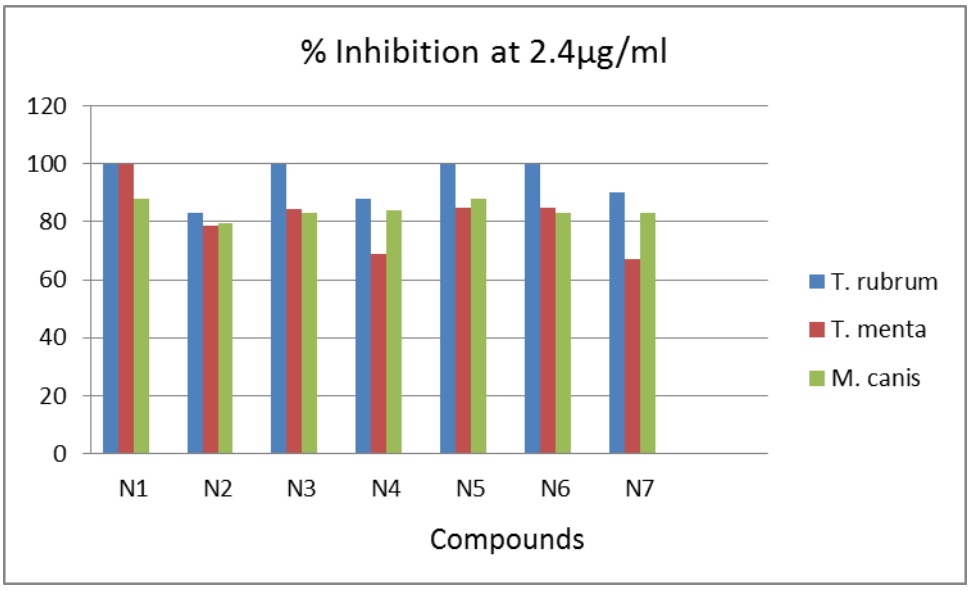


Figure 6.4 : % Inhibition of Schiff bases compounds against three fungus at 2.4 µg/m

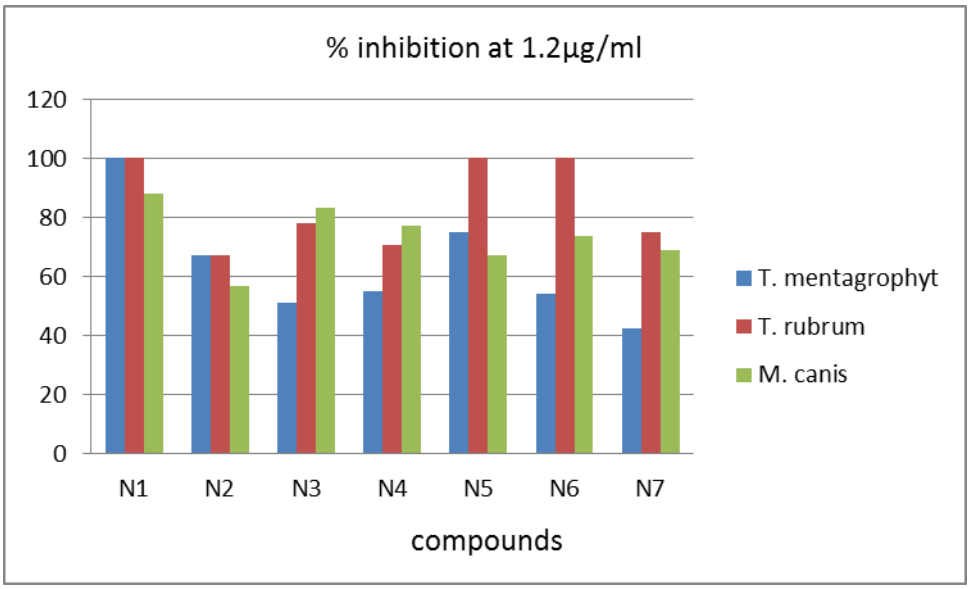


Figure 6.5: % Inhibition of Schiff bases compounds against three fungus at 1.2µg/ml

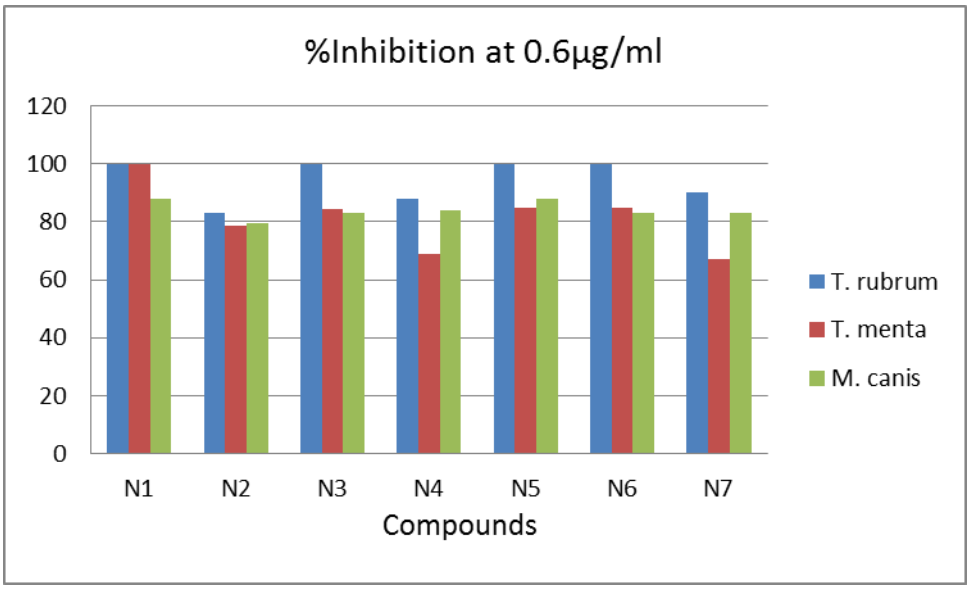


Figure 6.6 : % Inhibition of Schiff bases compounds against three fungus at 0.6µg/ml

6.2 Antioxidant Test

The compounds did not show any antioxidant activity at the concentration (10-50 µg/ml) compared with Gallic acid which have (IC_{50}= 1.6 µg/ml)

Fig. 6.7

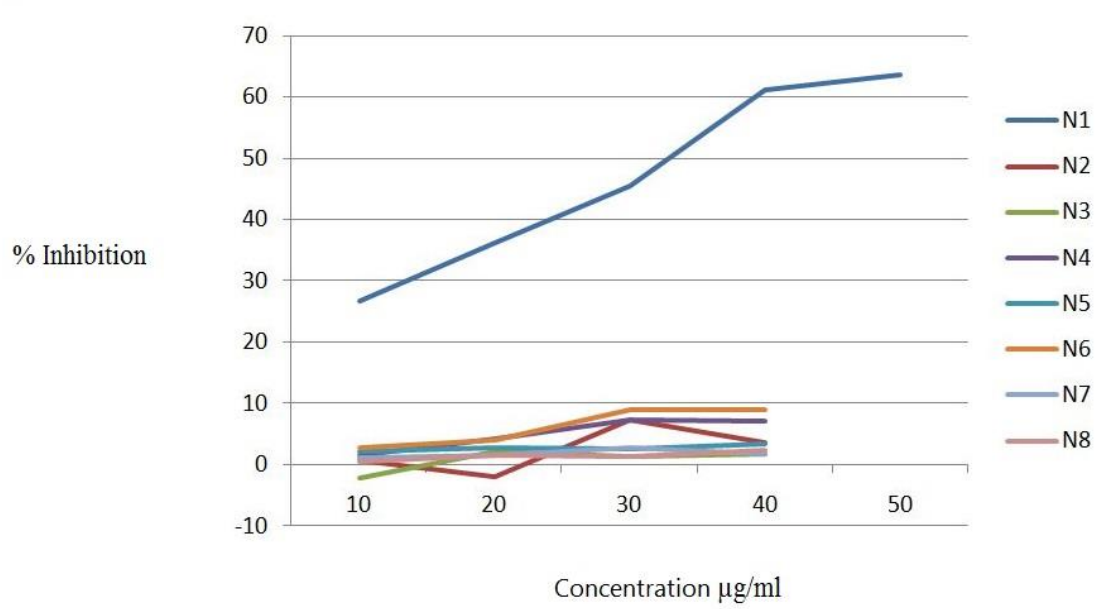


Figure 6.7 : % inhibition of Gallic acid Compared with Schiff Bases Compounds versus ConcentrationWhere :

N1 = Gallic acid

N2 = N'-(di(pyridin-2-yl)methylidene)-2-hydroxybenzoylhydrazide

N3= 2-hydroxy-N-(pyridine-2-ylmethylene)benzohydrazid

N4 = (Z)-N'-(furan-2-ylmethylene)nicotinoylhydrazide

N5 = N'-((5-bromothiophen-2-yl)methylene)nicotinoylhydrazide

N6 = N'-(thiophen-2-ylmethylene)nicotinoylhydrazide

N7 = (Z)-N'-(pyridin-2-ylmethylene)nicotinoylhydrazide

N8 = N'-((1H-pyrrol-2-yl)methylene)nicotinoylhydrazide

6.3 Antibacterial Test

The Schiff bases compounds were tested against five type of bacteria that cause dermic and mucosal infections . Results were negative and there was no activity against any of the tested types of bacteria at the concentration (5mg/ml) when compared with gentamicin **Fig 6.8, 6.9**

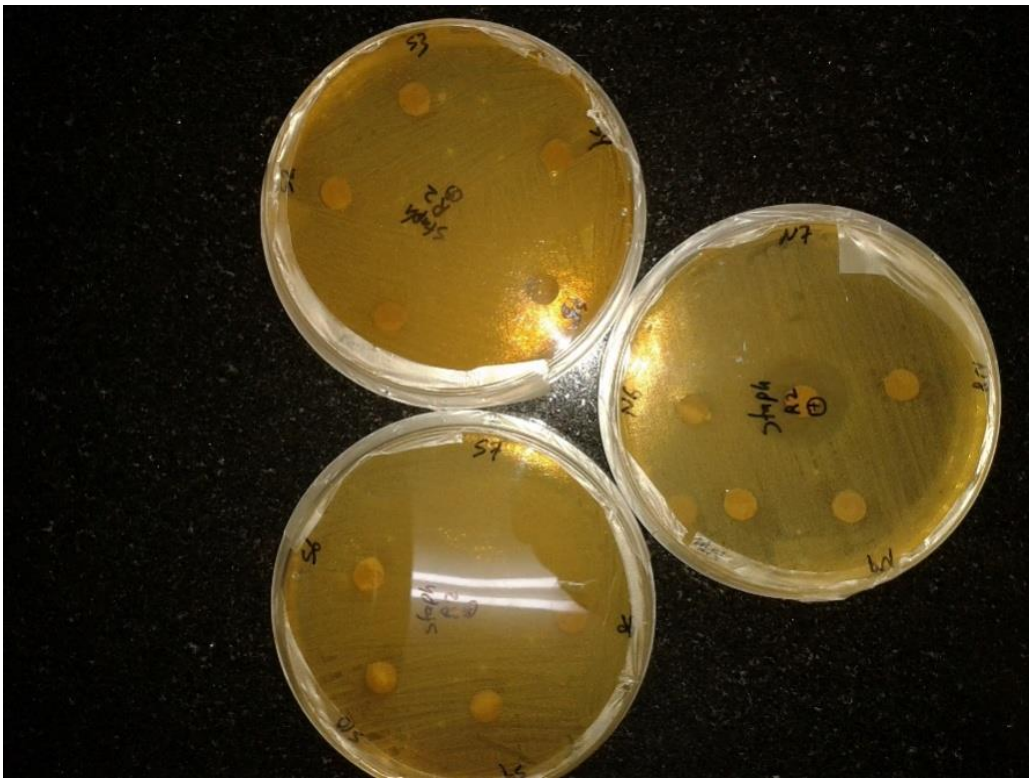


Figure 6.8 : Anti-bacterial Testing of Schiff Base N1, N2, N3 against *Staphylococcus aureus*

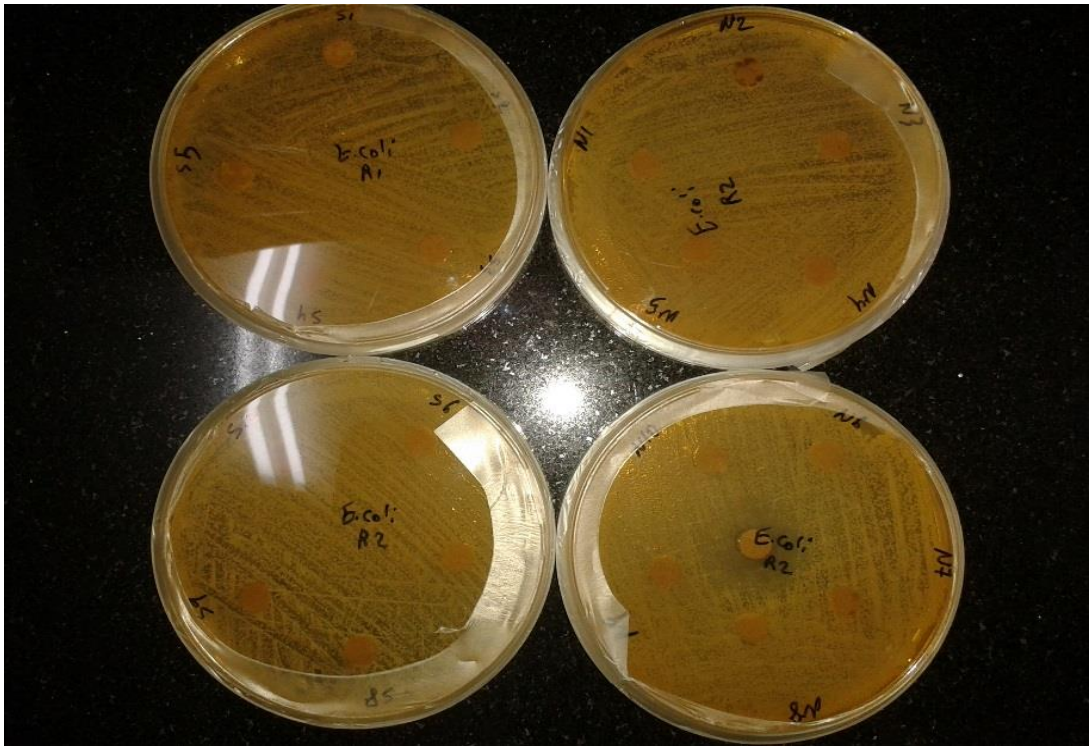


Figure 6.9: Anti-bacterial Testing of Schiff Base N4, N5, N6, N7 against *Escherichia coli*

Chapter Seven

Conclusion

Antifungal test :

***** N'-(dipyridin-2-yl)methylene)-2-hydroxybenzoylhydrazide showed complete inhibition against *T. mentagrophytes* at 1.2 µg/ml and at 2.4 µg/ml.

* (Z)-N'-(pyridin-2-ylmethylene)nicotinoylhydrazide, N'-(thiophen-2-ylmethylene)nicotinoylhydrazide and N'-(di(pyridin-2-yl)methylene)-2-hydroxybenzoylhydrazide shows complete inhibition at 1.2 and 2.4 µg/ml against *T. rubrum*.

* In general the seven compounds showed different inhibition at 0.6, 1.2 .2.4 µg/ml against *M. cains, T. rubrum* and *T. mentagrophet.*

Antioxidant test :

For our synthesized Schiff bases compounds there is no antioxidant effects comparing with Gallic acid, because our compounds are stable and unreactive to radicals such as $^{\cdot}OH$.

Antibacterial Test :

The Schiff bases compounds were tested, the results were negative and there was no activity against any of the tested types of bacteria when compared with gentamicin

References

[1] Hussien Z., Yousef E., Ahmed A., and Altaie A., **Org. Med. Chem. Lett**, 2014 , **4**, 1-4.

[2] Singh P., Goel R. L., Singh B. P. Si., **Journal of the Indian Chemical Society**, 1975, **52**, 958–959.

[3] Perry B. F., Beezer A. E., Miles R. J., Smith B. W., **Miller J., and Nascimento M. G.**, Microbios, 1988, **45**, 181–191.

[4] Elmali A., Kabak M and Elerman Y., **Journal of Molecular Structure**, 2000, **477**, 151–158.

[5] Ibrahim M. N., Sharif S. A., El-Tajory A. N., **Elamari A.A., Journal of Chemistry**, 2011, **8**, 212-216.

[6] Uematsu N., Fujii A., Hashiguchi S., Ikariya T., & Noyori R. ***Journal of the American Chemical Society***, 1996. ***118***, 4916-4917.

[7] Spichiger-Keller U. E., **John Wiley and Sons (*Ed.*)**, 2008, 259-319.

[8] Perry B. F., Beezer A. E., Miles R. J., Smith B. W., Miller J. and Nascimento M. G., **Microbois**, 1988, **45**, 179-181.

[9] Elmali A., Kabak M and Elerman Y., **J. Mol. Struct**, 2000, **477**, 145.

[10] Patel P. R., Thaker B. T., and Zele S., **Indian J. Chem**., 1999, **38**, 563.

[11] Metzler C. M., Cahill A., Metzler D. E., **J. Am. Chem. Soc**.,1980, **102**, 75- 82.

[12] Nalawade R., Nalawade A., Rajmane S., Shejwala R., **International Journal of Pharmaceutical Science Invention**,2015,**4**,01-04.

[13] Dudek G. O., **The Journal of Organic Chemistry**, 1965, **30**, 548-552.

[14] Karia F.D., Parsania P.H. , **Asian J. Chem**.,1999, **11**, 991.

[15] More P.G., Bhalvankar R.B., Pattar S.C., **J. Indian Chem. Soc**.,2001,**78**, 474.

[16] El-masry A.H. , Fahmy H.H., Abdelwahed S.H., **Molecules** ,2000, 5, 1429.

[17] Baseer M.A., Jadhav V.D. , Phule R.M., Archana Y.V., Vibhute Y.B., **Orient. J. Chem**. , 2000, **16**, 553-556.

[18] Pandeya S.N., Sriram D., Nath G., Clercq E. De**, IL Farmaco**,1999, **54**, 624-628.

[19] Singh W.M., Dash B.C., **Pesticides**,1988,**22**, 33-37.

[20] Hodnett E.M., Dunn W.J., **J. Med. Chem**.,1970,**13**, 768.

[21] Desai S.B, Desai P.B., Desai K.R., **Hetrocycl.Commun**, 2001, **7** ,83.

[22] Pathak P., Jolly V.S., Sharma K.P., **Orient. J. Chem**.,2000, 161-162.

[23] Samadhiya S., Halve A., **Orient. J. Chem**.,2001,**17**, 119.

[24] Aydogan F., Öcal N., Turgut Z., Yolacan C., **Bull.Korean Chem. Soc**.,2001, **22**, 476

[25] Bindu P., Kurup M.R.P., Satyakeerty T.R.E., **Polyhedron** ,1999, **18**, 321.

[26] Mahindra A.M., and Fisher J.M., Rabinovitz., **Nature (London),** 1983, **303**, 64.

[27] Pandeya S.N., Yogecswari P., Sriram D., **Chemotherap**y, 1999, **45**,192.

[28]Ibrahim N., SHARIF A., **Journal of Chemistry**, 2011, **8**, 180-184.

[29] Metzler C M, Cahill A and Metzler D E, **J. Am. Chem. Soc**., 1980, **102**, 6075-6082.

[30] Dudek G. O., Dudek E. P., **Chem. Commun**, 1965. 464.

[31] Dudek G. O., Dudek E. P., **J. Am. Chem. Soc**., 1966, **88**, 2407.

[32] Cimerman Z. and Stefanac Z., **Polyhedron**, 1985, **4**, 1755-1760.

[33] Desai K.R., Patel R.B., Desai P.S., And Chikhali K.H., **J Indian Chem Soc**.,2003,**80**, 138.

[34] Lanalia N.A and Thaker K.A, J. **Indian Chem Soc**., 1981,**59**, 1099.

[35] Solanki A and Patel J., **Indian J Chem**., 2004,**43b**, 1580.

[36] Husein A.,Ali-Shtayeh M.,Jamous R.,Abu Zaitoun S.,Jondi W.,Zatar N.,**African journal of microbiology research** ,2014, **8**, 3501-3507.

[37] Husein, A., Ali-Shtayeh, M., Jondi, W., Zatar, N., Abu-Reidah, I., & Jamous, R., ***Pharmaceutical biology***, 2014, ***52***, 1249-1255.

[38] Bhat R. M., Vidya K and Kamath G., **International Journal of Dermatology**,2001, **40**, 415-419.

[39] Bhalodia N.R and Shukla V.J.,**Journal of advanced pharmaceutical technology & research,**2011**, 2**, 104-109.

[40] Blair H. A., Dhillon S.., **American Journal of Cardiovascular Drugs**, 2014, **14**, 393-400.

[41] Hussain S., Abdul-Rahim S. and Farooqui M**., World Journal of Pharmacy and Pharmaceutical sciences**, 2014, **3**, 632-635.

[42] Noble S.L., and Forbes R.C., **American Family Physician**, 1998, **58**, 163-174.

[43] Diplock A.T., Charleux J.L., Crozier-Willi G., Kok F.J., Rice-Evans C., Roberfroid M., Stahl W and Vina-Ribes J., **British Journal of Nutrition**, 1998, **80**, S77-S112.

[44] Vaidya A.D.B. and Devasagayam T.P.A., **Journal of Clinical Biochemistry and Nutrition**, 2007, **41**, 1-11.

[45] Davies K.J.A. and Pryor W.A., **Free Radical Biology and Medicine**, 2005, **39**, 1263-1264.

[46] Husein, A., Al-Nuri M., Zatar N., Jondi W., Ali-Shtayeh M. , Warad I.,**IJRRAS**, 2012, 3,655-660 .

[47] Cheng L., Tang J., Luo H., Ji X., Dai F., Yang J.,& Zho,B. ***Bioorganic & medicinal chemistry letters***,2010, ***20***, 2417-2420.

[48] Bouayed J. and Bohn T.,**Oxidative Medicine and Cellular Longevity**, 2010, **3**, 228-237.

[49] Valko M., Leibfritz D., Moncol J., Cronin M.T.D., Mazur M., and Telser J., **International Journal of Biochemistry & Cell Biology**, 2007, 39, 44-84

[50] Ames B.N., **Scienc**e, 1983, **221**, 1256-1264.

[51] Demmig-Adams B., and Adams W.W., **Science**, 2002, **298**, 2149-2153.

جامعة النجاح الوطنية

كلية الدراسات العليا

تحضير وتحليل وقياس النشاط ضد البكتيريا والفطريات لمركبات قواعد شيف المبتكرة

اعداد

الاء عبد الرحيم جانم

اشراف

أ.د محمد النوري

أ.د اسماعيل وراد

قدمت هذه الاطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير بالكيمياء، بكلية الدراسات العليا ، جامعة النجاح الوطنية، نابلس – فلسطين

2016

تحضير وتحليل وقياس النشاط ضد البكتيريا والفطريات لمركبات قواعد شيف المبتكرة

اعداد

الاء عبد الرحيم جانم

اشراف

أ.د محمد النوري

أ.د اسماعيل وراد

الملخص

العمل الموصوف في هذه الأطروحة يتعلق بتحضير سبعة قواعد الشيف و قد تم تحضير هذه القواعد بتفاعل التكثيف بين الأمينات الأولية مع مركبات الكاربونيل في الايثانول النقي في ظروف خاصة. ونشير الى ان جميع المركبات درست خصائصها باستخدام القياسات الفزيائية التالية:

FT-IR, UV-visible, ^{1}H-NMR, ^{13}C-NMR.

و أيضا تم دارسة الأثر البيولوجي لقواعد الشيف ضد أنواع مختلفة من الفطريات

Trichophyton rubrum (CBS 392.58), *Trichophyton mentagophytes*

(CBS106.67)and *Microsporum canis* (CBS 132.88)

وكذلك ضد أنواع مختلفة من البكتيريا

Staphylococcus aureus (ATCC 25923), *Klebsiella*

pneumonia (ATCC 13883), *Proteus vulgaris* (ATCC 13315),

and *Pseudomonas aeruginosa* (ATCC 27853).