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Synthesis of some Pyrazolone and Pyrazole Derived from Adamantyl Chalcones Derivatives

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Dedication

Life is a dream and dream with persistence has to become a reality

To my beloved parents, who raised me to believe that anything is possible,

to my dearest husband, who encouraged me to go on every adventure, especially this one,

to my adorable daughter and sons, whose love gave me the strength to achieve my goal,

to my amazing brother and sisters, who supported me until the completion of this research,

and to my relatives, teachers, supervisors and friends at Hebron University.

I will always appreciate all they have done.

I dedicate this work.

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List of Abbreviations

Abbreviations	Definition
A	1-adamantyl-3-pyridyl-prop-2-en-1-one
RS-1	2-(3-((1s,3s)-adamantan-1-yl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine
RS-2	2-(3-((1s,3s)-adamantan-1-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine
RS-3	2-(3-((1s,3s)-adamantan-1-yl)-1-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine
RS-4	2-(3-((1s,3s)-adamantan-1-yl)-1-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine
RS-5	2-(3-((1s,3s)-adamantan-1-yl)-1-(o-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine
B1	2,4-dinitrophenylhydrazine
B2	Phenyl Hydrazine Hydrochloride
B3	2-chlorophenyl hydrazine hydrochloride
B4	4-methoxyphenyl hydrazine hydrochloride
B5	o-tolyl hydrazine hydrochloride
^{1}HNMR	Proton nuclear magnetic resonance
^{13}CNMR	Carbon-13 nuclear magnetic resonance
FT-IR	Fourier Transform Infrared
TLC	Thin Layer Chromatography
HOAc	Acetic acid
t-BuOK	Potassium tert-butoxide
t-BuOH	Tert-butyl alcohol
MCF-7	Breast adenocarcinoma cell line
CRD	Complete Randomize Design
MIC	Minimum Inhibitory Concentration
MGR	Mycelia Growth Rate
IZD	Inhibition Zone Diameters
$\boldsymbol{R_f}$	Retention factor
$CDCl_3$	Deuterated chloroform

DMSO-d6	Deuterated DMSO, dimethyl sulfoxide-d6
MHz	Megahertz
TMS	Tetra methyl silane
PTA	Phosphotungstic acid
Ppm	Parts per million
µg/ml	Micrograms/mil litter
cm^2/day	Square centimetres/day
^oC	Celsius unit of temperature
K	Kelvin unit of temperature
H	Hour
µl	Microliter
δ	Chemical shifts

Abstract

The chemistry of chalcone has generated intensive scientific interest due to their biological activities. Chalcones, precursors of open chain flavonoids present in edible plants, have displayed a broad spectrum of pharmacological activities. Changes in their structure have offered a high degree of diversity that has proven useful for the development of new medicinal agents with improved potency and lesser toxicity. Chalcones are used as intermediates in the synthesis of heterocyclic compounds. These compounds are widely distributed in nature and essential to life due to their wide variety of physiological and pharmacological activities. The chemistry of 5-membered heterocycles that contain more than one heteroatom such as pyrazolines and pyrazole derivatives have gained significance because of their pronounced bioactive nature. Pyrazolines derivatives display tremendous biological activities such as antimicrobial, anti-inflammatory, antitumor and antioxidant. In this study five adamantylated heterocyclic compounds (***RS 1-5)*** were synthesized by condensation of 1-adamantyl chalcone with substituted phenyl hydrazine leading to the formation of new pyrazoline ***(RS 1-5)*** compounds in good yields. All these compound were characterized by ^{1}HNMR, ^{13}CNMR and FT-IR spectroscopy. The microbiological activity of these new compounds was investigated against bacteria and fungi. The new compounds showed good to moderate activity against all microbial species used for screening.

Chapter one

Introduction

1.1 Overview

The pharmaceutical chemistry or the medicinal chemistry is at the intersection of chemistry and pharmacology involving the designing, synthesizing, identifying and developing of new chemical entities suitable for therapeutic use. In the early stages of the medicinal chemistry scientists isolated medicinal agents found in plants. Now, scientists in this field are creating new synthetic compounds as drugs (Foye, 2008).

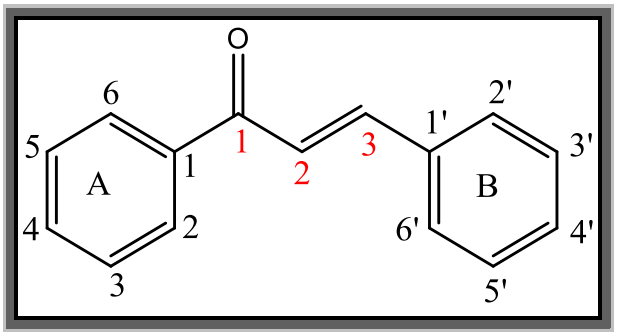
Pharmaceutical chemistry is almost always geared toward drug discovery and development (Beale, Block & Hill, 2010). Heterocyclic compounds are the most complex branches of organic chemistry that have been studied extensively and attracted attention of medicinal and pharmacological activities (Beale et al., 2010) (Wiley & Behr, 1967).

The chemistry of heterocyclic compounds is interesting because many natural products and drugs belong to this group. Heterocyclic compounds are cyclic organic substances which contain in the ring system at least one atom other than carbon. Many alkaloids, vitamins, antibiotics, synthetic medicines and dyestuffs are heterocyclic derivatives (Wiley & Behr, 1967).

α,β-Unsaturated ketone-chalcone is an important class of naturally occurring flavonoid compounds, Flavonoids and chalcones are natural antioxidants present in plants and preventing oxidative damages of the cell (Roy, Amdekar, Kumar & Singh, 2011). They exhibit a wide spectrum of biological activities such as anticancer, anti-inflammatory and antimicrobial activities. Chalcones are intermediates for the synthesis of a large number of bioactive molecules, such as pyrazolines and pyrazole derivatives (Jayaroopa, 2015).

1.2 Chemistry of Chalcone

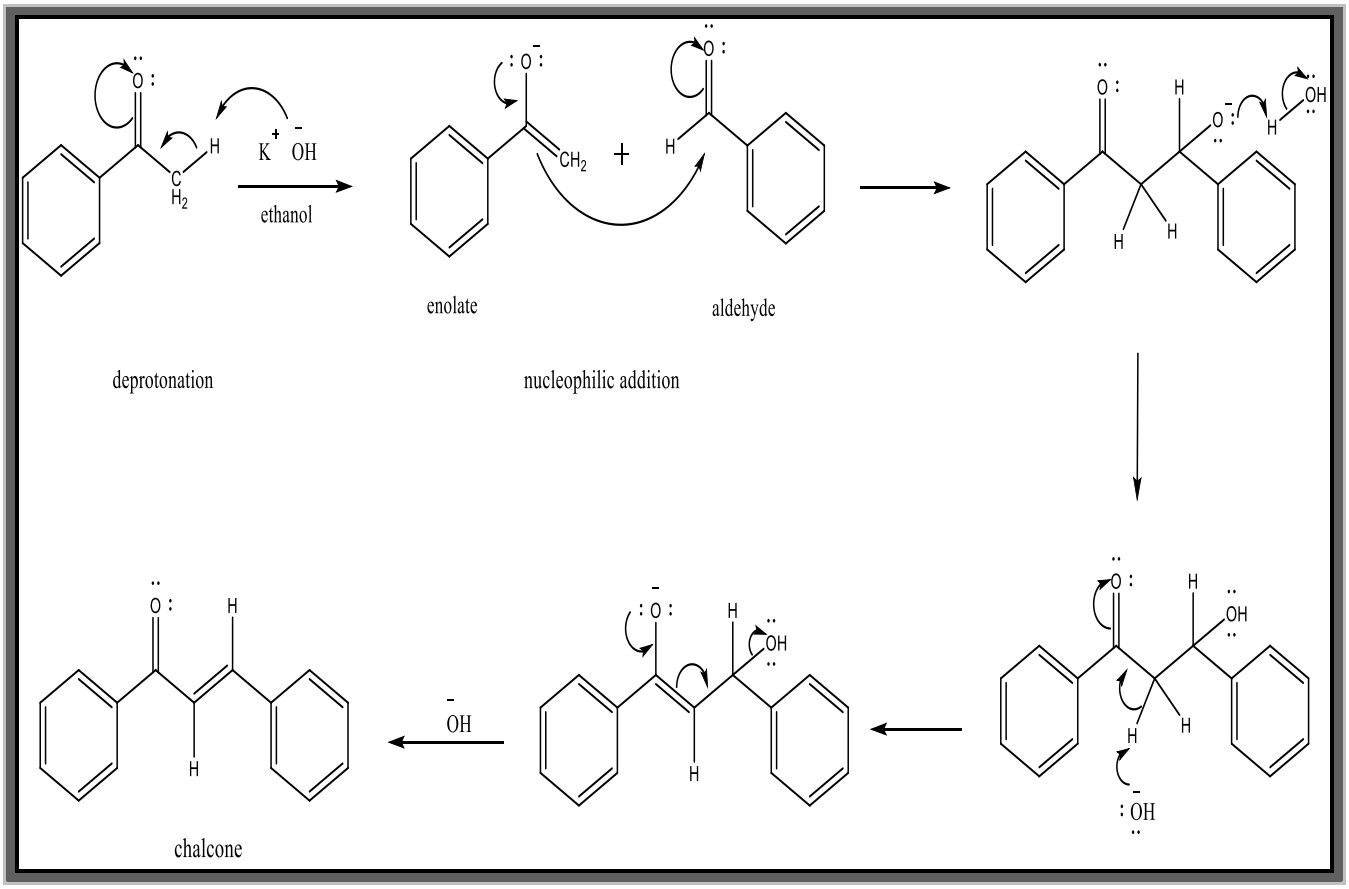
Chalcones (E)-1,3-diphenyl-2-propen-1-one ***(scheme 1.1)*** derivatives are open-chain flavonoids found in fruits and vegetables (Aksoz & Ertan, 2011), or prepared by condensation of phenyl methyl ketone with phenyl aldehyde in presence of suitable condensing agent (Elarfi & Al-Difar, 2012; Mishra et al., 2008). They undergo a chemical reaction that leads to preparation of heterocyclic compounds (Kalirajan, Sivakumar, Jubie, Gowramma & Suresh, 2009; Mandge, Singh, Gupta & Moorthy, 2007). These compounds have pharmacological activities such as anti-cancer, anti-inflammatory (Nowakowska, 2007), antibacterial, anti-fungal (Siddiqui, Praveen, Musthafa, Ahmad & Khan, 2012). Recently, a lot of research has been conducted about the chalcone due to its ease of synthesis and promising biological activities (Mahapatra, Bharti & Asati, 2015).



Scheme 1. 1: The general structure of chalcone (E)-1,3-diphenyl-2-propen-1-one.

1.2.1 Mechanism for Chalcone Synthesis

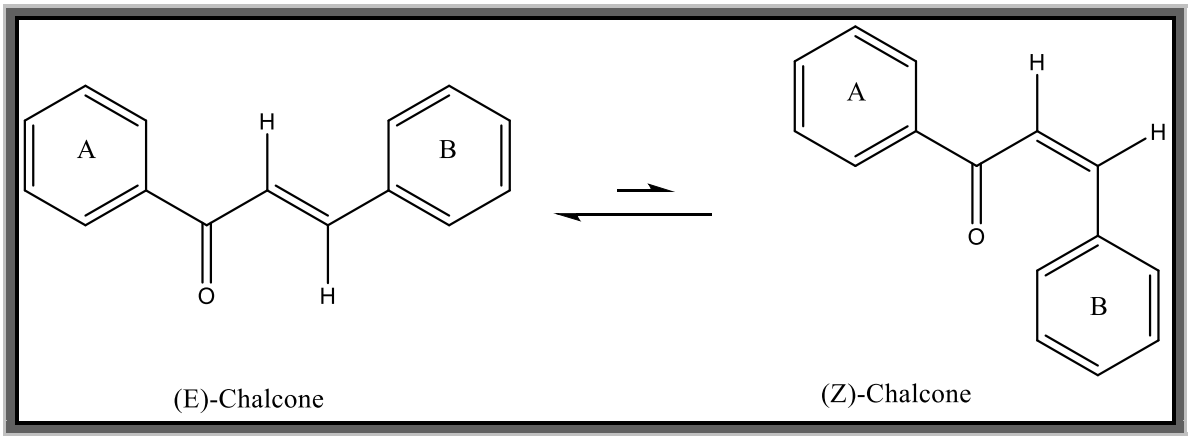
Many methods were used to synthesize the Chalcones. One of the most important methods is the use of the Claisen-Schmidt condensation of an aldehyde and a ketone by acid or base catalysis followed by dehydration to yield chalcones ***(scheme 1.2)***.



Scheme 1. 2: Synthesis of chalcone by Claisen-Schmidt condensation under basic condition.

1.2.2 Isomer of Chalcone

The chalcones are flexible molecules that have conformational properties. They exist as either (E) or (Z) configuration isomer ***(scheme 1.3)***. E−Z isomerism is a type of stereoisomerism that exists because of restricted rotation about double bonds depending on the position of the hydrogen atoms on the active α,β-unsaturated moiety. The most thermodynamically stable form is the (E) isomer, therefore, the majority of the chalcone isomer isolated is in this form. Conversely, the (Z) isomer is unstable due to the steric effect between the carbonyl group and the B-ring. The Recrystallization of a mixture E-Z chalcone yields only E-isomer (Albuquerque, Santos, Cavaleiro & Silva, 2014).



Scheme 1. 3: Configuration isomer of E and Z form of chalcone.

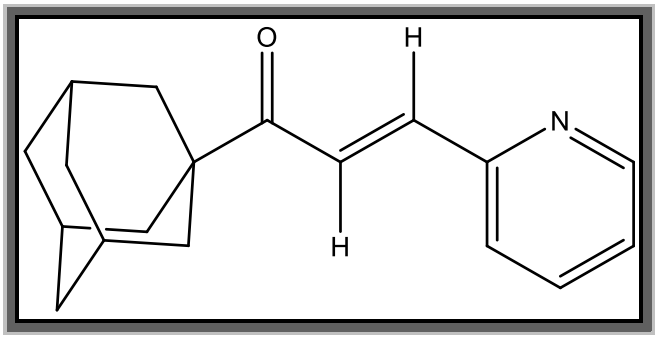
1.2.3 Pharmacological Activity of Chalcone

Chalcones have attracted attention because of their biological and pharmacological activities. Chalcones possess antibacterial (Siddiqui et al., 2012), antifungal (Siddiqui et al., 2012), antimicrobial (Nowakowska, Kędzia & Schroeder, 2008), anticancer (Bonesi et al., 2010), antioxidant (Bonesi et al., 2010) and anti-inflammatory (Nowakowska, 2007) properties.

1.2.4 Development of Chalcones

The discovery of chalcone compounds provides an outstanding case history of modern drug development and also emphasizes the unpredictability of biological activity from structural modification of a prototype drug molecule. Considerable interest has been focused on the chalcone structure, which is known to possess a broad spectrum of biological activities, such as antibacterial, anti-inflammatory, anticancer and anti-fungal, so the synthesis of chalcone derivatives is always a great challenge. *Anderson & Kaimari* ***(scheme 1.4)*** modulated the general chalcone structure ***(scheme 1.1)*** by replacement of the aryl ring A with a hydrocarbon moiety (adamantyl group) and the aryl ring B with hetero aryl moieties (pyridine) (Anderson & Kaimari, 2013).

1-Adamantyl chalcones have the general formula as shown in ***(scheme 1.4)***, in which adamantly and pyridine group are linked by a three carbon α,β-unsaturated carbonyl system.



Scheme 1. 4: The general structure of Adamantyl chalcone (E)-1-((3r,5r,7r)-adamantan-1-yl)-3-(pyridin-3-yl)prop-2-en-1-one.

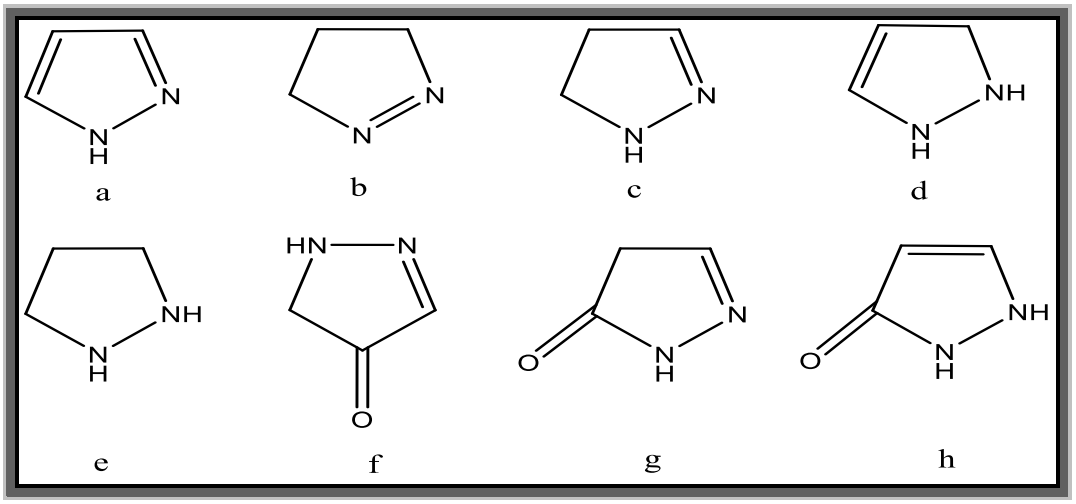
The structure has lipophilicity and hydrophobicity characters, adamantyl group ensures favourable condition for its transport through a biological membrane (Anderson & Kaimari, 2005, 2013) and the presence of pyridine and α,β-unsaturated groups improves their biological activities (Aramburu et al., 2016).

Adamantyl chalcone was prepared by condensation of 1-adamantyl methyl ketone with pyridine-2-carboxaldehydes in the presence of suitable condensing agent KOH (Anderson & Kaimari, 2013). Then they undergo a variety of chemical reactions leading to many heterocyclic compounds. In this research, pyrazolone and pyrazole derivatives of adamantyl chalcone compounds have been prepared, where in the chalcone moiety is an intermediate to new compounds with therapeutic value. Many reviews reveal that chalcone derivatives exhibit diverse pharmacological activities, such as anti-cancer, anti-inflammatory, anti-bacterial and antifungal (Nowakowska, 2007).

1.3 Chemistry of Pyrazoline and Pyrazole Derivative

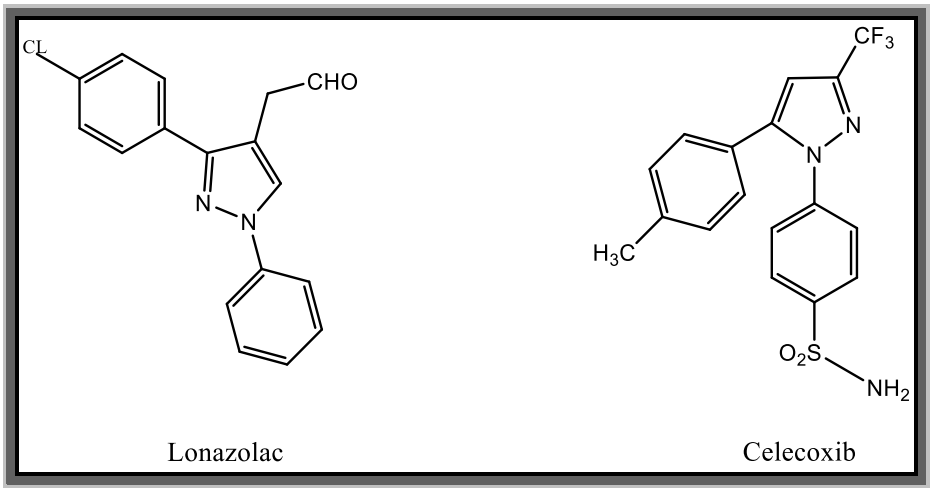
1.3.1 Chemistry of Pyrazole Compound

Pyrazole is an organic heterocyclic compound characterized by a 5-membered ring containing three carbon atoms and two adjacent nitrogen atoms with the formula $C_3H_4N_2$. It is a weak base with pKb 11.5. One of nitrogen atoms is neutral in nature and the other is basic (Eicher, Hauptmann & Speicher, 2013). Due to its planar conjugated ring structure, pyrazole is an aromatic molecule with six delocalized π- electrons. The aromatic nature arises from the unshared pair of electrons on the nitrogen (–NH) and the four π-electrons in the ring (Eicher et al., 2013). The partially reduced forms of pyrazole (***a***) are named as pyrazolines in ***(scheme 1.5 b, c, d)***. All have different positions of the double bonds. Pyrazoline derivatives have one endocyclic double bond. These derivatives play an important role in heterocyclic compounds history and possess considerable biological activities, thus making them an important pharmacophore for carrying out further drug development research. Among the various pyrazoline isomers, 2-pyrazolines appear to be the most frequently investigated compounds. The completely reduced form of pyrazole is pyrazolidine ***(scheme 1.5 e)*** (Jayaroopa, 2015) and the Oxo form of pyrazolines derivatives are pyrazolones ***(scheme 1.5 f, g, h)*** (Wiley & Behr, 1967).



Scheme 1. 5: Pyrazole (a), the reduced forms of pyrazole (b, c, d, e) and the Oxo form of pyrazolines derivatives (f, g, h).

Several pyrazoles are commercially available as pharmaceuticals. The pyrazole ring presents in a variety of drugs such as Lonazolac (is a non-steroidal anti-inflammatory drug) and Celecoxib (the brand name Celebrex) ***(scheme 1.6)***, which is the selective cyclooxygenase-2, (COX 2) inhibitor showing great promise as anti-inflammatory and analgesic agent (Gupton et al., 2002) (use in the treatment of rheumatoid arthritis, acute pain, osteoarthritis, painful menstruation) (Wiley & Behr, 1967). Moreover, Celecoxib has less undesirable side effect than the other known anti-inflammatory agents (Silverstein et al., 2000). Currently, researchers have been attracted toward designing more potent pyrazole derivatives that have wide range of biological activities.



Scheme 1. 6: Lonazolac and Celecoxib drugs.

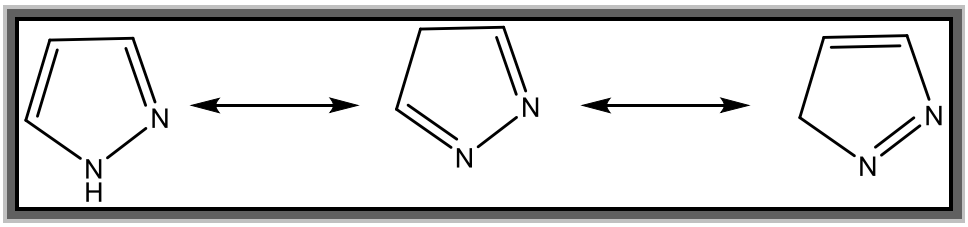
1.3.2 Chemistry of Pyrazoline Compounds

Pyrazoline is the partially reduced forms of pyrazole. It is a five membered heterocyclic having two adjacent nitrogen atoms within the ring with only one endocyclic double bond and is basic in nature. The pyrazoline derivatives are electron rich and play an important role in biological activities. These compounds occur in nature in the form of pigments, vitamins and constituents of plant and animal cell (Khanam, Dar, Siddiqui & Rehman, 2016; Yusuf & Jain, 2014). Pyrazoline and its substituted pyrazolone derivatives show biological activities such as antimicrobial, antitumor and anti-inflammatory properties (Yusuf & Jain, 2014). The versatility of new generation pyrazoline would represent a fruitful pharmacophore for further development of better medicinal agents. The synthesis of novel pyrazoline derivatives remains a main focus of modern drug discovery (Foye, 2008).

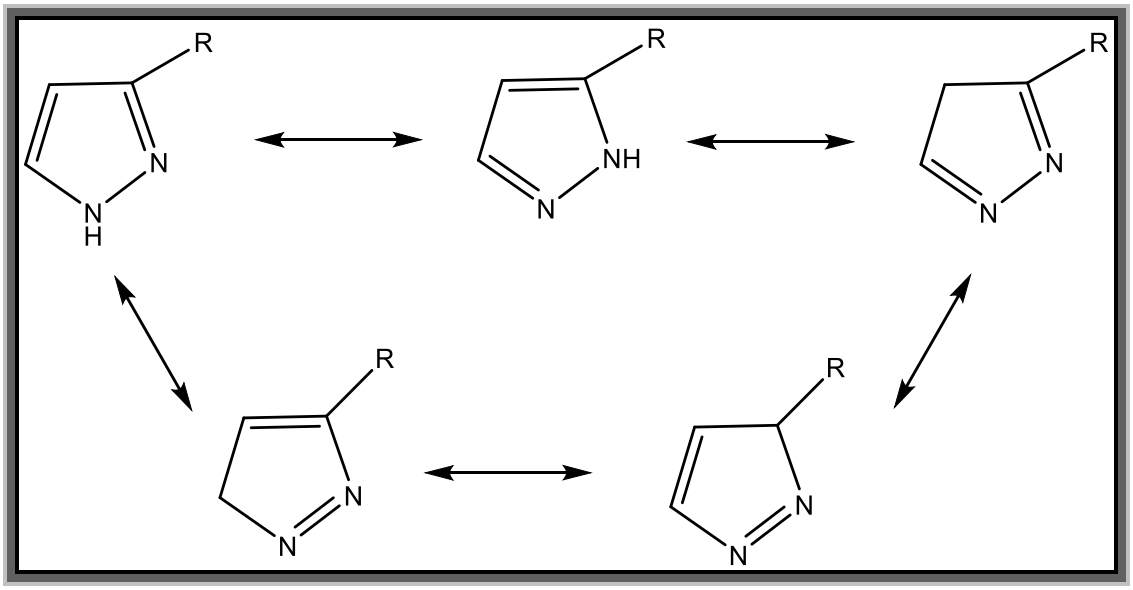
1.3.3 Tautomerism of Pyrazole

Pyrazole and pyrazole derivative are a tautomeric substance, in which unsubstituted pyrazole has three tautomeric forms ***(scheme 1.7)***, five tautomeric structures for the substituted pyrazole derivative ***(scheme 1.8)*** and two tautomeric forms of a disubstituted pyrazole derivative are possible ***(scheme 1.9)***. The existence of tautomerism cannot be demonstrated in pyrazole itself but it can be

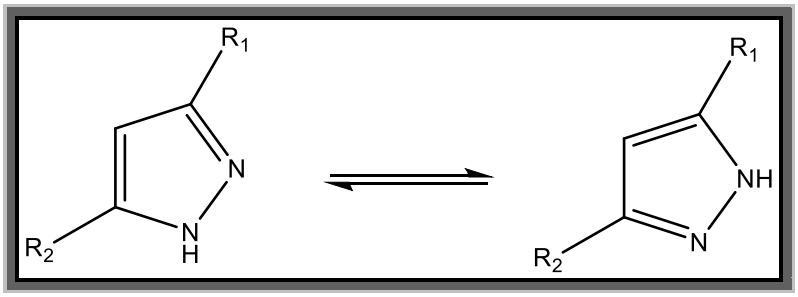
inferred by the consideration of pyrazole derivatives (Kumar & Jayaroopa, 2013a; Yusuf & Jain, 2014).



Scheme 1. 7: Three tautomeric forms of a unsubstituted pyrazole.



Scheme 1. 8: Five tautomeric forms of a substituted pyrazole derivative.

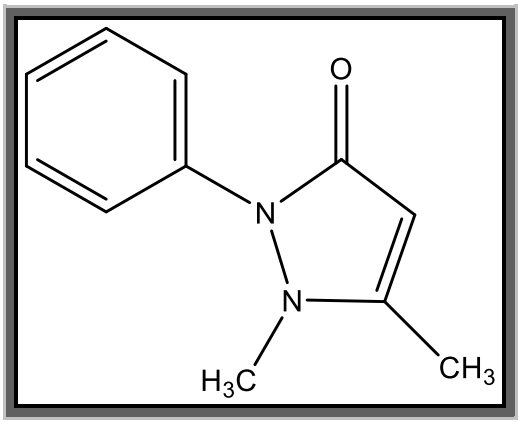


Scheme 1. 9: Two tautomeric forms of a disubstituted pyrazole derivative.

1.3.4 History of Pyrazole

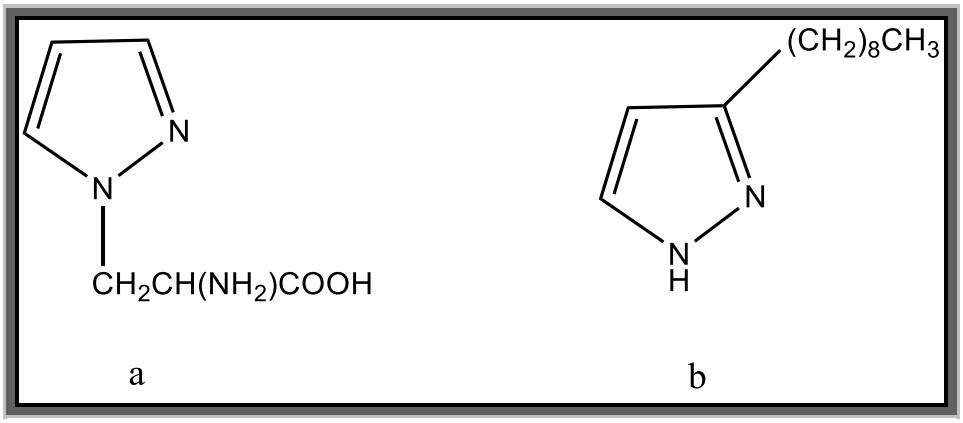
The history of pyrazoline shows that it attracted many chemists to explore pyrazoline as a biologically active molecule. The study of biological evaluation of pyrazoline derivatives has been an interesting field of pharmaceutical chemistry.

Ludwig Knorr, a German chemist was the first to discover the antipyretic action of pyrazole derivative in 1883. The compound named antipyrine. When he attempted to synthesize quinoline derivatives with antipyretic activity, he accidentally obtained antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) ***(scheme 1.10)*** which has analgesic, antipyretic and antirheumatic activity; which stimulated interest in pyrazole chemistry (Kumar & Jayaroopa, 2013a).



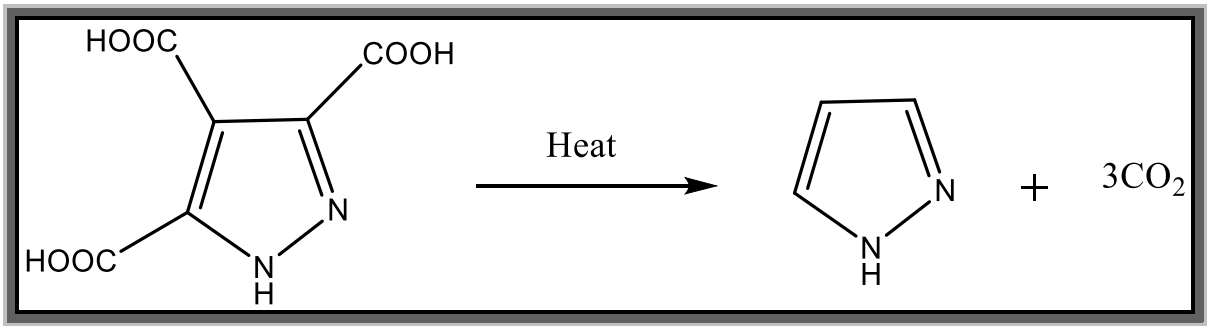
Scheme 1. 10: Antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one).

Kosuge and Okeda (1954) isolated the first natural pyrazole derivative. Until that discovery was made, it was thought that pyrazoles could not be obtained naturally. They isolated levo-β-(1-pyrazolyl) alanine ***(scheme 1.11 a)*** an amino acid from watermelon seeds, they also isolated 3-n-nonylpyrazole ***(scheme 1.11 b)*** from Houttuynia Cordata, a plant of the "piperaceae" family from tropical Asia, which showed antimicrobial activity (Citrullus Vulgaris) (Kumar & Jayaroopa, 2013b; Wiley & Behr, 1967).



Scheme 1. 11: Levo-β-(1-pyrazolyl) alanine (a) and 3-n-nonylpyrazole (b).

Buchner (1889) described pyrazole for the first time by decarboxylation of pyrazole-3,4,5-tricarboxylic acid ***(scheme 1.12)*** (Wiley & Behr, 1967).



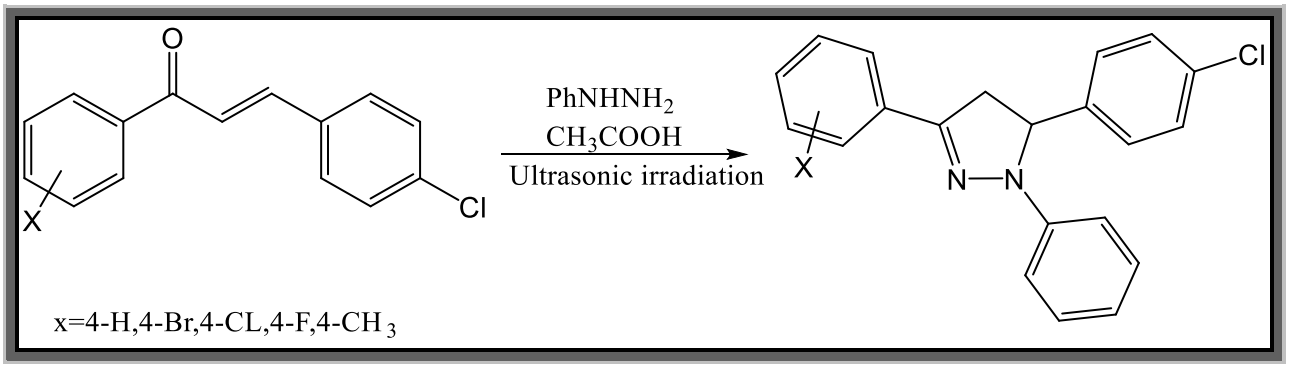
Scheme 1. 12: The first synthesis of pyrazole by the decarboxylation of pyrazole-3,4,5-tricarboxylic acid.

1.3.5 Literature Review for Synthesis Pyrazolines and Pyrazole Derivatives

The biological activities of pyrazole compounds have made them synthetic targets. There are several methods that have been developed for preparation of substituted pyrazole. One of the most important methods is the reaction between α,β-unsaturated chalcone with hydrazine derivatives. Several catalysts have been developed for the preparation of these heterocycles, including sodium acetate /acetic acid aqueous solution under ultrasound irradiation (Li, Zhang & Lin, 2007), hot acetic acid

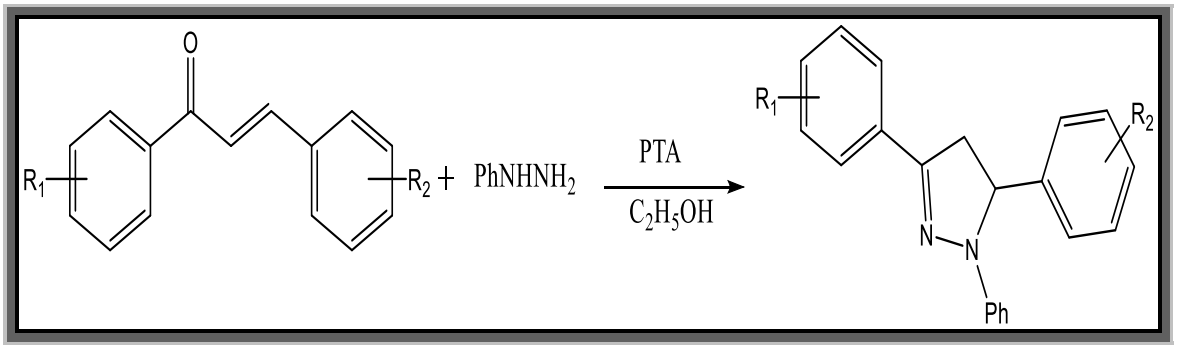
solution (Lévai, 2005; Yakovenko, Oganesyan, Zvolinskii & Zakharov, 1976), KOH/ethanol under refluxed (Yusuf & Jain, 2014), sodium acetate /ethanol under refluxed(Ibrahim, Al-Refai & El-Halawa, 2012), methanoic acid/ ethanol under reflux (Maleki, Moghaddam, Hojati, Gholizadeh & Saehabadi, 2009).

Synthesis of 2-pyrazolines has been performed by the cyclization of chalcones with phenyl hydrazine in glacial acetic acid under ultrasonic irradiation ***(scheme 1.13)***. These resulting compounds have been screened for their antimicrobial activity (Gupta & Jain, 2010). The electron donating group (CH_3) increased the reaction rate as well as the yield, whereas in the halogen series, as the electro negativity of halogen atom increased, the time required for the formation of pyrazoline increased. The main advantage of this method is milder reaction conditions, higher yields and shorter reaction time (Gupta et al., 2010).



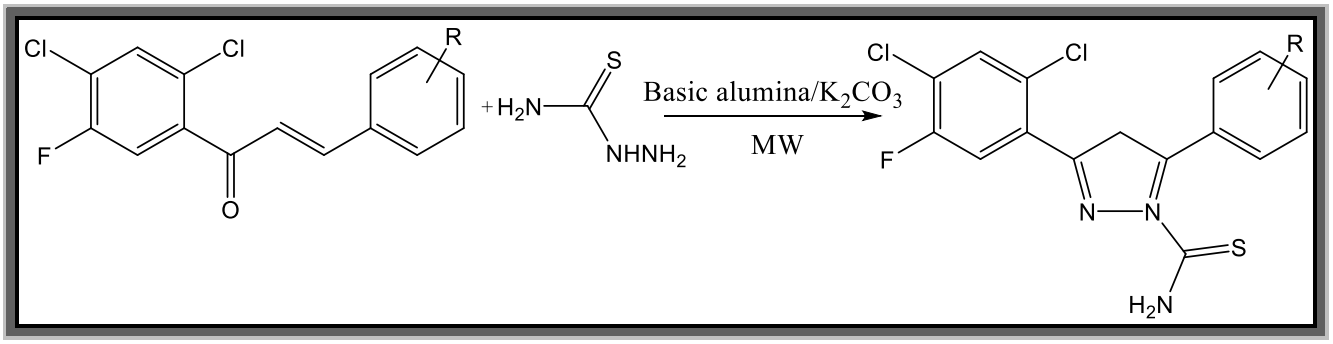
Scheme 1. 13: The synthesis of 2-pyrazolines by the cyclization of chalcones and phenyl hydrazine.

A series of novel 1,3,5-triaryl-2-pyrazoline compounds have been prepared using Phosphotungstic acid (PTA) as an eco-friendly, inexpensive and efficient catalyst. The advantages of this catalytic system are short reaction times, high product yields and non-toxicity of the catalysts ***(scheme 1.14)*** (Fazaeli, Aliyan, Bordbar & Mohammadi, 2010).



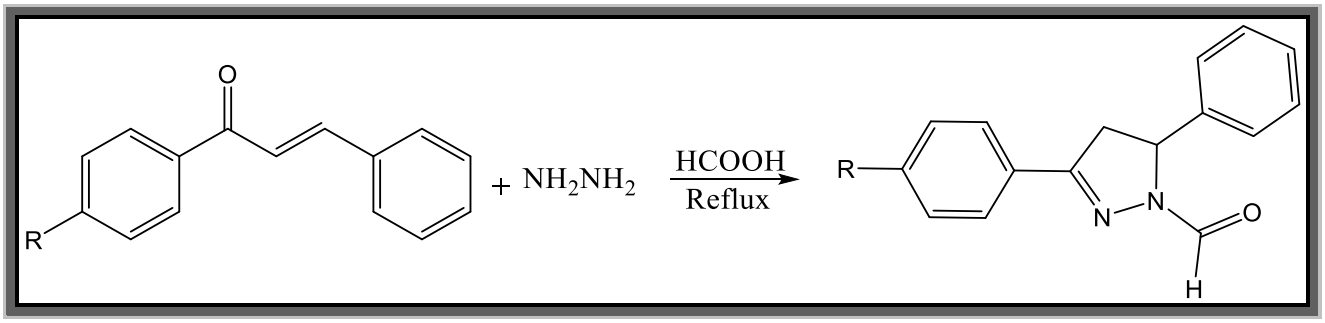
Scheme 1. 14: Synthesis of a series of novel 1,3,5-triaryl-2-pyrazoline using Phosphotungstic acid (PTA) ($H_3PW_{12}O_{40}$).

The synthesis of pyrazolines using microwave technique during a condensation of 2,4-dichloro-5-fluoro chalcones with thiosemicarbazide over potassium carbonate has been reported ***(scheme 1.15)*** (Kidwai, Kukreja & Thakur, 2006) (Neethu & Yusuf, 2014).



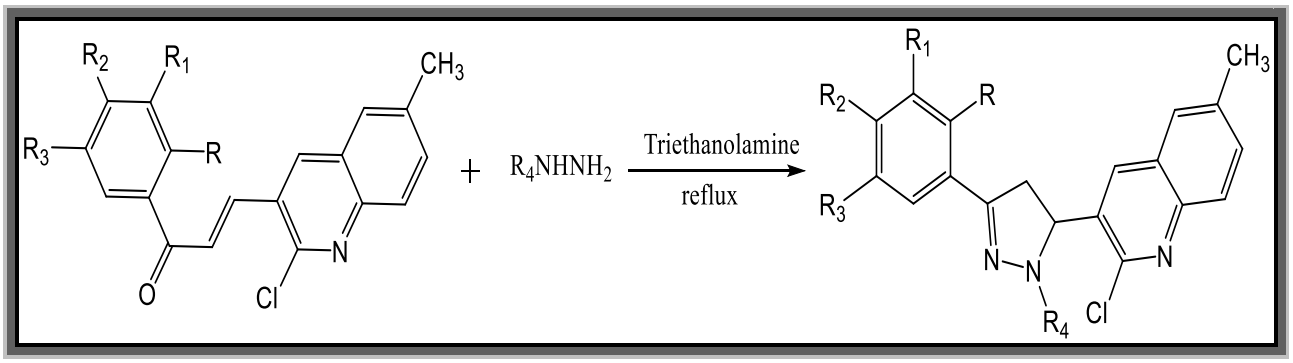
Scheme 1. 15 The synthesis of pyrazolines using microwave.

2-Pyrazolines were synthesized by the reaction of chalcone derivatives with hydrazine hydrate in the presence of formic acid (Sid, Lamara, Mokhtari, Ziani & Mosset, 2011) ***(scheme 1.16)***.



Scheme 1. 16: Synthesis of 2-pyrazolines in the presence of formic acid.

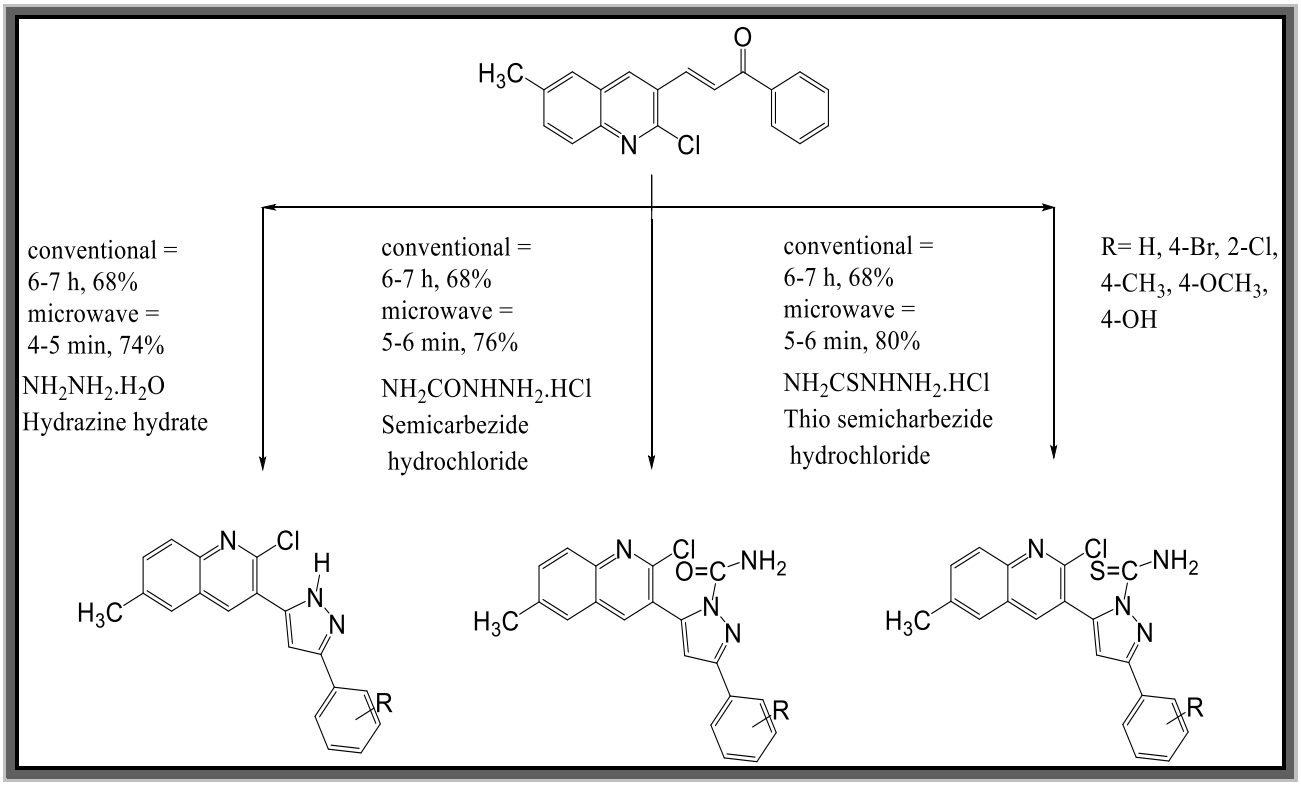
A series of novel 2-pyrazolines were synthesized by condensation of substituted chalcones with hydrazine hydrate/phenyl hydrazine in triethanolamine within 15-20*min*. The resulting compounds have been screened for their antibacterial activity ***(scheme 1.17)*** (Mcintosh, Donia & Schmidt, 2010).



Scheme 1. 17: Synthesis of 2-pyrazolines in the presence of triethanolamine.

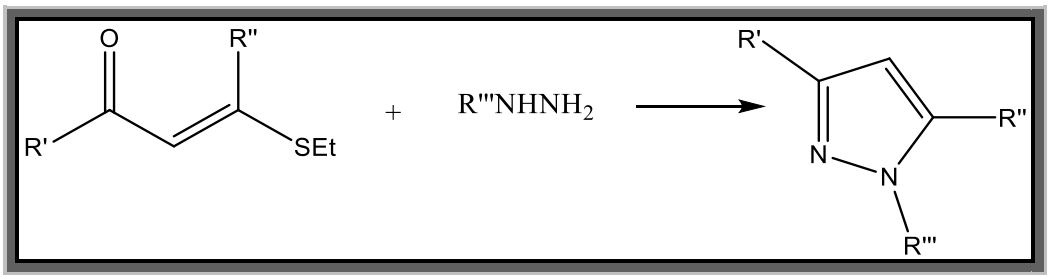
Microwave irradiation is a pollution free and eco-friendly technique used in organic synthesis. As microwave irradiation facilitates the polarization of the molecule, the reactions proceed much faster and with higher yields compared to conventional heating (Mistry, Desai, Patel & Patel, 2012). The synthesis of various pyrazole derivatives both by conventional and microwave-assisted synthesis have been achieved ***(scheme 1.18)***. It was found that the reaction carried out in acetone using conventional method requires about 6-7*h*, while microwave irradiation method requires only 4-6*min*.

The synthesized compounds have been tested of their antibacterial and antifungal activities (Kumar & Jayaroopa, 2013a; Mistry et al., 2012).



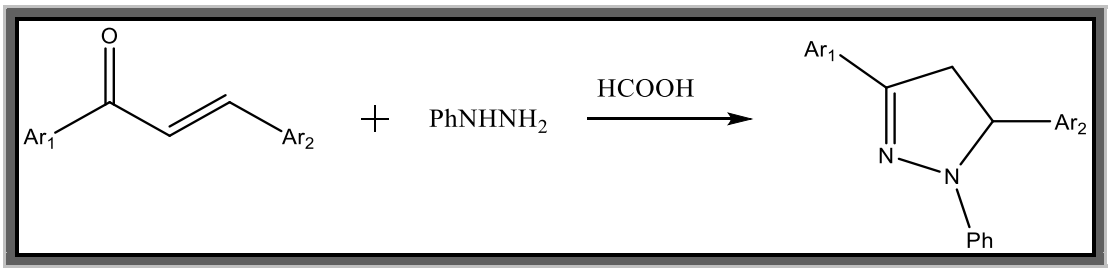
Scheme 1. 18: Synthesis of pyrazole derivatives by conventional and microwave.

Multisubstituted pyrazoles were synthesized by cyclo condensation of β-thioalkyl-α,β-unsaturated ketones with hydrazine in the presence of t-BuOK or HOAc in refluxing t-BuOH(Kumar & Jayaroopa, 2013a) ***(scheme 1.19)***.



Scheme 1. 19: Synthesis of pyrazoline in the presence of t-BuOK or HOAc in t-BuOH.

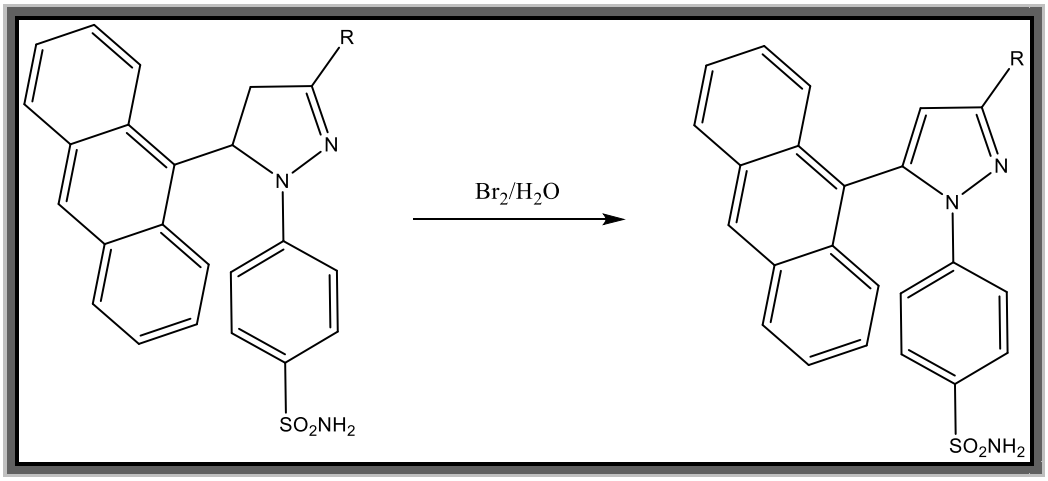
1,3,5-Trisubstituted 2-pyrazolines ***(scheme 1.20)*** have been synthesized through cyclization of phenyl hydrazine with α,β-unsaturated ketones using methanoic acid (formic acid) as catalyst under thermal condition (Maleki et al., 2009).



Scheme 1. 20: Synthesis of 1,3,5-Trisubstituted 2-pyrazolines using methanoic acid.

1.3.6 Reactions of Pyrazoline

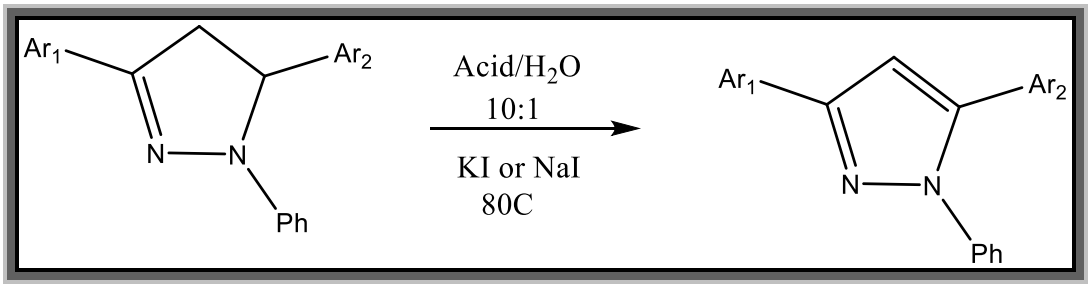
Mild oxidation of the pyrazoline derivatives with bromine water led to the formation of the corresponding pyrazoles ***(scheme 1.21)*** (Basaif, Faidllah & Hassan, 1997).



Scheme 1. 21: Oxidation of pyrazoline leads to the formation of pyrazole.

The aromatization of 1,3,5-trisubstituted-2-pyrazolines to the corresponding pyrazoles is performed under thermal condition in presence of KI or NaI and H_2O/CH_3COOH or sulfuric acid or oxalic

acid. The advantages of the method are shorter reaction time and high yields of the products ***(scheme 1.22)*** (Kumar & Govindaraju, 2015).



Scheme 1. 22: Aromatization of 1,3,5-trisubstituted-2-pyrazolines to the corresponding pyrazoles.

1.3.7 Pharmacological Activity of Pyrazoline and Pyrazole Derivative

Various chemists have designed several pyrazolines to examine their pharmacological influence. The compound of pyrazoline with benzene sulfonamide displayed remarkable anti-proliferative activity ***(scheme 1.23 a)*** (Rathore et al., 2014).

A series of 1,3-thiazolone derivatives bearing pyrazoline moiety ***(scheme 1.23 b)*** were synthesized and screened for their in vitro antitumor activity against human breast adenocarcinoma cell line (MCF-7). It was found that five of the tested compounds exhibited good antitumor activity in comparison to the reference drug, doxorubicin (Bhutani, Pathak, Husain, Kapoor & Kant, 2015).

Some 3-(pyrid-2-yl)-pyrazolines were synthesized and the anti-proliferative activity in two cancer cell lines reported ***(scheme 1.23 c)*** (Ciupa, Paul, Mahon, Wood & Caggiano, 2013).

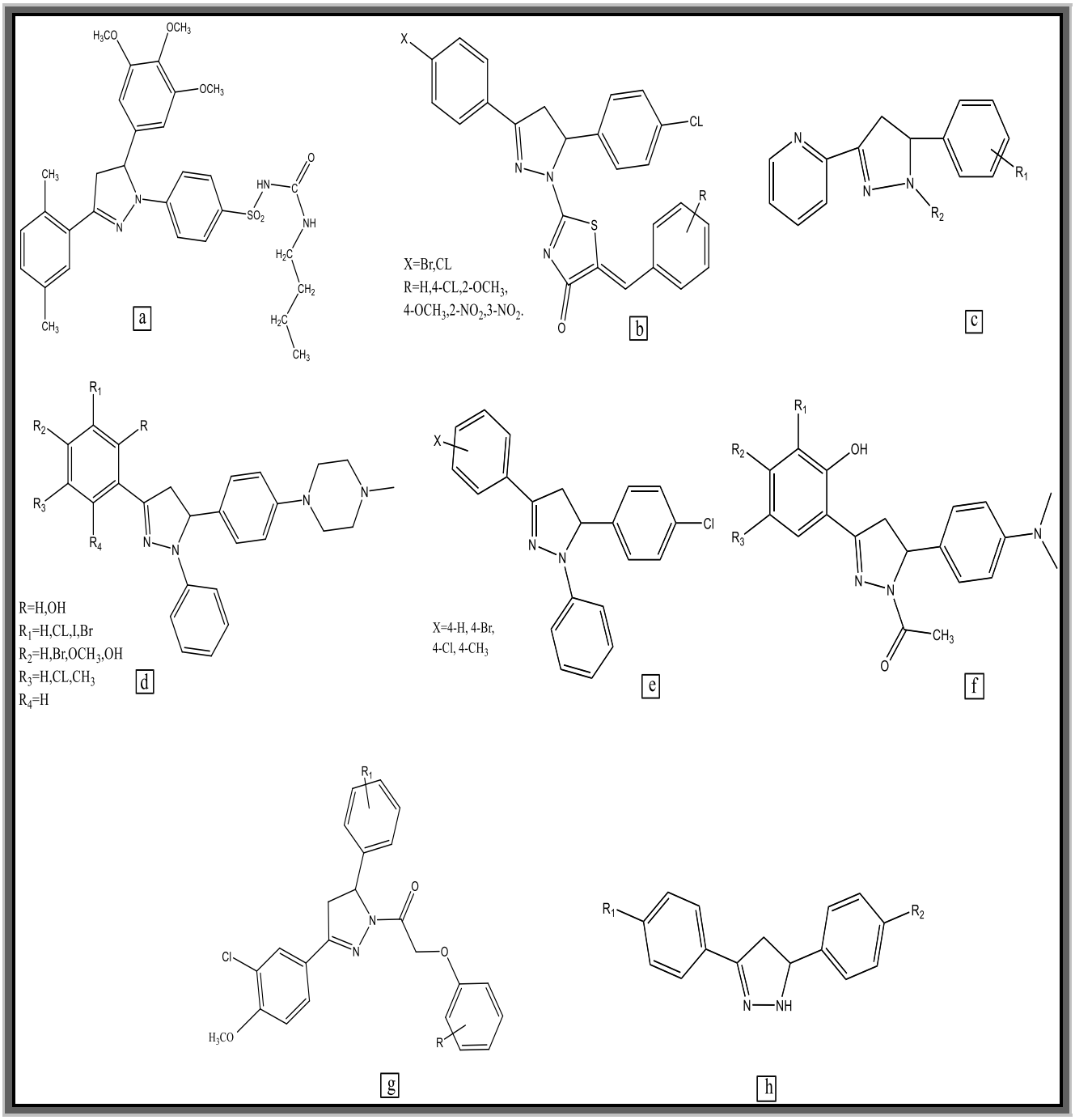
Novel pyrazolines were synthesized by reacting piperazine chalcones with phenyl hydrazine. These compounds possess moderate to good antimicrobial activity ***(scheme 1.23 d)*** (Shah, Ziauddin, Zameer, Hingole & Baseer, 2010).

Novel pyrazoline compounds were synthesized under ultrasonic irradiation and screened for their antimicrobial activity. Some of the compounds showed significant antimicrobial activity ***(scheme 1.23 e)*** (Gupta et al., 2010).

The synthesis of a new series of acetyl pyrazoline derivatives via cyclization reaction of chalcones with hydrazine hydrate by conventional methods has been reported to undergo in excellent yields and in less reaction time when using ethanol and few drops of glacial acetic acid ***(scheme 1.23 f)*** (Ansari, Ali & Asif, 2017). These newly synthesized compounds were screened for their antimicrobial activities reflecting moderate to good activity against different strains of bacteria and fungi. Compounds having functional groups such as chloro, bromo, iodo, hydroxyl and methyl groups exhibited the best antimicrobial activity.

The synthesis 1,5-disubstituted pyrazoline derivatives bearing p-methoxy-m-chloro phenyl moiety and their antimicrobial activity has been reported ***(scheme 1.23 g)*** (Singh, Ram & Sodhi, 2013).

Piperazine-Pyrazoline merged compounds were studied for their antibacterial activity ***(scheme 1.23 h)*** (Saundane, Katkar & Vaijinath, 2013).



Scheme 1. 23: pyrazoline derivatives exhibiting biological activity.

1.4 Biological Activity

Biological activity or pharmacological activity describes the beneficial or adverse effects of a drug on living matter. Nitrogen containing azole ring has attracted attention for the discovering of novel and potent anti-microbial agents. Pyrazole derivatives are an important class of heterocyclic

compounds, found in many potent biologically active molecules. The increasing clinical importance of drug-resistant fungal and bacterial pathogens has lent additional urgency to microbiological research and new antimicrobial compound development. For this purpose new series of pyrazole derivative were synthesized and evaluated their Minimum Inhibitory Concentration (MIC), depending on the Complete Randomize Design (CRD). MIC may be defined, as the lowest concentration of antimicrobial agent requires to inhibit the growth of microorganism. The MIC values of the synthesized compound were evaluated against gram-positive bacteria, gram-negative bacteria and fungal strain.

1.4.1 Antimicrobial Activity

Microorganisms require nutrition for all their metabolic activities. They draw the nutrition from their surrounding area. The growth of the microorganisms depends on the type of the nutrition they utilize. Resistance of pathogenic bacteria to available antibiotics is quickly becoming a major problem in the community and hospital based healthcare settings. Antimicrobials are one of the very important categories of drug. It is quite clear from its wide use that these categories of drugs are very important from the medical point of view. However, microbial resistance towards the drug creates a very serious problem because of development of resistance; many drugs are now useless which were very effective in the past. Moreover, the toxic effects produced by these antibiotics are also reducing their significance (Kumar et al., 2012). There is a need for new antimicrobial agents for resisting microbial infections. Antimicrobial (antibacterial and antifungal) activity of the synthesized compounds ***RS 1-5*** was done by disc diffusion method (Govindaraju et al., 2012).

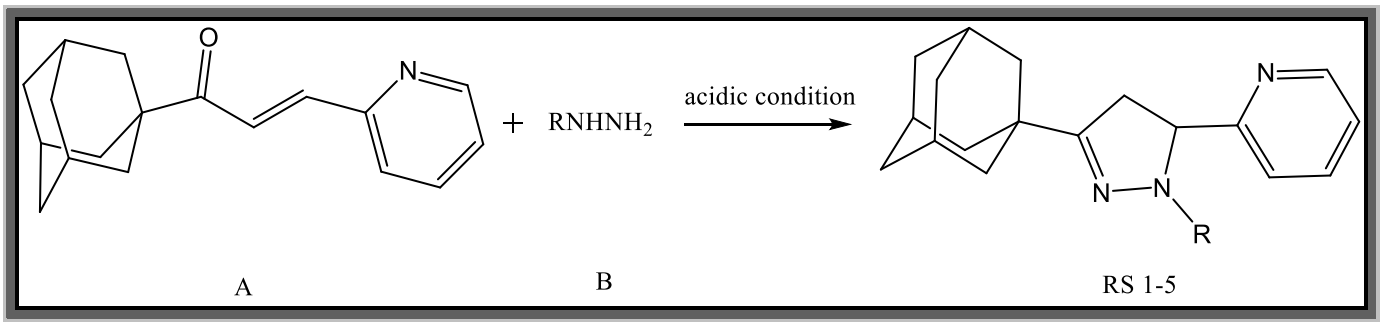
1.4.2 Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that inhibit the visible growth of a microorganism after overnight incubation. MIC values can be determined by a number of standard test procedures. The most commonly employed methods are the broth dilution method and agar dilution methods. In agar dilution method, the diluted compounds were inoculating

into bacterial growth media, incubated and scored for growth. This procedure is a standard assay for antimicrobials. MIC is important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Clinically, the minimum inhibitory concentrations are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents (Govindaraju et al., 2012).

1.5 Aim of The Present Work

The present work deals with the synthesis of new heterocyclic compounds of pyrazole derivatives using adamantly chalcone ***A*** (1-adamantyl-3-pyridyl-prop-2-en-1-one) as a precursor. A series of compounds ***RS 1-5*** were synthesized, characterized by ^{1}H NMR, ^{13}C NMR and FT-IR and evaluated for their antimicrobial activities ***(scheme 1.24)***.



Scheme 1. 24: General scheme for the synthesized compounds.

Chapter two

Materials and Methods

2.1 Materials and Instrumentation

2.1.1 Materials used in Synthesis of Compounds

1-Adamantyl methyl ketone (99%), pyridine-2-carboxaldehyde (99%), potassium hydroxide (90%), 2,4-dinitrophenylhydrazine (97%), phenyl hydrazine hydrochloride(99%), 2-chlorophenyl hydrazine hydrochloride (97%), 4-methoxyphenyl hydrazine hydrochloride(98%), o-tolyl hydrazine hydrochloride (97%), concentrate sulfuric acid , glacial acetic acid, sodium acetate, ethyl acetate, hexane, methanol and ethanol obtained from Sigma-Aldrich.

2.1.2 Materials used in Microbiology Assays

2.1.2.1 Materials used in Bacterial Assays

Gram-negative bacteria species *Pseudomonas aeruginosa (ATCC/27853), Klebsiella pneumonia, Salmonella typhimurium* and *Escherichia coli (ATCC/25922).* Gram-positive bacteria species *Bacillus subtilis* and *Staphylococcus aureus (ATCC/25923).* All microorganisms were obtained from Agricultural biological laboratory. (10µg) of gram-negative bacteria (*Meropenem, Gentamycin)* and (10µg) of gram-positive bacteria *(Meropenem, Ampicillin) are used as antibiotic standards*, normal saline 0.9%, Mueller Hinton agar media (MHA).

2.1.2.2 Materials used in Fungal Assays

Potato dextrose agar (PDA), chloramphenicol (99%), *Fusarium oxysporum fungus* obtained from Agricultural laboratory.

2.1.3 Instrumentation used to Determine the Compounds

1) ^{1}H-NMR apparatus used to determine the proton spectra of compounds. The spectra recorded in a deuterated solvent $CDCl_3$ in 400 MHz at 25 °C or by DMSO-d6 solvent in 300MHz at 21 °C. Chemical shifts (δ) are given in parts per million (ppm) downfield relative to tetramethyl silane TMS.

2) ^{13}C-NMR apparatus utilised to identify the carbon atoms in an organic molecule, it is an important tool in chemical structure. ^{13}C NMR detects only the ^{13}C isotope of carbon, whose natural abundance is only 1.1%, because the main carbon isotope ^{12}C is not detectable by NMR since it has zero net spin. The spectra recorded in a deuterated solvent $CDCL_3$ in 400 MHz at 25 C^0 . The chemical shift reference standard for ^{13}C is the carbons in TMS, whose chemical shift is considered to be 0.0 ppm. The typical range of chemical shifts is much larger than for ^{1}HNMR .

3) FT-IR spectrometer apparatus used to provide detailed information about the structural changes. KBr thin disc used to determine IR spectrum for the solid compounds in the region (4000–400 cm^{-1}).

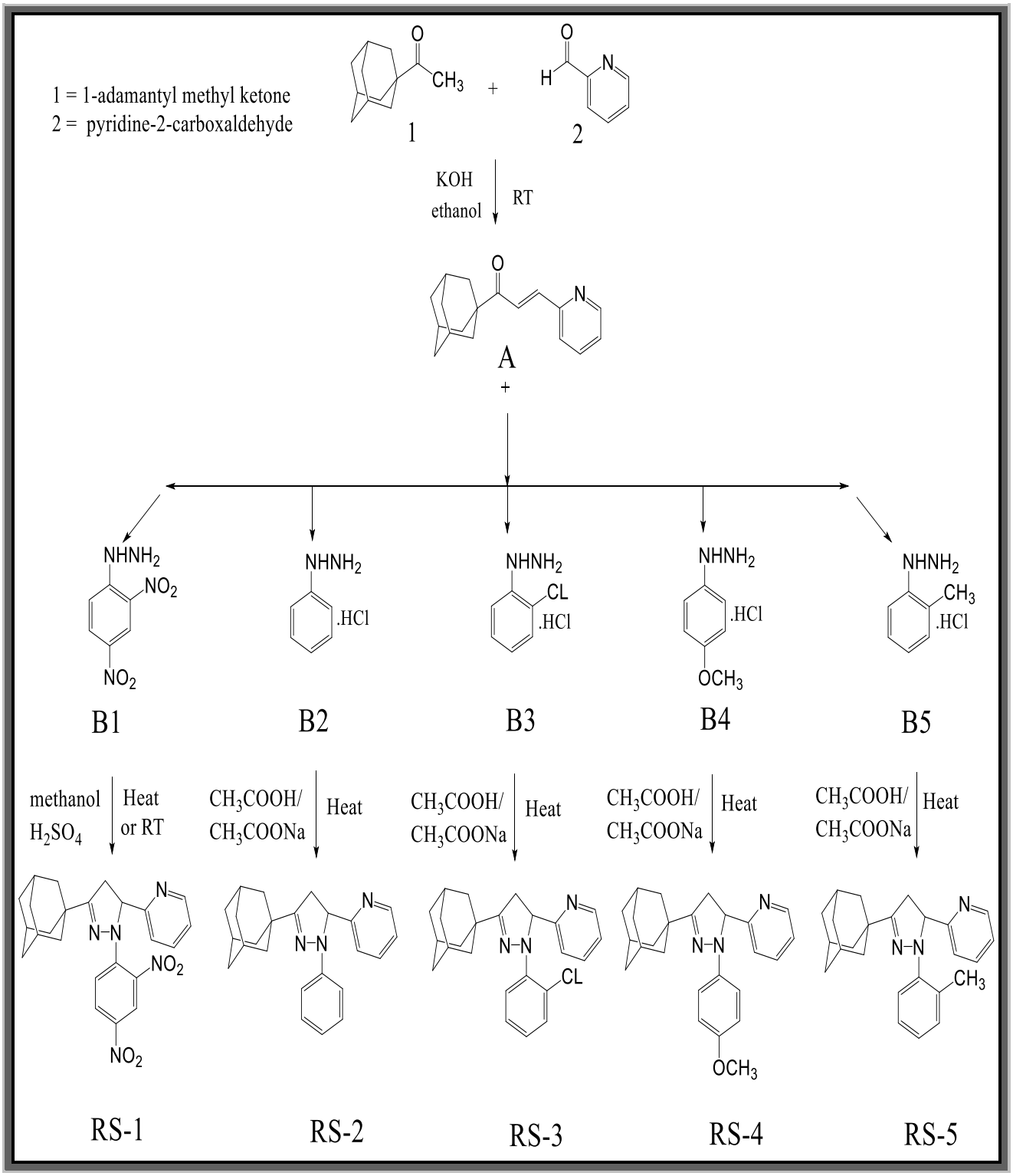
4) TLC (Thin-layer chromatography) plastic sheets silica gel, 20*20 cm, layer thickness 0.2 mm, was carried out on eluted with an ethyl acetate/hexane mixture, the spots were detected by UV light which monitored the progress of the reaction.

5) The open capillary was used to determine the melting points of the compound use Electro Thermal Stuart SMP3 advanced melting point apparatus.

2.2 The Experiments Leading to The Synthesis of Pyrazolines

One of the most important reactions in the organic synthesis is the Michael addition. It might be expected that the reaction of α,β-unsaturated compounds with phenyl hydrazine or other substituted hydrazines would proceed through ring closure to give pyrazolines (Bhatnagar & George, 1968; Elkanzi, 2013).

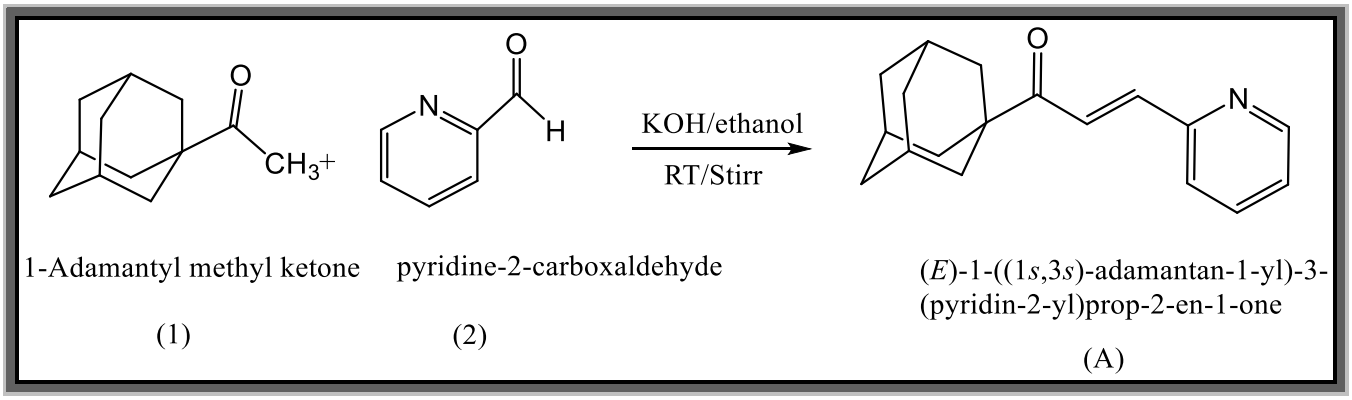
The 1-adamantyl-3-pyridyl-prop-2-en-1-one (**A**) reacts with substituted phenyl hydrazine (**B 1-5)** to form trisubstituted 4,5-dihydropyrazoles (**RS 1-5**) as shown in ***(scheme 2.1)***.



Scheme 2. 1: Summarized reactions of five synthesised pyrazoline compounds.

2.2.1 Synthesis of Adamantyl Chalcone (E)-1-((1s,3s)-adamantan-1-yl)-3-(pyridin-2-yl)prop-2-en-1-one (A)

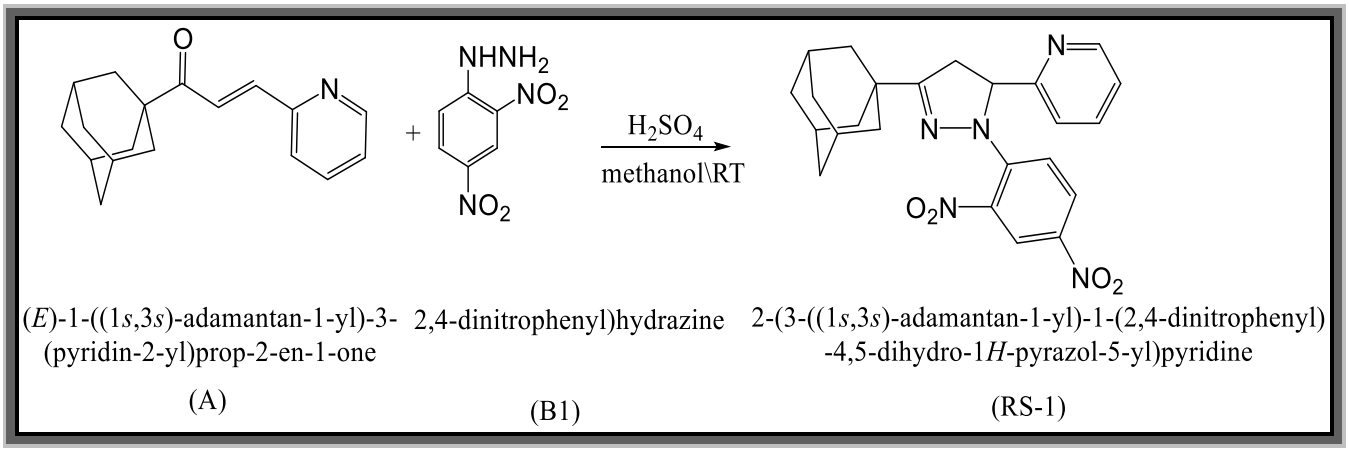
A (0.0028 mol) of 1-adamantyl methyl ketone **(1) (*scheme 2.2)*** was added to an ethanolic solution (0.0028 mol of KOH in 40 mL ethanol 96%) and stirred for 15 *min*, then (0.0028 mol) of pyridine-2-carboxaldehyde **(2)** was added drop wise to the solution and stirred at room temperature for 48 *h*. The TLC used to monitored the reaction progress. The reaction mixture was poured into ice water, the yellow solid was formed, filtered, then dried. The adamantly chalcone **(A)** was obtained according to published method (Anderson & Kaimari, 2013).



Scheme 2.2: The reaction synthesis of the 1-adamantyl-3-pyridyl-prop-2-en-1-one (A).

2.2.2 Synthesis of 2-(3-((1s,3s)-adamantan-1-yl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-1)

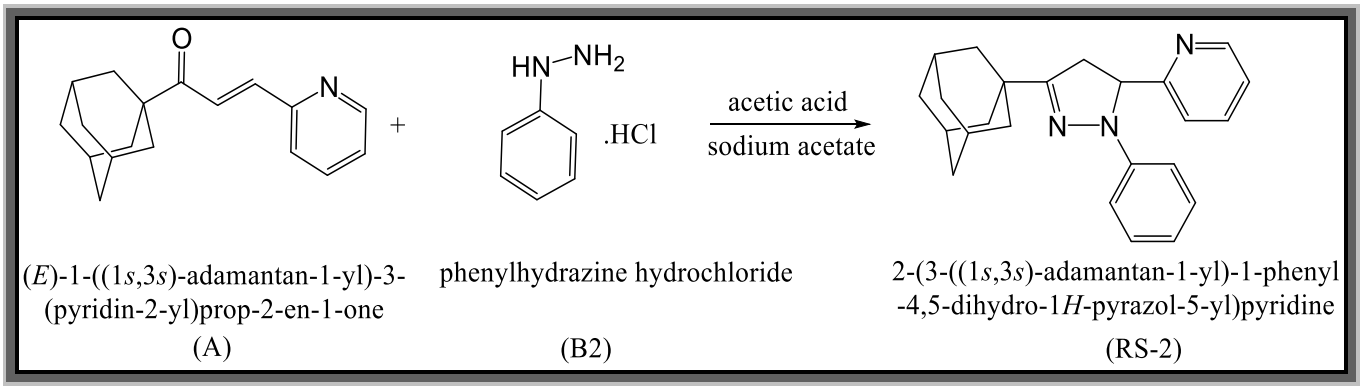
Adamantly chalcone (**A**, 0.5 mmol) was added into 3ml of methanol under stirring, 2,4-dinitrophenyl hydrazine (**B1,** 0.5 mmol) was added into 5ml of methanol and 0.3ml of concentrated sulfuric acid, the adamantly chalcone was added under stirring for 48*h* at room temperature to the solution (***scheme 2.3)***.



Scheme 2.3: The reaction synthesis of 2-(3-((1s,3s)-adamantan-1-yl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-1).

2.2.3 Synthesis of 2-(3-((1s,3s)-adamantan-1-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-2)

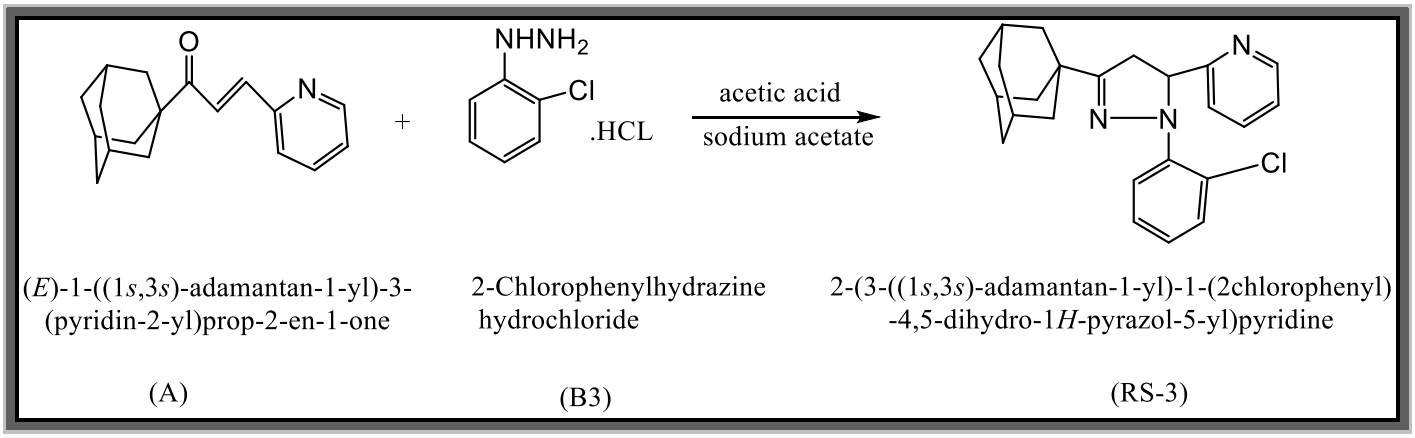
A mixture of adamantly Chalcone (0.75 mmol), phenyl hydrazine hydrochloride (**B2**, 0.75 mmol) and sodium acetate (0.15 mmol) were dissolved in acetic acid aqueous solution (6 mL, HOAc /H_2O=2/1,v/v). The mixture was stirred and heated at 80°C for 48*h*. The reaction mixture was poured into crushed ice, then the solution was evaporated to obtain the pyrazoline product (***scheme 2.4)***



Scheme 2.4: The reaction synthesis of 2-(3-((1s,3s)-adamantan-1-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-2).

2.2.4 Synthesis of 2-(3-((1s,3s)-adamantan-1-yl)-1-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-3)

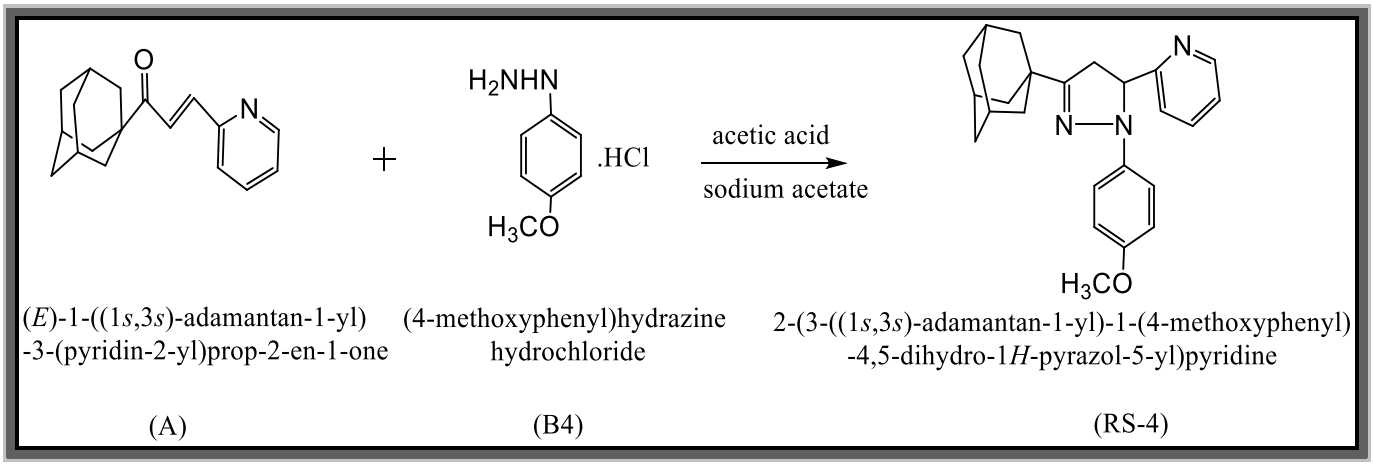
A mixture of adamantly Chalcone (0.75 mmol), 2-chloro phenyl hydrazine hydrochloride (**B3**, 0.75 mmol) and sodium acetate (0.15 mmol) were dissolved in acetic acid aqueous solution (6 mL, HOAc/H_2O=2/1,v/v). The mixture was stirred and heated at 80°C for 48*h*. The reaction mixture was poured into crushed ice, then in the rotovap the solution was evaporated in order to obtain the pyrazoline product (***scheme 2.5)***.



Scheme 2.5: The reaction synthesis of 2-(3-((1s,3s)-adamantan-1-yl)-1-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-3).

2.2.5 Synthesis of 2-(3-((1s,3s)-adamantan-1-yl)-1-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-4)

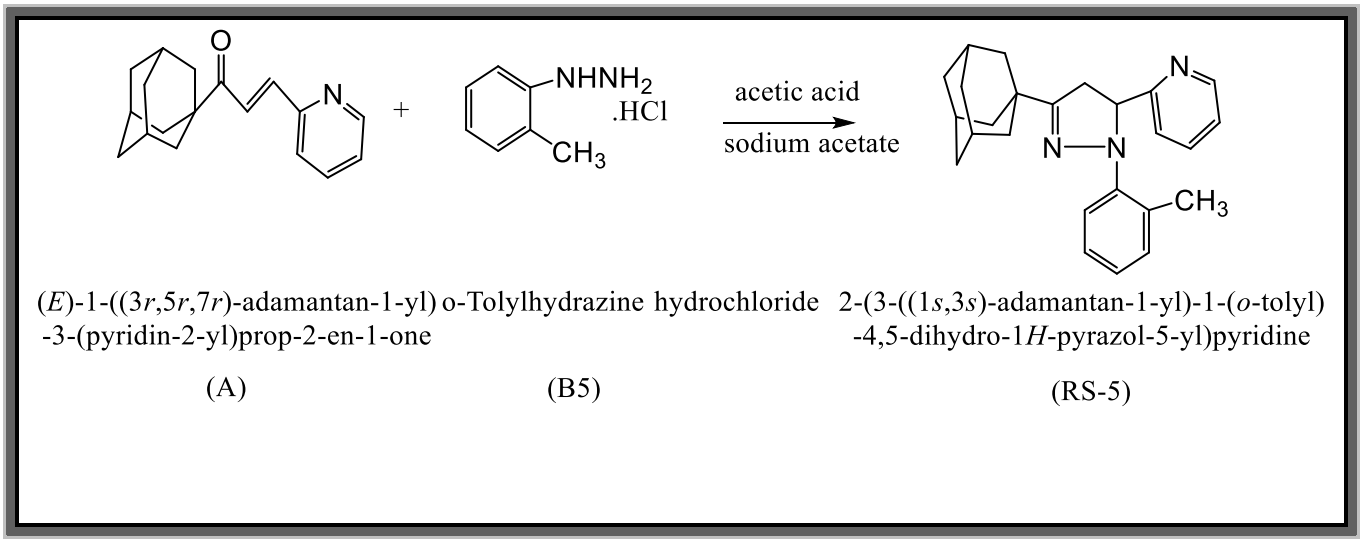
A mixture of adamantly Chalcone (0.75 mmol), 4-methoxy phenyl hydrazine hydrochloride (**B4**, 0.75 mmol) and sodium acetate (0.15 mmol) were dissolved in acetic acid aqueous solution (6 mL, HOAc/H_2O=2/1,v/v). The mixture was stirred and heated at 80°C for 48*h*. The reaction mixture was poured into crushed ice, then the solution was evaporated in the rotovap to obtain the pyrazoline product (***scheme 2.6)***.



Scheme 2.6: The reaction synthesis of 2-(3-((1s,3s)-adamantan-1-yl)-1-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-4).

2.2.6 Synthesis of 2-(3-((1s,3s)-adamantan-1-yl)-1-(o-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-5)

A mixture of adamantly Chalcone (0.75 mmol), o-tolyl hydrazine hydrochloride (**B5**, 0.75 mmol) and sodium acetate (0.15 mmol) were dissolved in acetic acid aqueous solution (6 mL, HOAc /H_2O=2/1,v/v). The mixture was stirred and heated at 80°C for 48*h*. The reaction mixture was poured into crushed ice, then the solution was evaporated in the rotovap to obtain the pyrazoline product ***(scheme 2.7)***.



Scheme 2.7: The reaction synthesis of 2-(3-((1s,3s)-adamantan-1-yl)-1-(o-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-5).

2.3 Test Microorganisms

The synthesized compounds were tested for their antimicrobial activity against gram-negative bacteria species (*Psedomonas aeruginosa, Klebsiella pneumonia, Salmonella typhimurium* and *Escherichia coli),* gram-positive bacteria species (*Bacillus subtilis* and *Staphylococcus aureus),* and fungi species *Fusarium oxysporum.*

2.3.1 Preparation of Stock Solution

Against gram-negative and gram positive bacteria species, there are four concentrations (1000, 500, 250, 125) µg/ml in DMSO were prepared. The prepared protocol depends on the complete randomize design CRD. Five concentration (20, 40, 80, 120, 160) µg/ml in DMSO were prepared for fungus depends on CRD.

2.3.2 Antibacterial Activity by Disc Diffusion Method

Gram-negative and gram-positive bacteria species were used as antibacterial test strains. The compounds ***RS 1-5*** were screened at the concentration (500µg/mL) in DMSO on the agar media for all bacterial strains. The antibiotic *Meropenem and Gentamycin* were used as standard drugs against gram negative bacteria. *Meropenem and Ampicillin* were used as standard drugs against gram positive bacteria. The plate inoculated with bacteria were incubated for 24 *h* at 37^0C. After the period of incubation, the zone of inhibition produced by the test compounds was measured in *mm*. The screening tests were performed in triplicate and the results were taken as a mean of three determinations. ***Fig-2.1*** shows the evaluation of antibacterial activity by disc diffusion method.

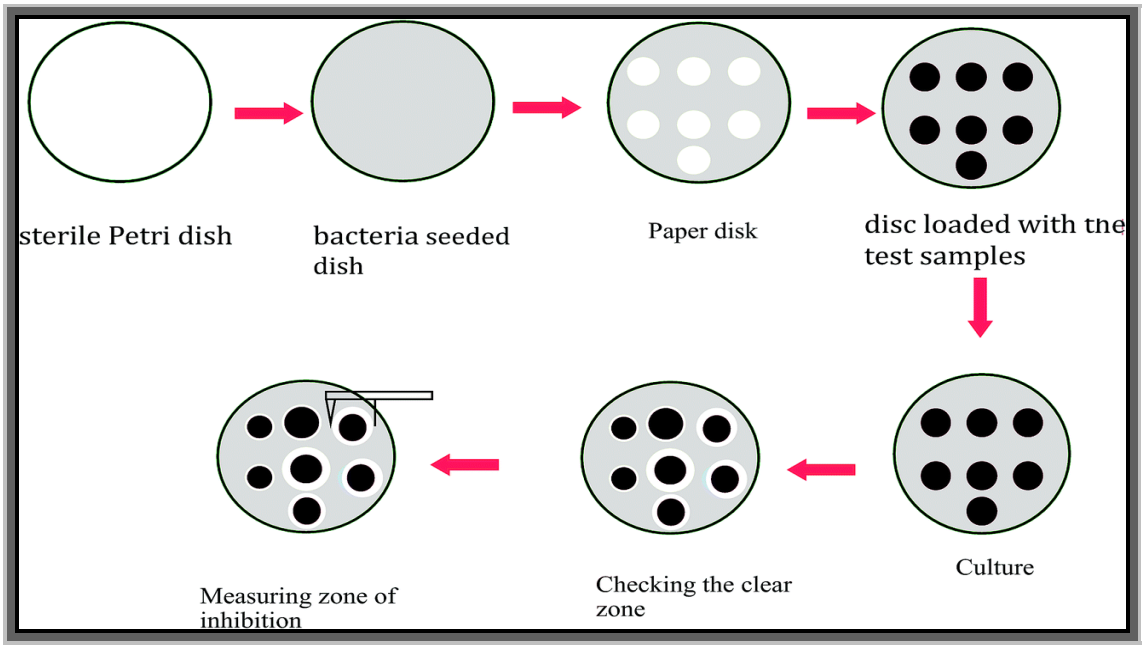


Fig-2. 1: Evaluation of antibacterial activity by disc diffusion method.

The tested samples diffuse through the agar around its disks and inhibits germination of the microorganism by a characteristics zone of inhibition depending on the microorganism sensitivity to the test sample, then measuring the inhibition zones diameters in *mm*.

2.3.3 Antibacterial Assay

The prepared compounds were screened for their minimum inhibitory concentration activity by ager diffusion method (Dar, Khanam & Gatoo, 2014). Mueller Hinton agar media was prepared by dissolving 38.0 g in 1000 ml distilled water, heating to boil in order to dissolve the medium completely, sterilized by autoclaving at (121bs). The melted agar medium was poured in sterile petri plates. After the solidification of agar, the bacterial was cultured by transferring a loop full of organisms from a laboratory culture, immersed into 2ml of normal saline 0.9% for preparation of bacterial suspension. The prepared microorganisms were spreading on the Mueller Hinton agar plates. Then cups were prepared on the plates surface using inoculating loop or borer, the cups were filled with solution of suitable concentration of sample. Another way was preparing antibiotic discs by using filter paper No.3. I used the office hole punching machine. The holes were approximately

6mm diameter. The discs were then autoclaved at (121bs) pressure for 15 minutes. Sterile discs were placed in petri dishes approximately 5mm apart. A fixed volume of 20µl was loaded on each disc one by one using a pipette. After the inoculums were prepared from the cultures and were matched for turbidity with 0.5 McFarland solutions. The prepared antibiotic discs were placed on the inoculated agar plate, then incubated at 37^0C overnight. After incubation, the zone of inhibition was measured for each of the antibiotic disc, and the lowest concentration (highest dilution) required to arrest the growth of bacteria was calculated.

2.3.4 Antifungal Assay (Mycelial Growth Rate)

The activity of the synthesized compounds ***RS 1-5*** on *Fusarium oxysporum* isolate was carried out *in vitro* on potatoes dextrose agar (PDA). The medium 1 liter contained 39.0 g of PDA and 0.3 g of chloramphenicol, a standard volume of medium was heated to dissolve the components. Flasks containing 100 ml PDA were autoclaved, then allowed to cool to 55-60^{o}C. Appropriate volumes of **A** and ***RS 1-5*** compounds stock solutions (10000 µg ml^{-1}, active ingredient of each in DMSO) were added to the media to give final concentrations of 20, 40, 80, 120 and 160 µg ml^{-1}. Growth media without compounds were used as control. Growth media were dispensed into standard disposable Petri dishes (90 *mm* diameter) with 14 ml of medium in each dish. Petri plates were inoculated in the center with 5 *mm* mycelium disks of 5 days old culture *Fusarium oxysporum* and incubated at 25 ^{o}C. Fungal colonies diameters were measured after 24 and 72 h and the mycelium growth rate (MGR, cm^2 day^{-1}) was calculated by using the following equation:

$$MGR = \frac{\left(\frac{d_2}{2}\right)^2 - \left(\frac{d_1}{2}\right)^2 * \pi}{T}$$

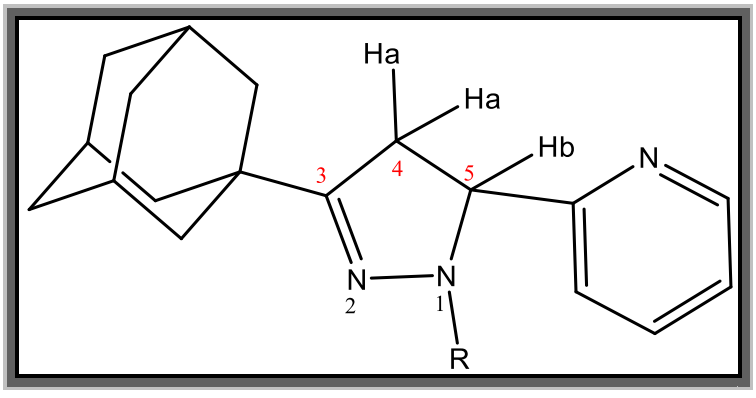
where MGR- mycelium growth rate, d_2 - average diameter of colony (cm) after 72 *h*, d_1 - average diameter of colony (cm) after 24 *h*, π - 3.14, and T- time of incubation (day) (Barakat & Al-Masri, 2017). The experimental design was completely randomized design (CRD) with five replicates.

Chapter Three

Results and Discussion

3.1 Spectroscopic Analysis of Pyrazole compounds (RS 1-5)

The pyrazoline compounds ***RS 1-5*** were characterized by using different spectroscopic techniques including ^{1}H NMR ,^{13}C NMR and FT-IR. These techniques provide the structure of the products. The structural assignments were made by NMR analysis by all compounds. In its ^{1}H NMR spectra, all the three protons, Ha and Hb are non-equivalent and therefore have different chemical shifts ***(scheme 3.1)***. All the three protons, Ha and Hb attached to the C-4 and C-5 carbon atoms of the pyrazoline ring gave a peaks. The methylene protons of pyrazoline ring Ha appeared in the region approximately δ 2.870-3.340 ppm and Hb appeared in the region δ 3.404-3.830 ppm, is the most de shielded due to its close proximity to pyridine ring. the peaks are observed for these three protons which supported the formation of compounds ***RS 1-5***. A signal at δ 2.026-1.691 ppm is assigned to adamantyl protons attached to pyrazoline ring at C-3. Moreover, a collection of signal observed in the aromatic region δ 8.809-7.122 ppm is due to aromatic protons at 1st and 5th position of the pyrazoline ring.



Scheme 3. 1: General scheme of the pyrazoline compound.

3.1.1 [1]HNMR of Adamantyl Chalcone (A)

The [1]HNMR of 1-((1s,3s)-adamantan-1-yl)-3-(pyridin-2-yl)prop-2-en-1-one ***(Fig-3.1 a, b)*** shows the chemical shifts and splitting peaks. The peak appeared at δ 7.67 attributed to **α** proton and at δ 7.75 to **β** proton. A collection of signal observed in the aromatic region δ 8.58-7.36 ppm is due to aromatic protons, the peaks at δ 2.75-1.90 ppm correspond to adamantyl group and at 4.9,3.58 indicate the presence of ethanol. Position of the chemical shift elucidated in ***(scheme 3.2 a)***.

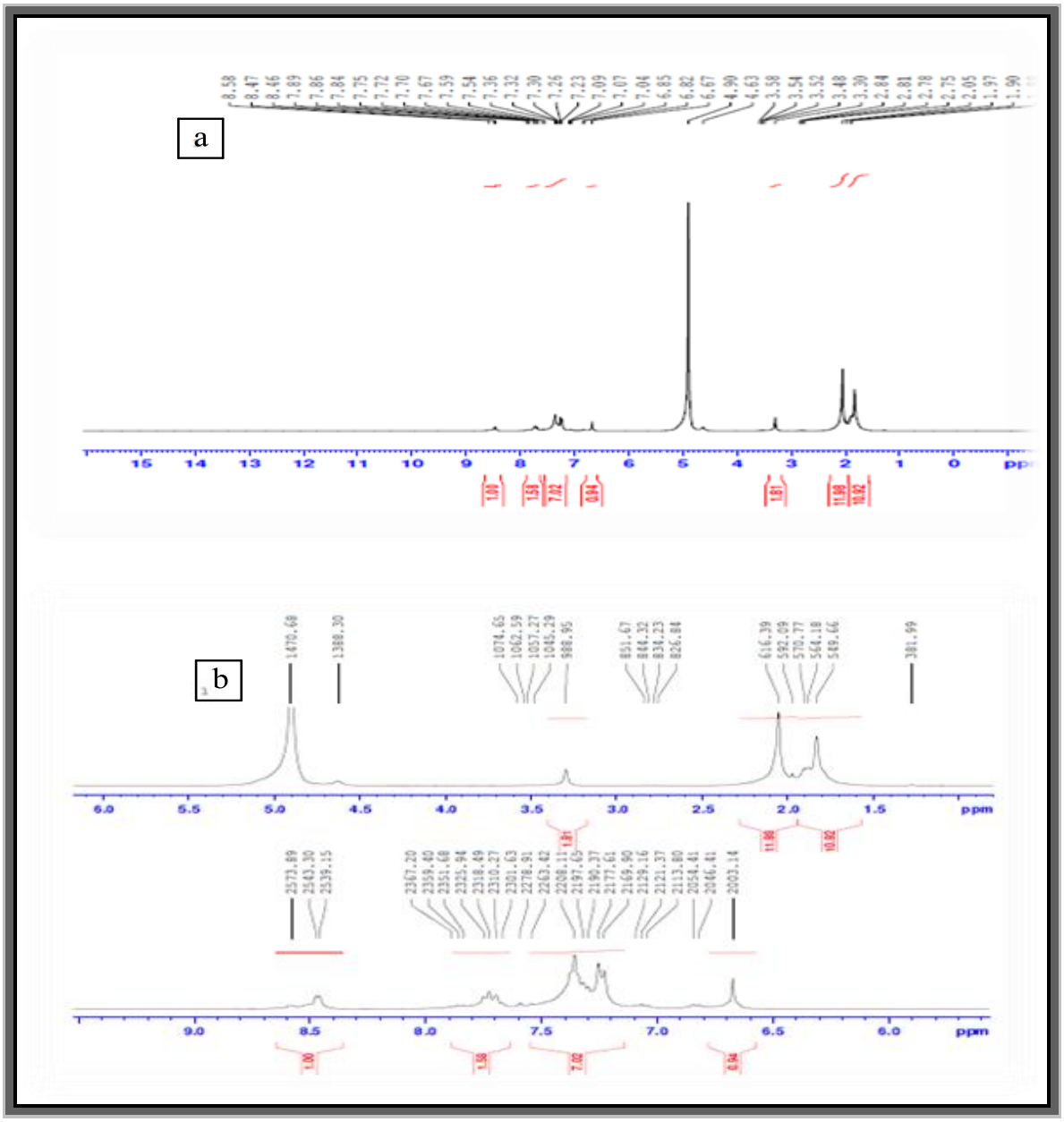


Fig-3. 1 a, b: [1]HNMR spectra of (a) adamantyl chalcone (b) spreading. [1]H-NMR (400 MHz, CDCl3, δ ppm) δ = 8.58, 7.59, 7.54, 7.36 (m, 4H, CH=CH-CH=CH), 7.67 (d, 1H, C-2, CH), 7.75 (d, 1H, C-3, CH), 2.75-1.90 (m, 15H, $C_{10}H_{15}$).

3.1.2 ¹HNMR of 2-(3-((1s,3s)-adamantan-1-yl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-1)

The ¹HNMR of ***RS-1*** 2-(3-((1s,3s)-adamantan-1-yl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3.2 a, b)*** shows the chemical shifts and splitting peaks. The peak appeared at 2.47 indicate to ***Ha*** proton and at 3.304 to ***Hb*** proton. There are no double bond in carbon 4 and 5 because the spectrum appeared at low chemical shift in high field region, where if there are double bond it should appear at down field approximately at(7.56 - 7.12). The peak at δ 11.43 ppm for NH due to the ring open in position 1. Position of the chemical shift elucidated in ***(scheme 3.2 b)***.

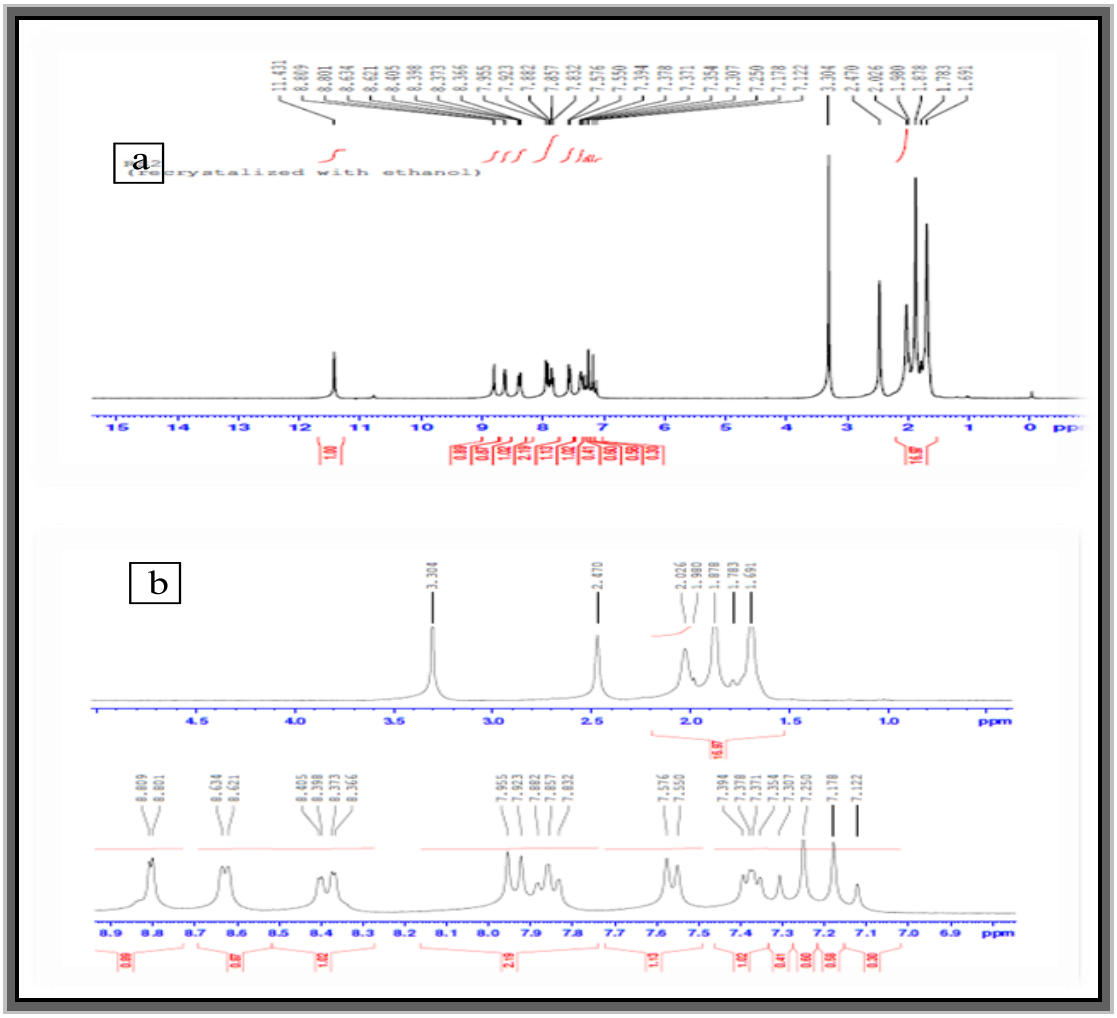


Fig-3. 2 a, b: ¹HNMR spectra of (a) RS-1 compound (b) spreading. ¹H-NMR (300 MHz, DMSO-d6, δ ppm) δ = 8.621, 7.576, 7.25, 7.1٧٨ (m, 4H, CH=CH-CH=CH), 8.809, 8.373, 7.55 (m, 3H, Ar-H), 2.470 (d, 2H, C-4, CH2), 3.304 (d, 1H, C-5, CH), 2.026-1.691 (m, 15H, $C_{10}H_{15}$), 11.431 (s, 1H, NH).

3.1.3 ¹HNMR of 2-(3-((1s,3s)-adamantan-1-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-2)

The ¹HNMR of ***RS-2*** 2-(3-((1s,3s)-adamantan-1-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3.3 a, b)*** shows the chemical shifts and splitting peaks. The methylene protons of pyrazoline ring ***Ha*** appeared in the region δ 2.81-3.30 ppm and ***Hb*** appeared in the region δ 3.52 ppm, is the most de shielded due to its close proximity to pyridine ring. The peaks are observed for these three protons which supported the formation of compound ***RS-2***. A signal at δ 2.05-1.83 ppm is assigned to adamantyl protons attached to pyrazoline ring at C-3. Moreover, a collection of signal observed in the aromatic region δ 8.47-6.84 ppm is due to aromatic protons at 1st and 5th position of the pyrazoline ring, the intense peak at 4.90 refer to ethanol. Position of the chemical shift elucidated in ***(scheme 3.2 c)***.

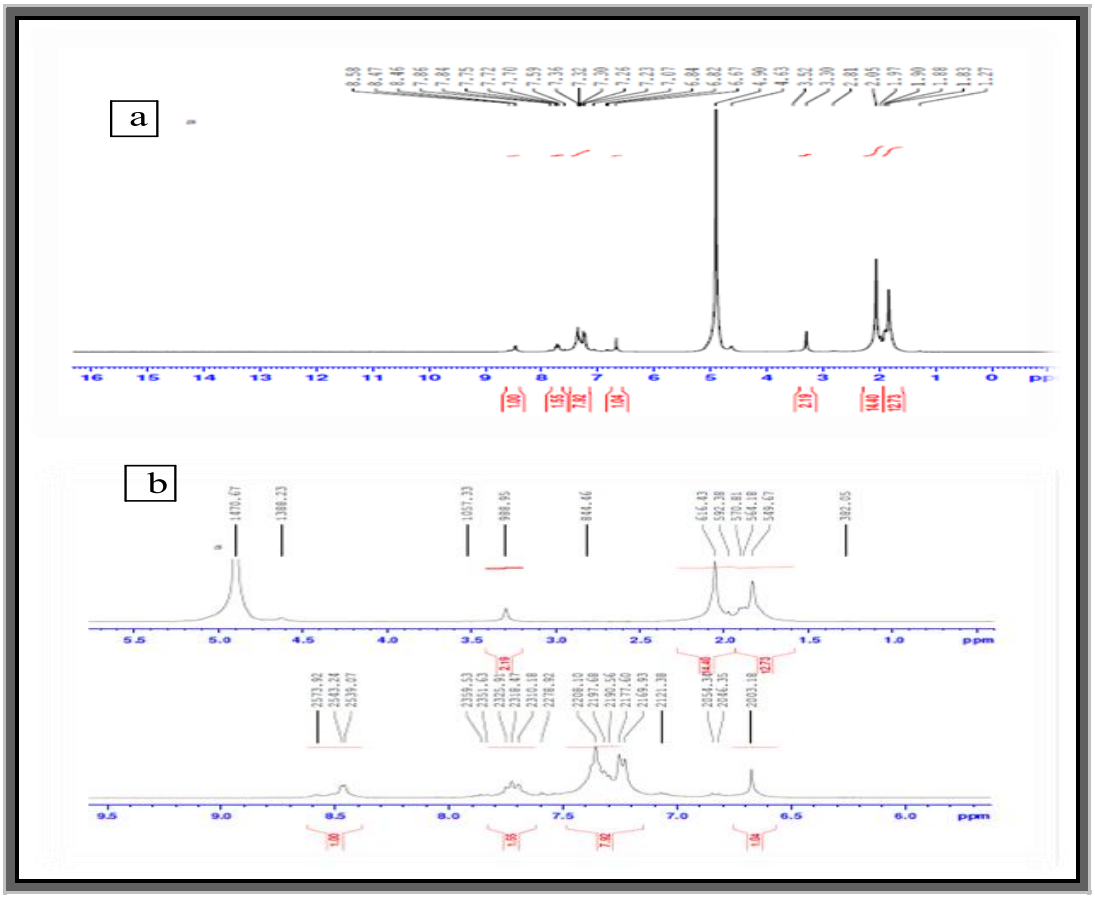


Fig-3. 3 a, b: ¹HNMR spectra of (a) RS-2 compound (b) spreading. ¹H-NMR (400 MHz, CDCl3, δ ppm) δ = 8.47, 7.75, 7.59, 7.26 (m, 4H, CH=CH-CH=CH), 7.23, 6.82, 6.84 (m, 5H, Ph-H), 3.30, 2.81 (d, 2H, C-4, CH2), 3.52 (d, 1H, C-5,CH), 2.05-1.83 (m, 15H, $C_{10}H_{15}$), 4.90 (s, 1H, OH, C_2C_5OH).

3.1.4 ¹HNMR of 2-(3-((1s,3s)-adamantan-1-yl)-1-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-3)

The ¹HNMR of ***RS-3*** 2-(3-((1s,3s)-adamantan-1-yl)-1-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3.4 a, b)*** shows the chemical shifts and splitting peaks. The methylene protons of pyrazoline ring ***Ha*** appeared in the region δ 3.02 -2.99 ppm and ***Hb*** appeared in the region δ 3.45ppm, is the most de shielded due to its close proximity to pyridine ring. The peaks are observed for these three protons which supported the formation of compound ***RS-3***. A signal at δ 2.05-1.76 ppm is assigned to adamantyl protons attached to pyrazoline ring at C-3. Moreover, a collection of signal observed in the aromatic region δ 8.40-7.40 ppm is due to aromatic protons at 1st and 5th position of the pyrazoline ring, the intense peak at 4.90 refer to OH in ethanol. Position of the chemical shift elucidated in ***(scheme 3.2 d)***.

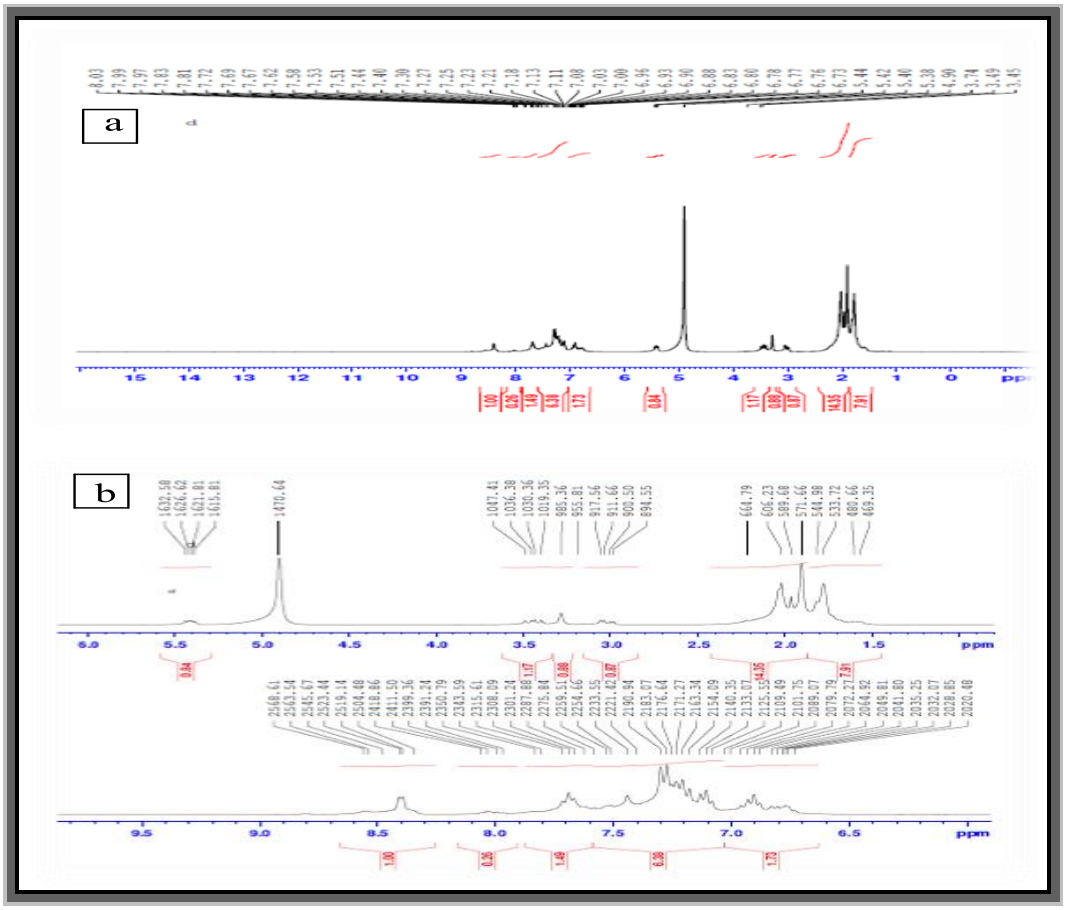


Fig-3. 4 a, b: ¹HNMR spectra of (a) RS-3 compound (b) spreading. ¹H-NMR (400 MHz, CDCl3, δ ppm) δ = 8.40, 7.72, 7.51, 7.23 (m, 4H, CH=CH-CH=CH), 7.03, 7.53, 7.49, 7.40 (m, 4H, Ph-H), 3.02, 2.99 (d, 2H, C-4, CH2), 3.45 (d, 1H, C-5, CH), 2.05, 1.90, 1.87, 1.76 (m, 15H, $C_{10}H_{15}$), 4.90 (s, 1H, OH, C_2H_5OH).

3.1.5 ¹HNMR of 2-(3-((1s,3s)-adamantan-1-yl)-1-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-4)

The ¹HNMR of ***RS4*** 2-(3-((1s,3s)-adamantan-1-yl)-1-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3.5 a, b)*** shows the chemical shifts and splitting peaks. The methylene protons of pyrazoline ring ***Ha*** appeared in the region δ 3.30-2.83 ppm and ***Hb*** appeared in the region δ 3.38 ppm, is the most de shielded due to its close proximity to pyridine ring. The peaks are observed for these three protons which supported the formation of compound ***RS-4***. A signal at δ 2.02-1.76 ppm is assigned to adamantyl protons attached to pyrazoline ring at C-3. Moreover, a collection of signal observed in the aromatic region δ 8.52-6.71 ppm is due to aromatic protons at 1st and 5th position of the pyrazoline ring, the single peak at 3.67 back to proton of methoxy group, the intense peak at 4.94 refer to ethanol. Position of the chemical shift elucidated in ***(scheme 3.2 e)***.

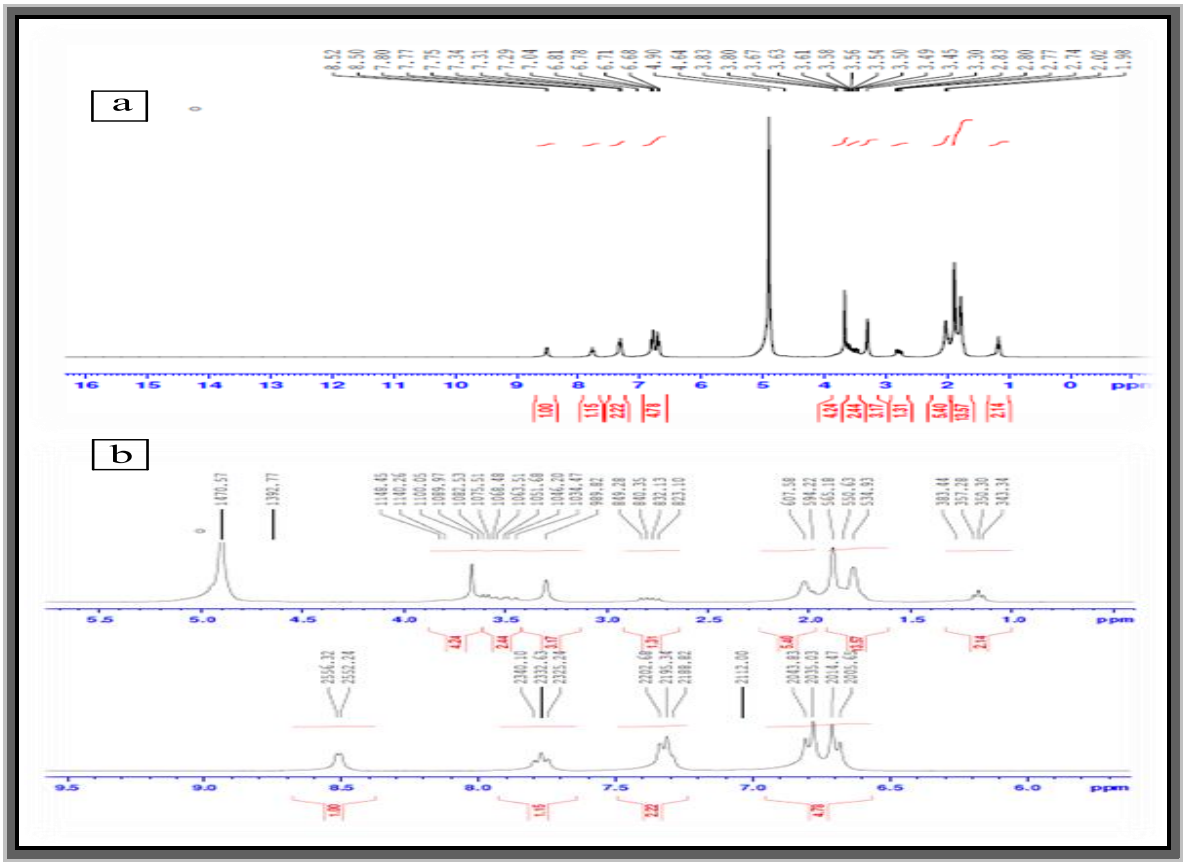


Fig-3. 5 a, b: ¹HNMR spectra of (a) RS-4 compound (b) spreading. ¹H-NMR (400 MHz, $CDCl_3$, δ ppm) δ = 8.52, 7.80, 7.75, 7.34 (m, 4H, CH=CH-CH=CH), 6.78, 6.71 (m, 4H, Ph-H), 3.30, 2.83 (d, 2H, C-4, CH2), 3.83 (d, 1H, C-5,CH), 2.02, 1.99, 1.87, 1.76 (m, 15H, $C_{10}H_{15}$), 3.67 (s, 3H, CH_3), 4.64 (s, 1H, OH, C_2H_5OH).

3.1.6 ¹HNMR of 2-(3-((1s,3s)-adamantan-1-yl)-1-(o-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-5)

The ¹HNMR of the ***RS-5*** compound 2-(3-((1s,3s)-adamantan-1-yl)-1-(o-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3.6 a, b)*** shows the chemical shifts and splitting peaks. The methylene protons of pyrazoline ring ***Ha*** appeared in the region δ 3.14 - 2.97 ppm and ***Hb*** appeared in the region δ 3.43ppm, is the most de shielded due to its close proximity to pyridine ring. The peaks are observed for these three protons which supported the formation of compound ***RS-5***. A signal at δ 2.01-1.76 ppm is assigned to adamantyl protons attached to pyrazoline ring at C-3. Moreover, a collection of signal observed in the aromatic region δ 8.45-7.06 ppm is due to aromatic protons at 1st and 5th position of the pyrazoline ring, a single peak at 2.15 back to methyl group in phenyl ring, the intense peak at 4.90 refer to ethanol. Position of the chemical shift elucidated in ***(scheme 3.2 f)***.

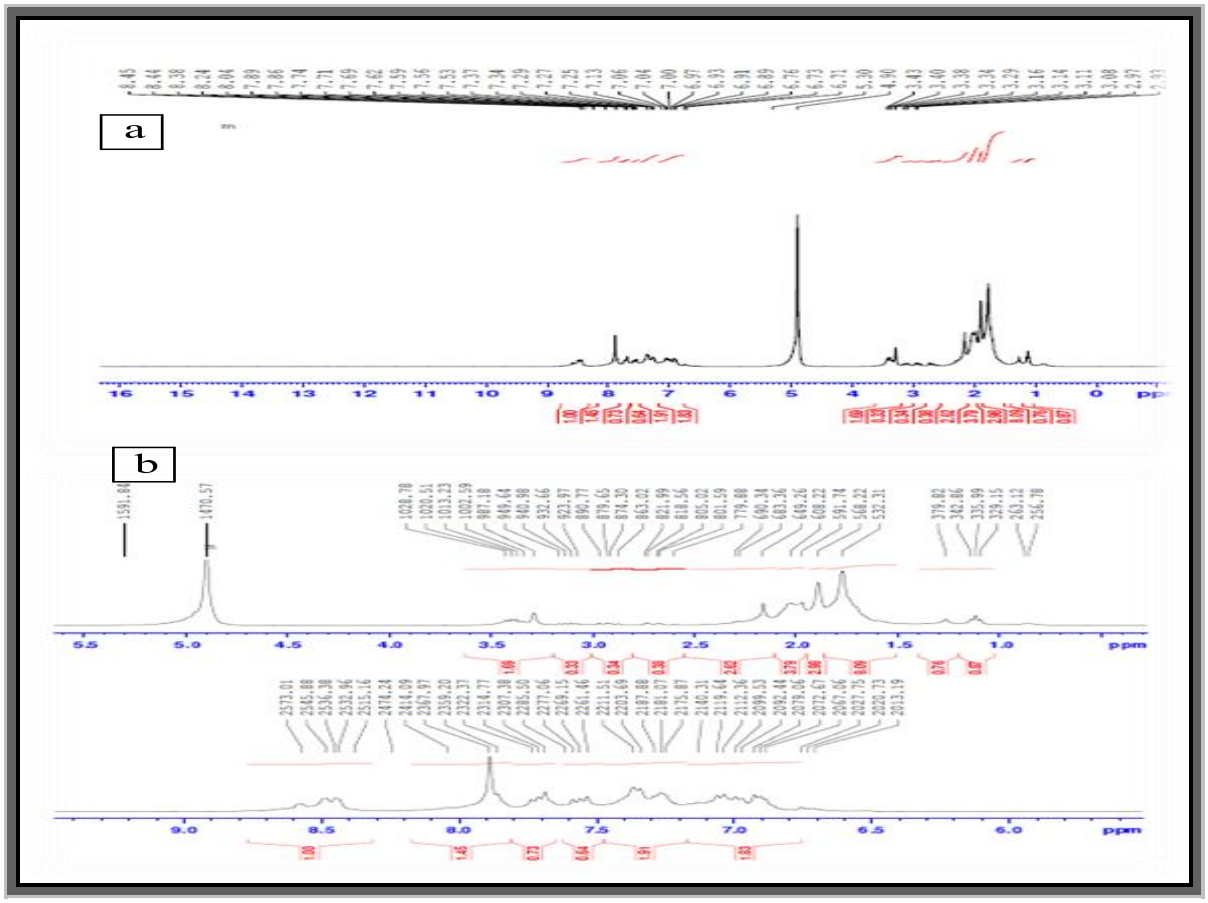
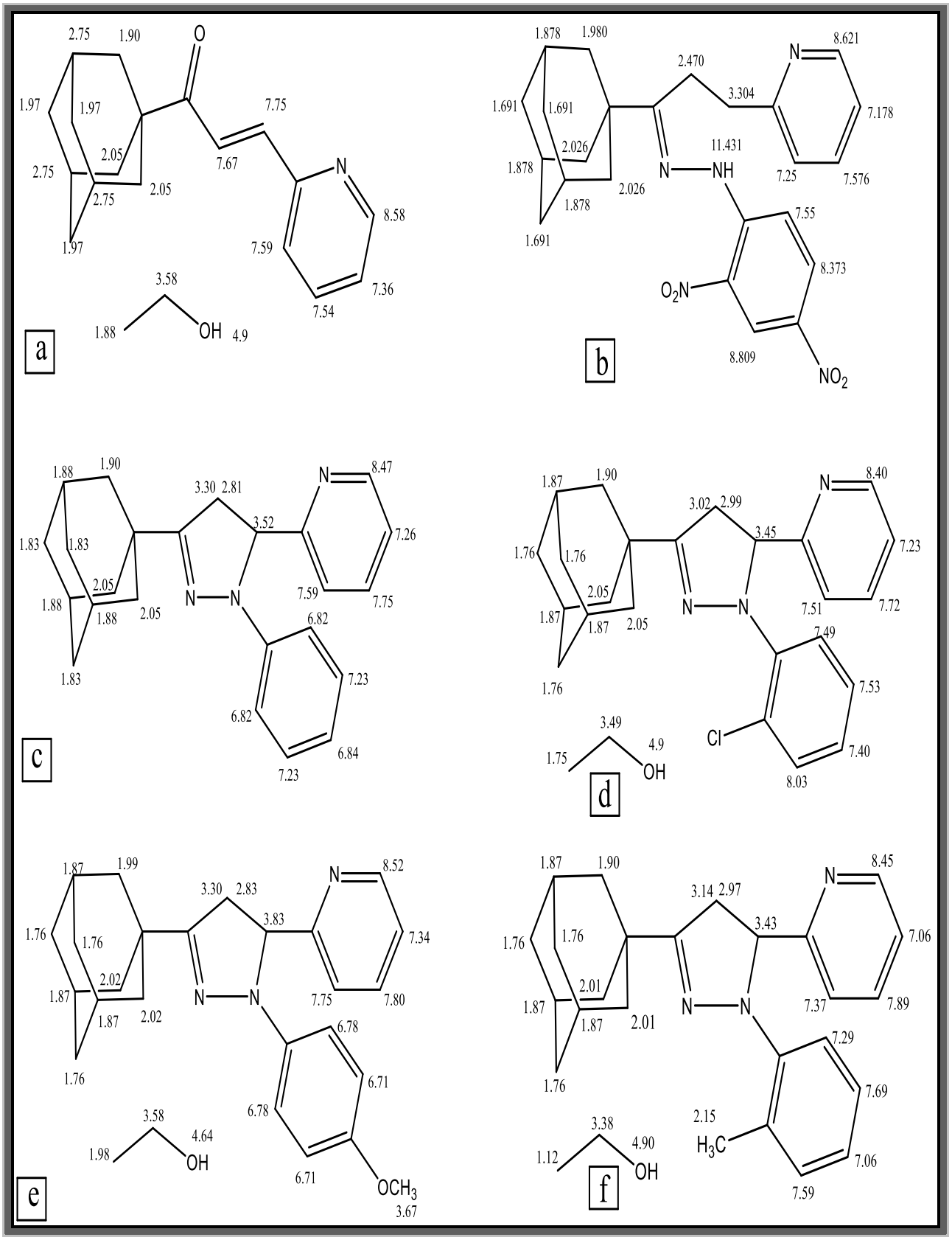


Fig-3. 6 a, b: ¹HNMR spectra of (a) RS-5 compound (b) spreading. ¹H-NMR (400 MHz, CDCl3, δ ppm) δ = 8.45, 7.89, 7.37, 7.06 (m, 4H, CH=CH-CH=CH), 7.69, 7.59, 7.29, 7.06 (m, 4H, Ph-H), 3.14, 2.97 (d, 2H, C-4, CH2), 3.43 (d, 1H, C-5, CH), 2.01, 1.90, 1.87, 1.76 (m, 15H, $C_{10}H_{15}$), 2.15 (s, 3H, CH_3), 4.90 (s, 1H, OH, C_2H_5OH).



Scheme 3. 2: Position of the chemical shift in ¹HNMR for the synthesized compounds.

3.1.7 ^{13}CNMR Analysis of adamantyl chalcone

In ^{13}C NMR spectrum of 1-((1s,3s)-adamantan-1-yl)-3-(pyridin-2-yl)prop-2-en-1-one ***(Fig-3.7 a, b)*** shows the chemical shifts and splitting peaks. The peak appeared at δ 127.1 attributed to carbon 2 and at 139.9 to carbon 3. A collection of signal observed in the aromatic region δ 155.4-123.8 ppm are due to pyridine carbon and the peaks at region δ 40.2-30.5 ppm correspond to adamantyl carbon. Position of chemical shift elucidated in **(scheme 3.3 a)**.

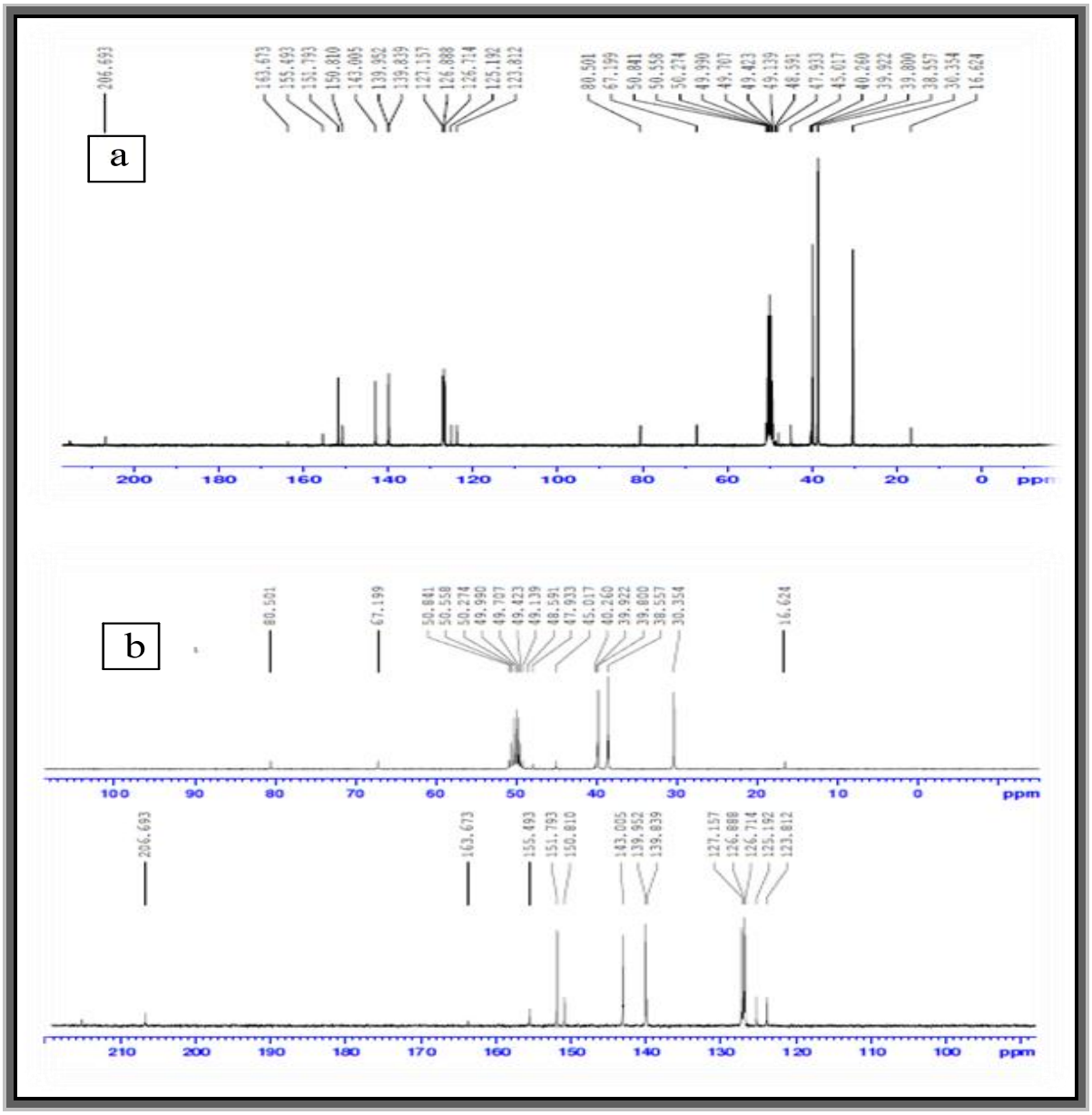


Fig-3. 7 a, b: ^{13}CNMR spectrum for (a) adamantyl chalcone (b) spreading. ^{13}CNMR (400 MHz, CDCl3, δ ppm) δ = 40.2-30.5(10C, $C_{10}H_{15}$), 143.0, 139.8, 125.1, 123.8(4C, C_5H_4N), 127.1 (1C, C-2, C=C), 139.9 (1C, C-3,C=C), 155.4 (1C, C-C-N), 206.6 (1C, C=O), 80, 67 (C, CH_3CH_2OH).

3.1.8 ^{13}CNMR of 2-(3-((1s,3s)-adamantan-1-yl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-1)

In ^{13}C NMR spectrum of compound ***RS-1*** 2-(3-((1s,3s)-adamantan-1-yl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3.8 a)*** shows the chemical shifts and splitting peaks. A signal at δ 59.0-37.5 ppm is assigned to adamantyl carbon attached to azole ring at C-3. Two signals at δ 28.1and δ 40.8 ppm are assigned to C-4 and C-5 respectively. One signal at δ 155.6 ppm is attributed to C-3 in the azole ring which is a carbon attached to electronegative nitrogen by a double bond is de shielded due to its sp^2 hybridization and electro negativity of nitrogen. A collection of signals appeared in the region δ 148.3-121.0 ppm which are assigned to aryl carbons, the intense peak at δ 80 ppm back to carbon in CH_3OH. Position of chemical shift elucidated in **(scheme 3.3 b)**.

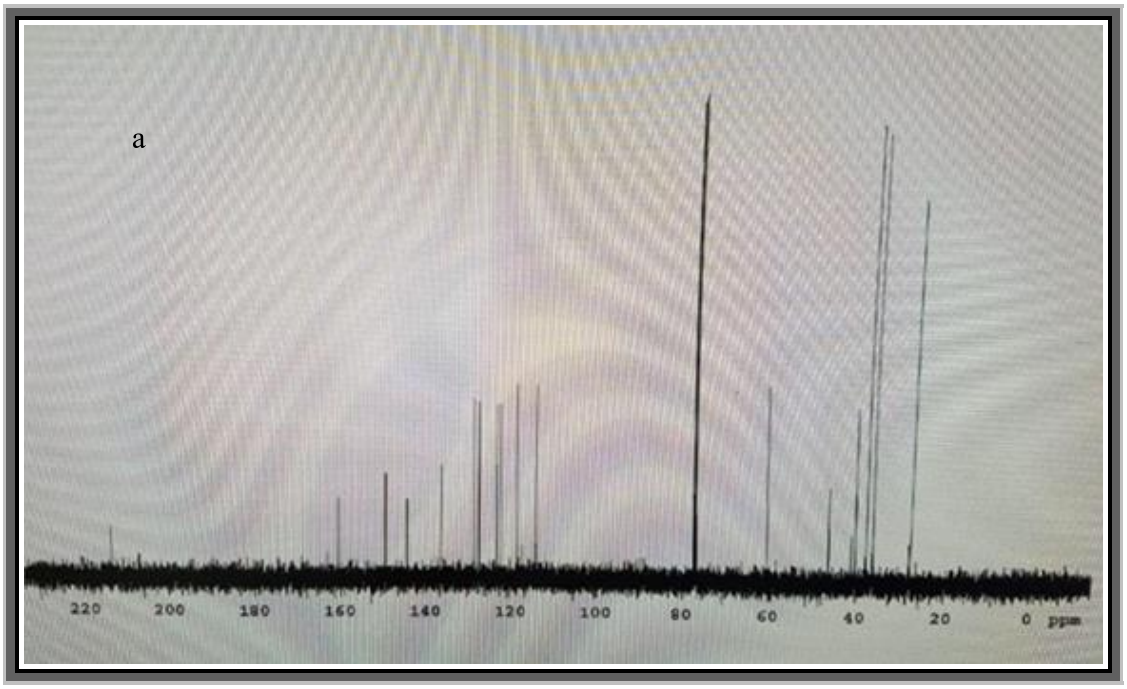


Fig-3. 8 a: ^{13}C NMR spectrum for compound (RS-1) (a). ^{13}CNMR (400MHz, $CDCL_3$, δ ppm) δ = 155.6 (C-3, C=N), 28.1 (C-4, CH_2), 40.8 (C-5, CH), 148.3, 136.4, 122.9, 121.0 (4C, C_5H_4N), 160 (1C, sp^2, C-C-N), 142 (1C, p C-NO_2), 129.0 (1C, o C-NO_2), 151.5 (1C, C-NH), 130.8, 123.4, 116.6 (3C, C_6H_3), 59.0-37.5(10C, $C_{10}H_{15}$), 80(1C, CH_3OH), 215 (1C, C=O).

3.1.9 ^{13}CNMR of 2-(3-((1s,3s)-adamantan-1-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-2)

In ^{13}C NMR spectrum of compound ***RS-2*** 2-(3-((1s,3s)-adamantan-1-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3.8 a, b)*** shows the chemical shifts and splitting peaks. A signal at δ 44.62-30.37 ppm is assigned to adamantyl carbon attached to azole ring at C-3. Two signals at δ 38.8 and δ 67.7 ppm are assigned to C- 4 and C- 5 respectively. One signal at δ 165.2 ppm is attributed to C-3 in the azole ring which is a carbon attached to electronegative nitrogen by a double bond is de shielded due to its sp² hybridization and electro negativity of nitrogen. A collection of signals appeared in the region δ 144.4-107.0 ppm which are assigned to aryl carbons, the intense peak at δ 50.5 ppm back to carbon in ethanol. Position of chemical shift elucidated in **(scheme 3.3 c)**.

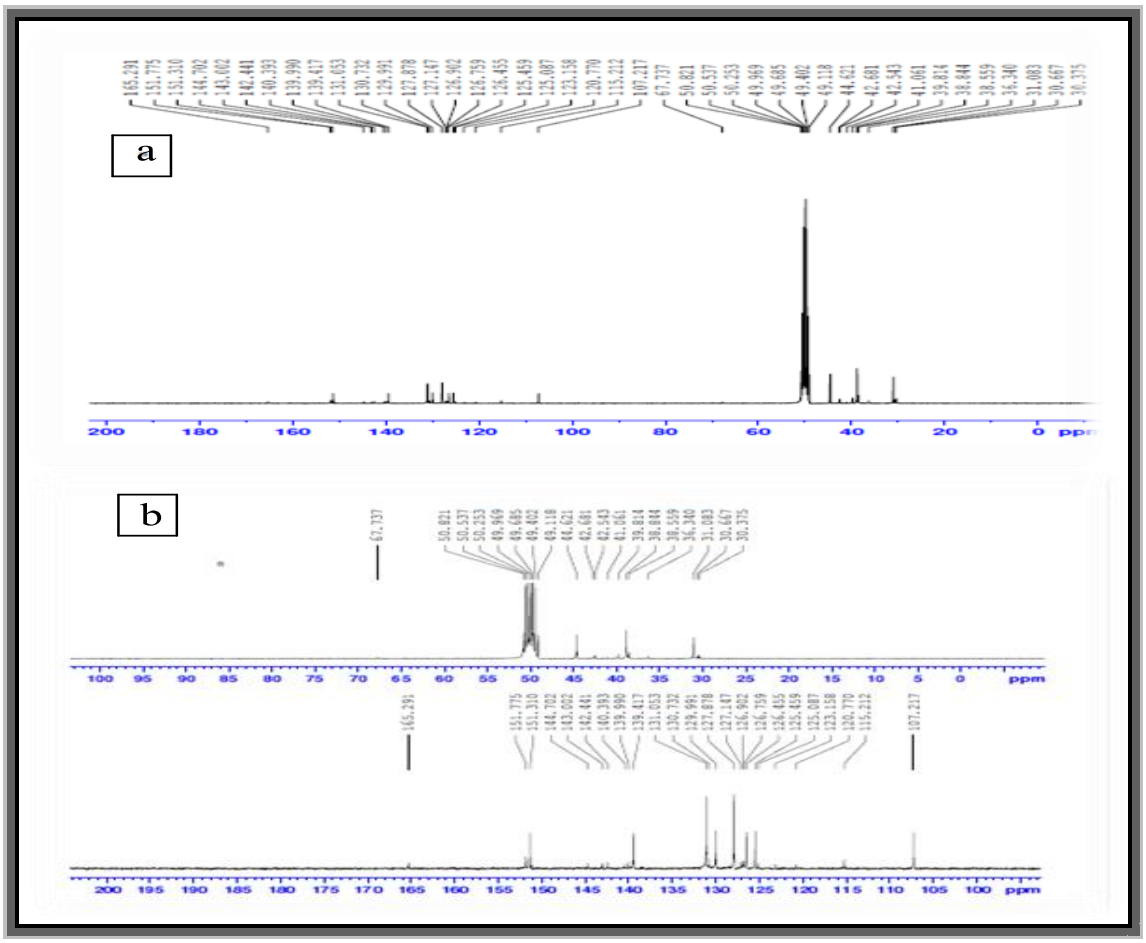


Fig-3. 9 a, b: ^{13}C NMR spectrum for compound (RS-2) (a). spreading (b). ^{13}C NMR (400 MHz, $CDCL_3$, δ ppm) δ = 165.2 (C-3, C=N), 38.8 (C-4, CH_2), 67.7 (C-5, CH), 144.4, 129.9, 127.8, 126.4 (4C, C_5H_4N), 151.3 (1C, sp², C-C-N), 143.0, 131.0, 125.4, 107 (6C, C_6H_5), 44.62, 41.06, 31.08, 30.37 (10C, $C_{10}H_{15}$), 50.5 (1C, C_2H_6O).

3.1.10 ^{13}CNMR of 2-(3-((1s,3s)-adamantan-1-yl)-1-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-3)

In ^{13}CNMR spectrum of compound ***RS-3*** 2-(3-((1s,3s)-adamantan-1-yl)-1-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3.10 a, b)*** shows the chemical shifts and splitting peaks. A signal at δ 49.5-30.7 ppm is assigned to adamantyl carbon attached to azole ring at C-3. Two signals at δ 39.7and δ 69.9 ppm are assigned to C-4 and C-5 respectively. One signal at δ 166.1 ppm is attributed to C-3 in the azole ring which is a carbon attached to electronegative nitrogen by a double bond is de shielded due to its sp² hybridization and electronegativity of nitrogen. A collection of signals appeared in the region δ 150.2-105.7 ppm which are assigned to aryl carbons, the intense peak at δ 50.5 ppm back to carbon in ethanol. Position of chemical shift elucidated in **(scheme 3.3a)**.

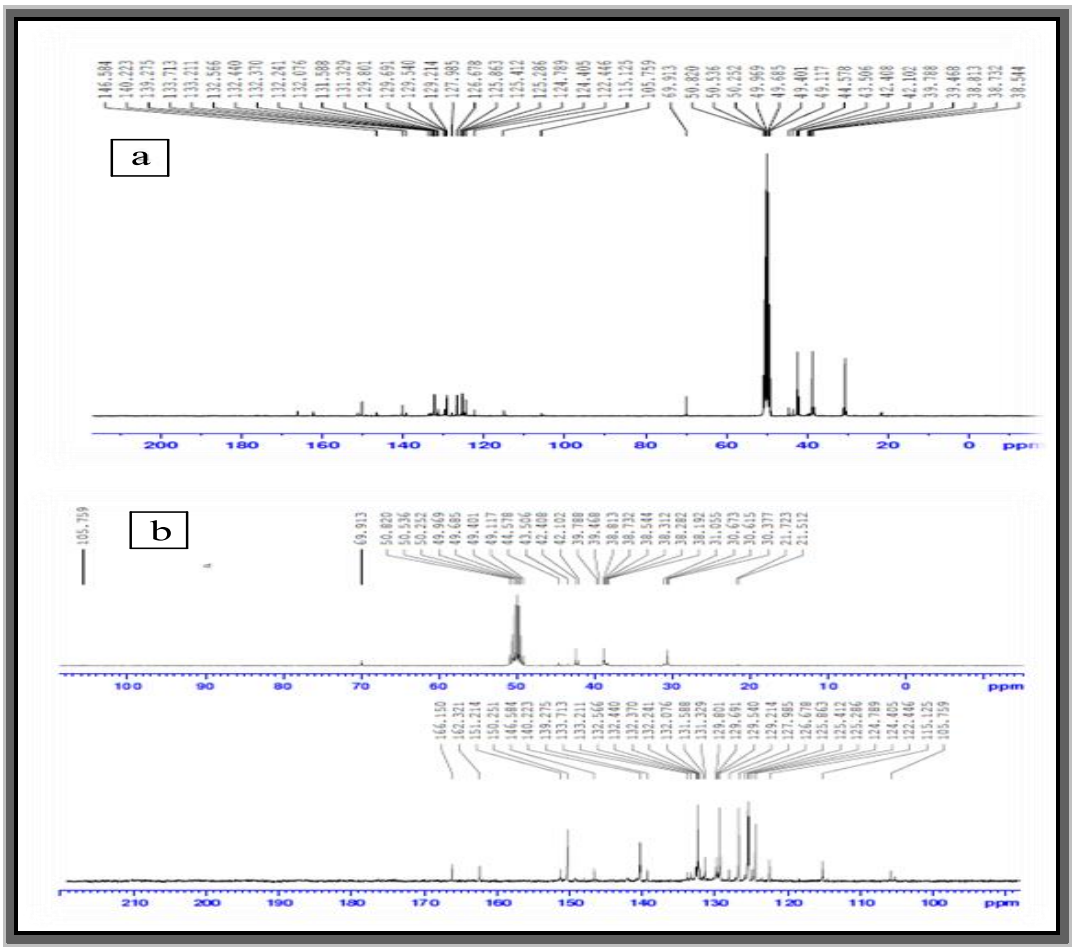


Fig-3. 10 a, b: ^{13}C NMR spectrum for compound (RS-3). ^{13}C NMR (400 MHz, $CDCL_3$, δ ppm) δ = 166.1 (C-3, C=N), 39.7 (C-4, CH_2), 69.9 (C-5, CH), 162.3 (1C, C-C-N) 150.2, 129.8, 127.4, 115.1 (4C, C_5H_4N), 146.2 (1C, sp2, C-N), 132.5, 126.6, 124.4, 105.7 (4C, C_6H_4), 122.4 (1C, C-CL) 49.5, 42.4, 38.8, 30.7 (10C, $C_{10}H_{15}$), 50.5 (1C, C_2H_6O).

3.1.11 ^{13}CNMR of 2-(3-((1s,3s)-adamantan-1-yl)-1-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-4)

In ^{13}C NMR spectrum of compound ***RS-4*** 2-(3-((1s,3s)-adamantan-1-yl)-1-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3.11 a, b)*** shows the chemical shifts and splitting peaks. A signal at δ 50.5-30.6 ppm is assigned to adamantyl carbon attached to azole ring at C-3. Two signals at δ 42.5 and δ 69.5 ppm are assigned to C- 4 and C- 5 respectively. One signal at δ 164.4 ppm is attributed to C-3 in the azole ring which is a carbon attached to electronegative nitrogen by a double bond is de shielded due to its sp² hybridization and electro negativity of nitrogen. A collection of signals appeared in the region δ 155.0-116.2 ppm which are assigned to aryl carbons. The methoxy peak appeared at 56.9 and the intense peak at δ 50.5 ppm back to carbon in ethanol. Position of chemical shift elucidated in **(*scheme 3.3 a*)**.

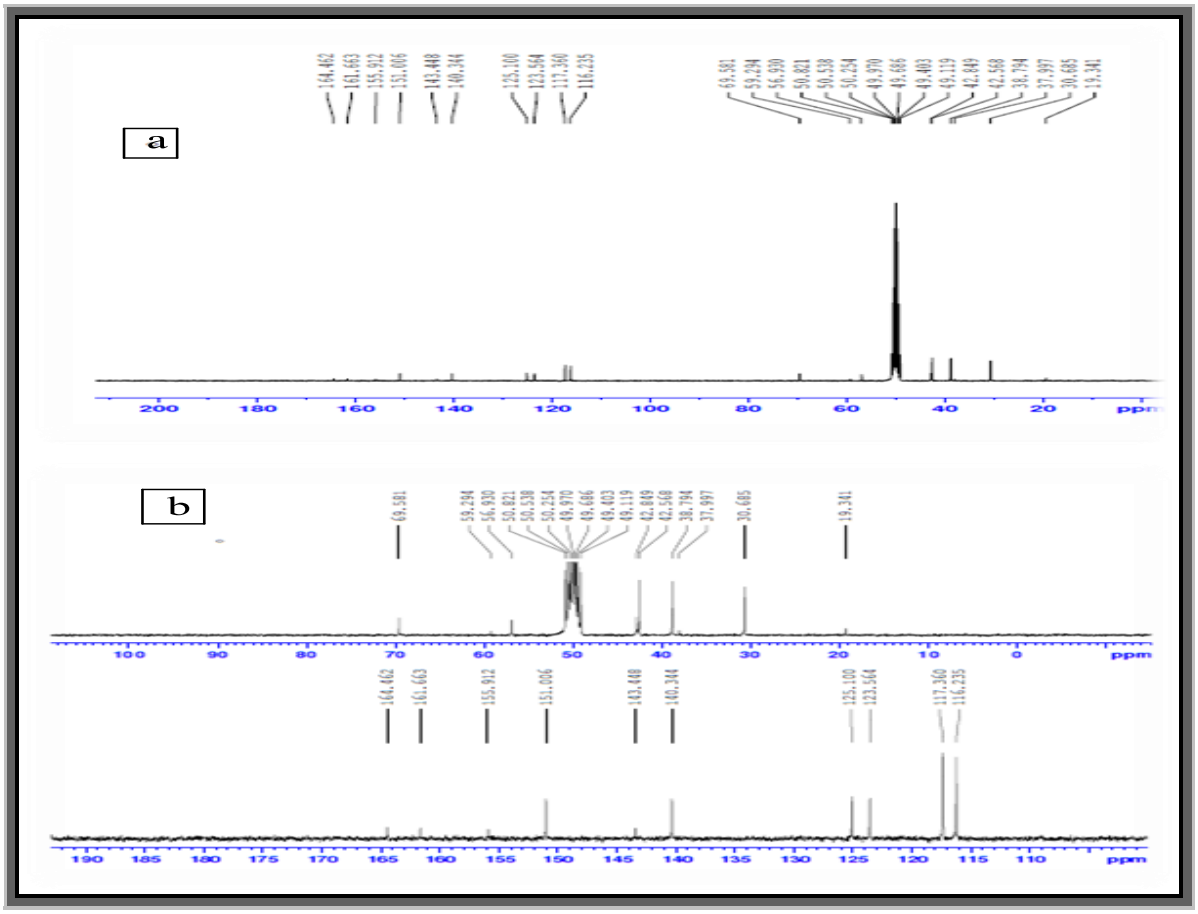


Fig-3. 11 a, b: ^{13}C NMR spectrum for compound (RS-4)(a). spreading (b). ^{13}C NMR (400 MHz, $CDCL_3$, δ ppm) δ = 164.4 (C-3, C=N), 42.5 (C-4, CH_2), 69.5 (C-5, CH), 151.0, 140.3, 125.1, 123.5 (4C, C_5H_4N), 161.6 (1C, sp², C-C-N), 155.0, 143.4, 117.3, 116.2 (6C, C_6H_4), 50.5, 49.6, 38.7 (10C, $C_{10}H_{15}$), 155.0 (1C, sp², C-C-O), 56.9 (1C, OCH_3), 50.5, 30.1 (1C, C_2H_6O).

3.1.12 ^{13}CNMR of 2-(3-((1s,3s)-adamantan-1-yl)-1-(o-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (RS-5)

In ^{13}CNMR spectrum of compound **RS-5** 2-(3-((1s,3s)-adamantan-1-yl)-1-(o-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3.12 a, b)*** shows that chemical shifts and splitting peaks. A signal at δ 42.4 - 20.8 ppm is assigned to adamantyl carbon attached to azole ring at C-3. Two signals at δ 38.7 and δ 67.3 ppm are assigned to C- 4 and C- 5 respectively. One signal at δ 164.3 ppm is attributed to C-3 in the azole ring which is a carbon attached to electronegative nitrogen by a double bond is de shielded due to its sp² hybridization and electro negativity of nitrogen. A collection of signals appeared in the region δ 142.9-121.9 ppm which are assigned to aryl carbons. At 16.5 it back to methyl group in phenyl ring, the intense peak at δ 50.5 ppm back to carbon in ethanol. Position of chemical shift elucidated in **(scheme 3.3 a)**.

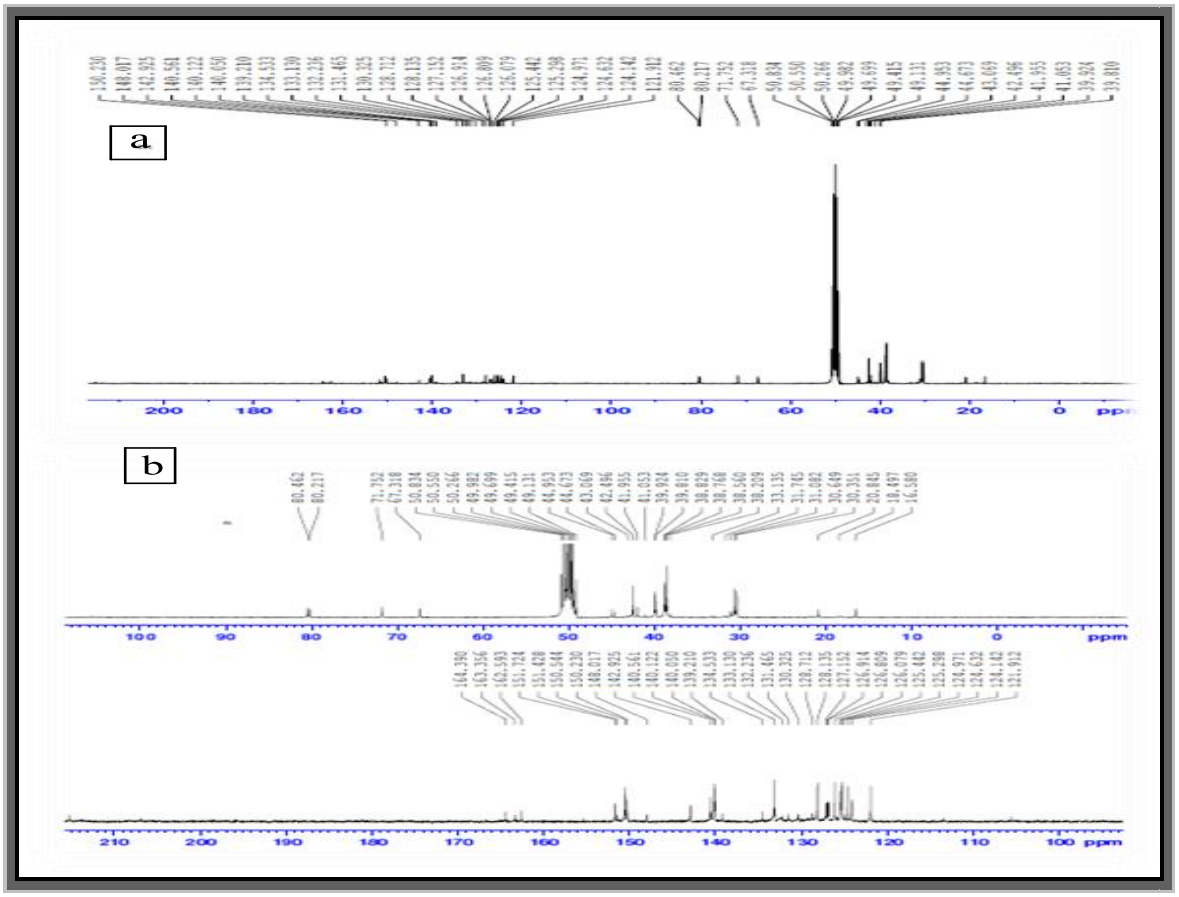
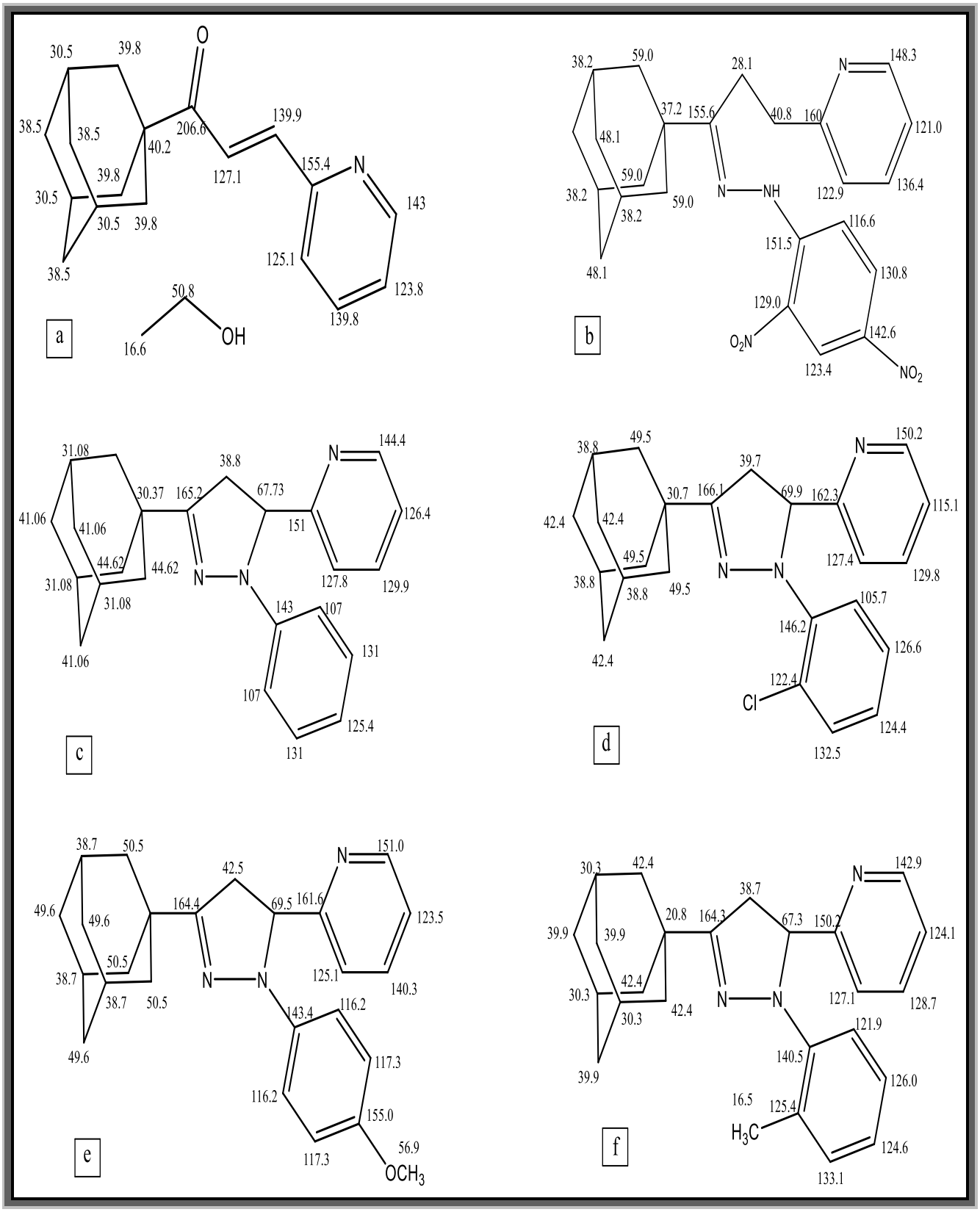


Fig-3. 12 a, b: ^{13}C NMR spectrum for compound (RS-5)(a). spreading (b). ^{13}C NMR (400 MHz, $CDCL_3$, δ ppm) δ = 164.3 (C-3, C=N), 38.7 (C-4, CH_2), 67.3 (C-5, CH), 142.9, 128.7, 127.1, 124.1 (4C, C_5H_4N), 150.2 (1C, sp2, C-C-N), 140.5, 133.1, 126.0, 125.4, 124.6, 121.9 (6C, C_6H_5), 16.5 (1C, CH_3), 42.4, 39.9, 30.3, 20.8 (10C, $C_{10}H_{15}$), 50.5 (1C, C_2H_6O).



Scheme 3. 3: Position of the chemical shift in ^{13}CNMR for the synthesized compound.

3.1.13 FTIR of adamantyl chalcone

In FT-IR spectrum of 1-((1s,3s)-adamantan-1-yl)-3-(pyridin-2-yl)prop-2-en-1-one ***(Fig-3.13)*** showed sharp peaks at 1716 cm^{-1} correspond to C=O, the peak appeared at 2904 cm^{-1} (C–H stretching of aliphatic adamantyl sp^3 hybridization), 3200 cm^{-1} (C–H stretching of aromatic ring sp^2 hybridization), 3393 cm^{-1} to O-H from ethanol, 1617 cm^{-1} back to C=C, C-C aromatic, 1223 cm^{-1} to C-H bend in pyridine and 1449 cm^{-1} to C=N.

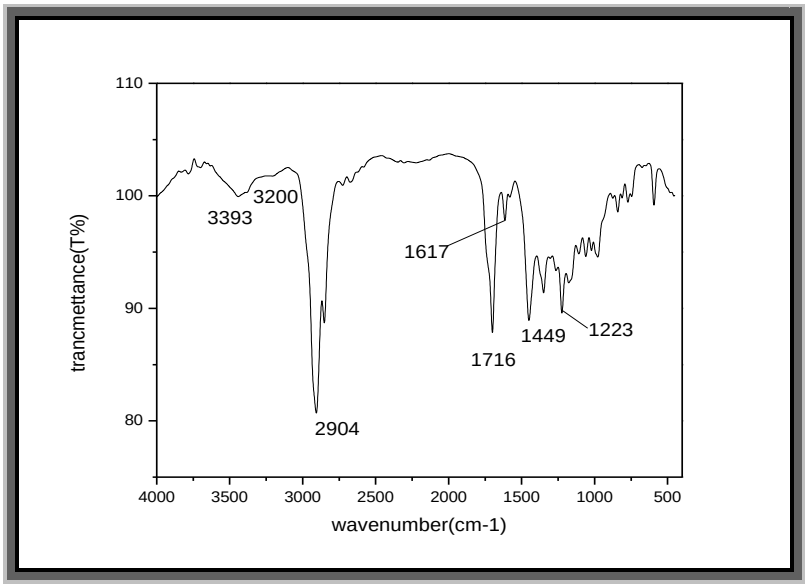


Fig-3. 13: FT-IR spectrum of adamantyl chalcone. FT-IR (KBr, cm^{-1}) for A : (3200, C-H, sp^2, Ar), **(**2904, C-H, sp^3, $C_{10}H_{15}$), (1716, C=O), (1617, C=C,C-C, C_5H_4N), (1449, C=N), (1223, C-H bend).

3.1.14 FTIR of pyrazole Compound (RS-1)

In FT-IR spectrum of compound ***RS-1*** 2-(3-((1s,3s)-adamantan-1-yl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine (***Fig-3.14)*** showed sharp peaks at 3286 cm^{-1} (C–H stretching of aromatic ring sp^2 hybridization), 2899 cm^{-1} (C–H stretching of aliphatic adamantyl sp^3 hybridization), The bands appeared at 3446 cm^{-1} confirm the presence of N-H functional group which indicate the ring open, the peaks appeared at 1737, 1616 cm^{-1} back to C=C, C-C aromatic, at 1447 cm^{-1} to C=N, 1134 cm^{-1} C-H bend, and at (1512, 1334 cm^{-1} N-O) The absorption bands due to the nitro group: N–O asymmetric stretch from 1512 $cm^{-1,}$ N–O symmetric stretch from 1334 cm^{-1}.

They are at lower wavenumbers than usual (1550-1360 cm^{-1}) because the nitro group is conjugated with the benzene ring.

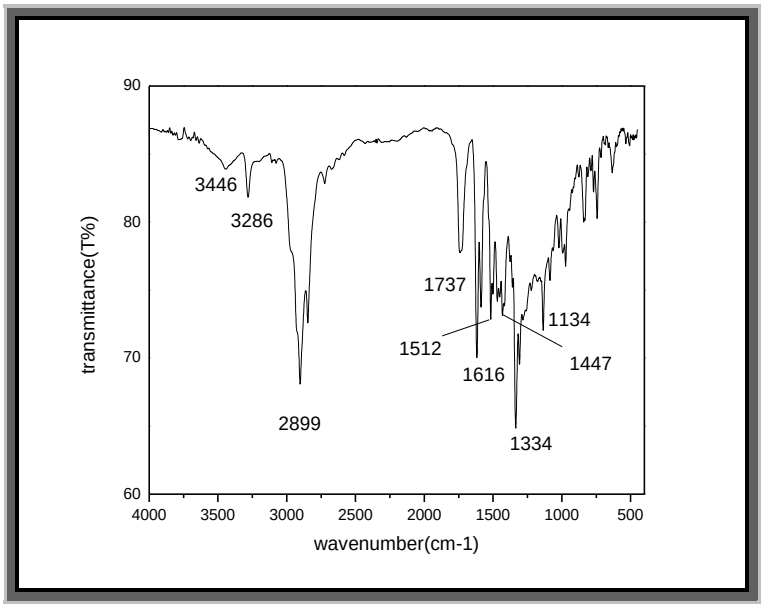


Fig-3. 14: FT- IR spectrum for compound (RS-1). IR (KBr, cm^{-1}) for RS-1: (3286, C–H,sp^2, Ar), (2899, C–H, sp^3, $C_{10}H_{15}$), (3446, N-H), (1737, 1616,C=C, C-C), (1447, C=N),(1512, 1334, N-O), (1134, C-H bend).

3.1.15 FTIR of pyrazole Compound (RS-2)

In FT-IR spectrum of compound ***RS-2*** 2-(3-((1s,3s)-adamantan-1-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine (***Fig-3.15)*** showed sharp peaks at 3059 cm^{-1} (C–H stretching of aromatic ring sp^2 hybridization), 2901 cm^{-1} (C–H stretching of aliphatic adamantyl sp^3 hybridization), The bands appeared at 1675,1597 cm^{-1} confirm the presence of C=C, C-C aromatic, at 1491 cm^{-1} to C=N and at 1307 cm^{-1} to C-H bend.

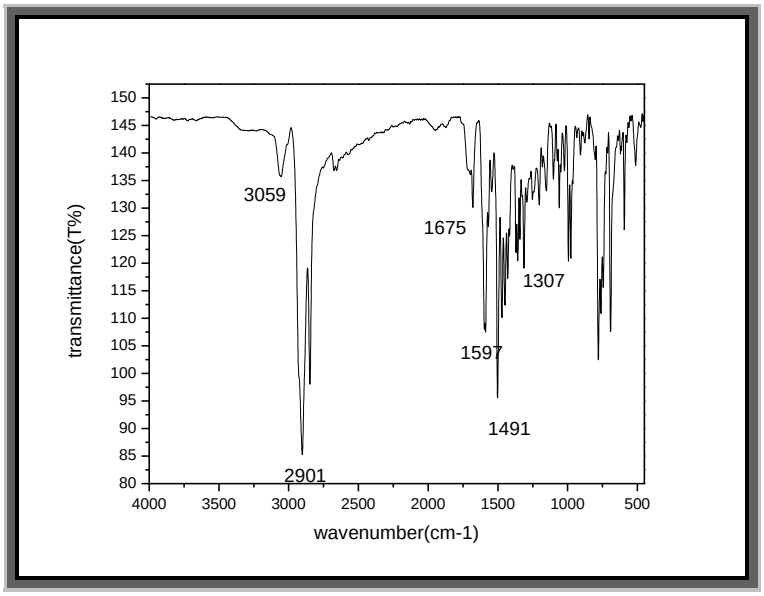


Fig-3. 15: FT-IR spectrum of compound (RS-2).FT- IR (KBr, cm^{-1}) for RS-2: (3059, C–H,sp^2, Ar), (2901,C–H, sp^3, $C_{10}H_{15}$), (1675, 1597, C=C, C-C, Ar), (1491, C-H bend), (1307, C–N).

3.1.16 FTIR of pyrazole Compound (RS-3)

In FT-IR spectrum of compound ***RS-3*** 2-(3-((1s,3s)-adamantan-1-yl)-1-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3-16)*** showed peaks at 3066 cm^{-1} (C–H stretching of aromatic ring sp^2 hybridization), 2901 cm^{-1} (C–H stretching of aliphatic adamantyl sp^3 hybridization). The bands appeared at 1688, 1597 cm^{-1} confirm the presence of C=C, C-C aromatic, at 1464 cm^{-1} to C=N and at 1301 cm^{-1} to C-H bend and the peak at 740 cm^{-1} ppm confirm the presence of C-Cl.

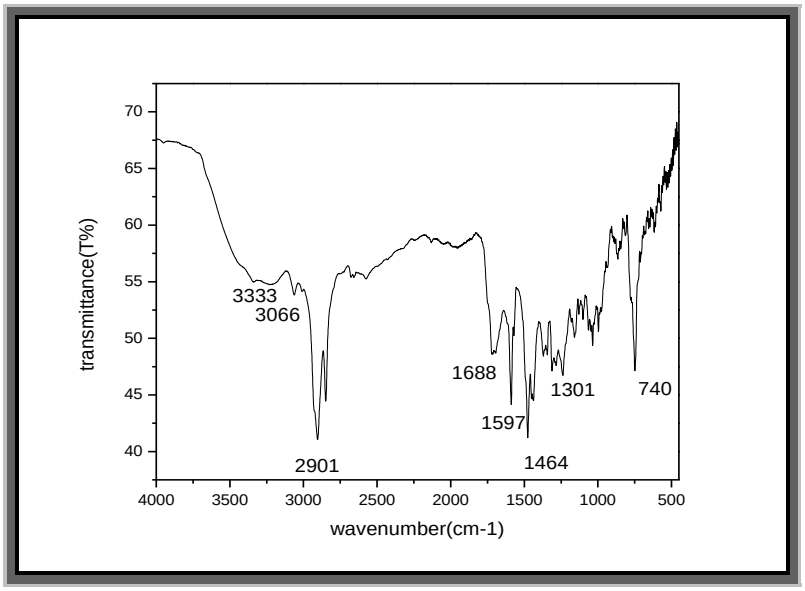


Fig-3. 16: FT-IR spectrum for compound (RS-3). FT-IR (KBr, cm^{-1}) for RS-3: (3066, C–H,sp^2, Ar), (2901, C–H, sp^3, $C_{10}H_{15}$), (1688, 1597,C=C, C-C, Ar), (1464, C=N), (1301, C-H bend), 740 (C-CL), (3333, OH, C_2H_5OH).

3.1.17 FTIR of Pyrazole Compound (RS-4)

In FT-IR spectrum of compound ***RS-4*** 2-(3-((1s,3s)-adamantan-1-yl)-1-(4-methoxyphenyl) -4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3-17)*** showed peaks at 3060 cm^{-1} (C–H stretching of aromatic ring sp^2 hybridization), 2914 cm^{-1} (C–H stretching of aliphatic adamantyl sp^3 hybridization), The bands appeared at 1695,1597 confirm the presence of C=C, C-C aromatic, at 1517 cm^{-1} to C=N and at 1241 cm^{-1} to C-H bend, the peak at 3336 cm^{-1} due to OH in ethanol.

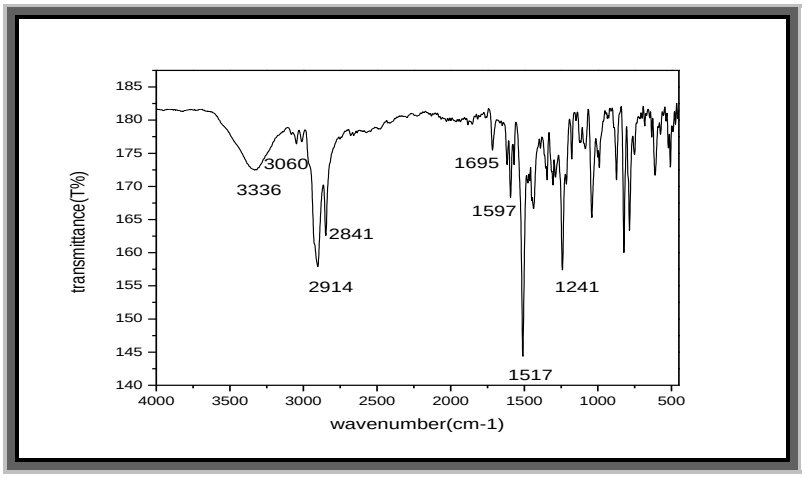


Fig-3. 17: FT-IR spectrum for compound (RS-4). FT-IR (KBr, cm^{-1}) for RS-4: (3060, C–H,sp^2, Ar), 2914 (C–H, sp^3, $C_{10}H_{15}$), 2841 (C-H, OCH_3), 1695, 1597 (C=C, C-C, Ar), 1517 (C=N), 1241 (C-H bend), 3336 (OH ,C_2H_5OH).

3.1.18 FT-IR of Pyrazole Compound (RS-5)

In FT-IR spectrum of compound ***RS-5*** 2-(3-((1s,3s)-adamantan-1-yl)-1-(o-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(Fig-3-18)*** showed peaks at 3052 cm^{-1} (C–H stretching of aromatic ring sp^2 hybridization), 2887 cm^{-1} (C–H stretching of aliphatic adamantyl sp^2 hybridization). The bands appeared at 1688,1583 confirm the presence of C=C, C-C aromatic, at 1449 cm^{-1} to C=N and at 1301 cm^{-1} to C-H bend.

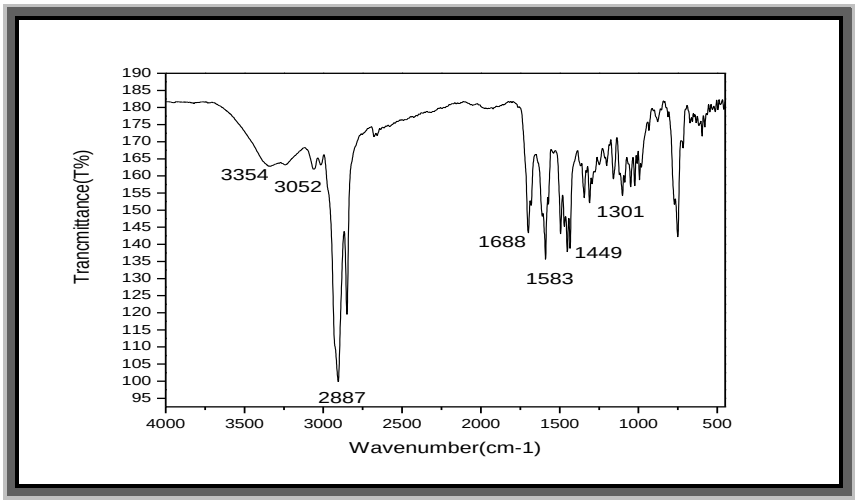


Fig-3.18: FT- IR spectrum for compound (RS-5). FT-IR (KBr, cm^{-1}) for RS-5: (3052, C–H, sp^2, Ar), 2887 (C–H, sp^3, $C_{10}H_{15}$), 1688, 1583 (C=C, C-C, Ar), 1449 (C=N), 1301 (C-H bend), (3354, OH, C_2H_5OH).

3.2 Thin Layer Chromatography (TLC)

The progress of the reaction was monitored by TLC. It was performed to ensure that the products are pure. Ethyl acetate: Hexane (2:5) is the mobile phase which was used. The TLC shows that a pure product was obtained for all the synthesised compounds ***RS 1-5***. The Retention Factor (R_f) values for the starting material and the products are included in ***Table-3.1***.

Table-3. 1: Retention factor (R_f) values for the prepared compounds RS 1-5.

Synthesized compounds	**R_f for A**	**$R_f$for (B 1-5)**	**R_f for (RS 1-5)**
RS-1	0.54	0.18	0.62
RS-2	0.54	0.66	0.60
RS-3	0.54	0.78	0.58
RS-4	0.54	0.56	0.38
RS-5	0.54	0.74	0.56

3.3 Melting Point and Present Yield

Determining the melting point of a new synthesised compound is one way to test if the substance is pure. (***Table-3.2)*** includes the melting, colour and present yield for ***RS 1-5*** compounds. The colour compounds is due to the presence of chromophore conjugation. It is a region in the molecule where the energy differs between two separate molecular orbitals and falls within the range of the visible spectrum. Visible light that hits the chromophore can thus be absorbed by exciting an electron from its ground state into an excited state. The electrons jump between energy levels that are extended pi orbitals, created by a series of alternating single and double bonds, in aromatic systems.

The molecular formula and molecular weight for synthesized pyrazole compound are listed in (***Table-3.3)***.

Table-3. 2: Melting point, colour and present yield for the new RS 1-5.

Synthesised Compounds	The melting point(^{0}C)	Colour	Present yield
A	95-97	Bright yellow	70.4%
RS-1	182-184	Orange	88.3%
RS-2	107-109	Dark yellow	78.7%
RS-3	Oily	Yellowish brown	71.7%
RS-4	82-83	Brownish orange	75.1%
RS-5	70-72	Yellowish orange	89.6%

Table-3. 3: Molecular formula and molecular weight for RS 1-5.

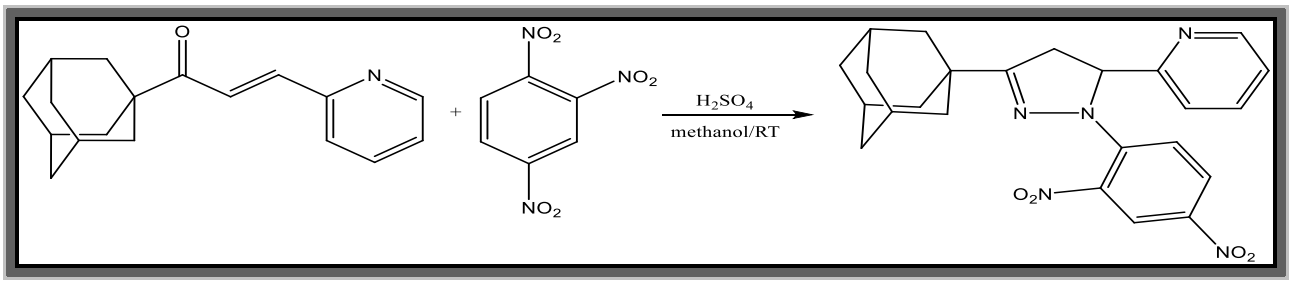
The synthesized compounds	The molecular formula	The molecular weight
RS-1	$C_{24}H_{25}N_5O_4$	447.49 g/mol
RS-2	$C_{24}H_{26}N_3$	357.48g/mol
RS-3	$C_{24}H_{26}N_3CL$	391.93 g/mol
RS-4	$C_{25}H_{29}N_3O$	387.47g/mol
RS-5	$C_{25}H_{29}N_3$	371.51g/mol

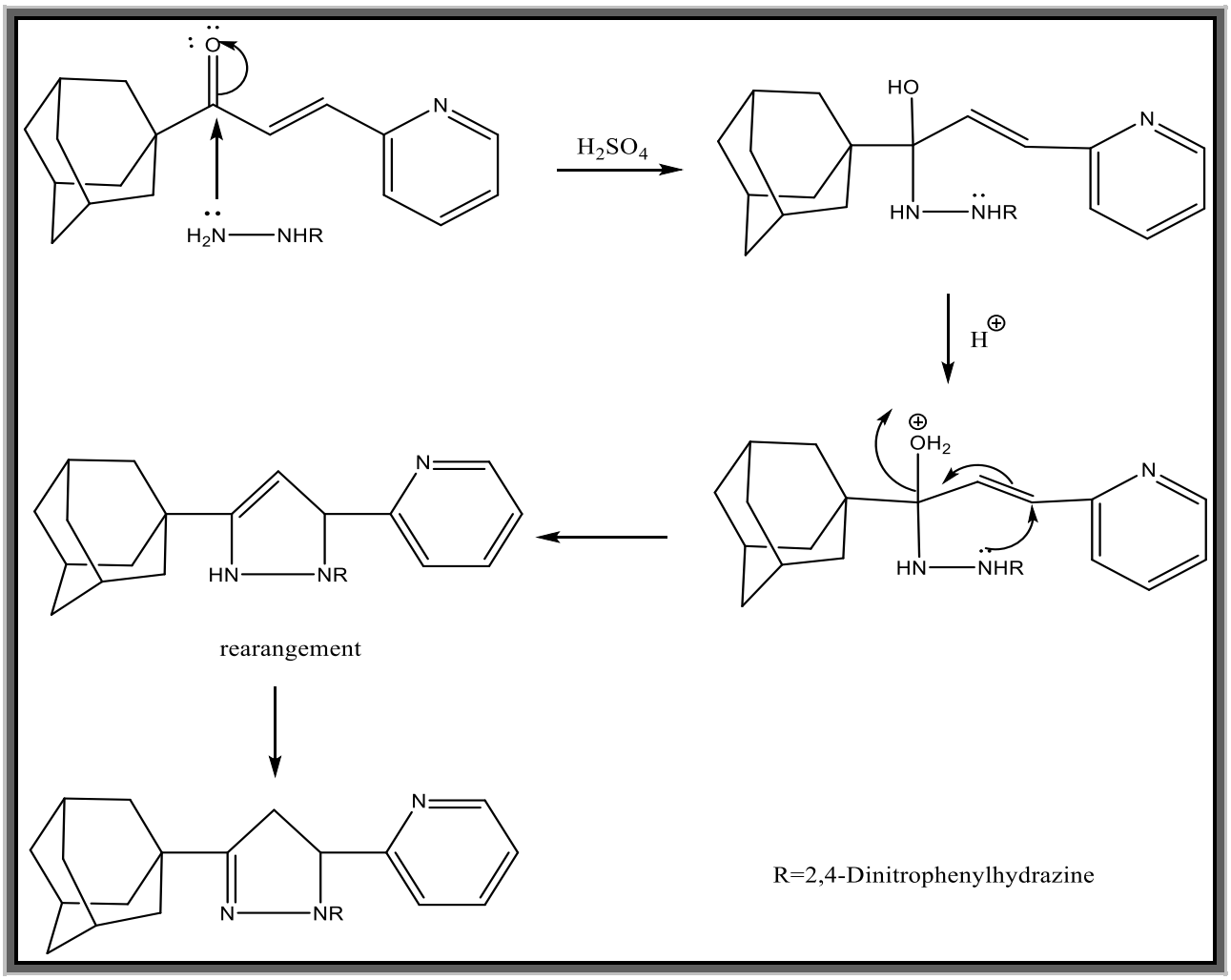
3.4 The Expected Mechanism for The Synthesized Compounds

3.4.1 Mechanism for RS-1 Compound

The proposed general mechanism of this reaction is shown in ***(scheme 3.4)***. In this cyclization, first the amine nucleophilic attack of carbonyl carbon of the chalcone by nitrogen atom of 2,4-dinitrophenylhydrazine and the carbonyl oxygen gets hydroxylation. This reaction involves the initial formation of an aryl hydrazone, another end of the nitrogen atom of hydrazine bonded with β-

carbon of chalcone leads to cyclization, the unsaturation was shifted between carbonyl and α carbon of the chalcones. The hydroxylated group was eliminated as water molecule. Migration of proton and the π-bond was shifted to N-2 and C-3 of the azole ring (Thirunarayanan & Sekar, 2013) *(Kitawat & Singh, 2014) (Sunil, 2012) (Li, 2006).*



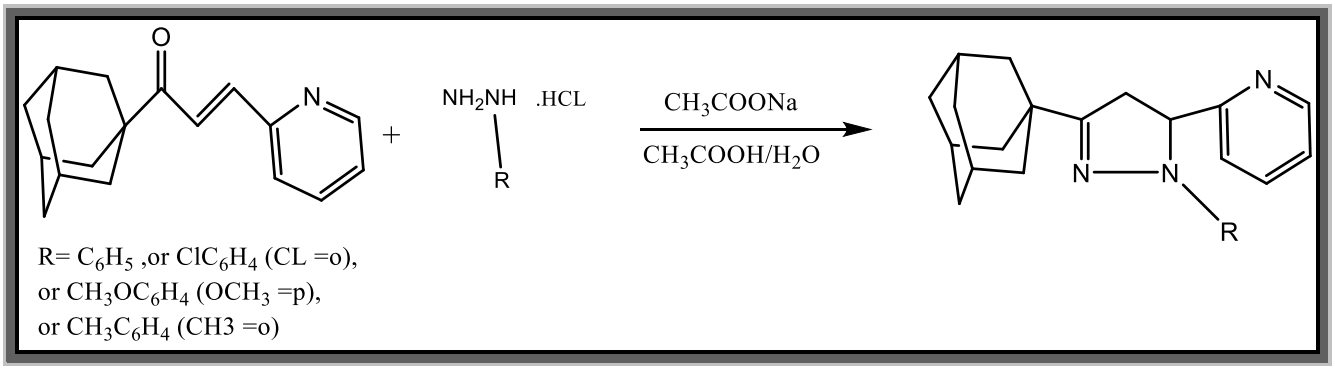


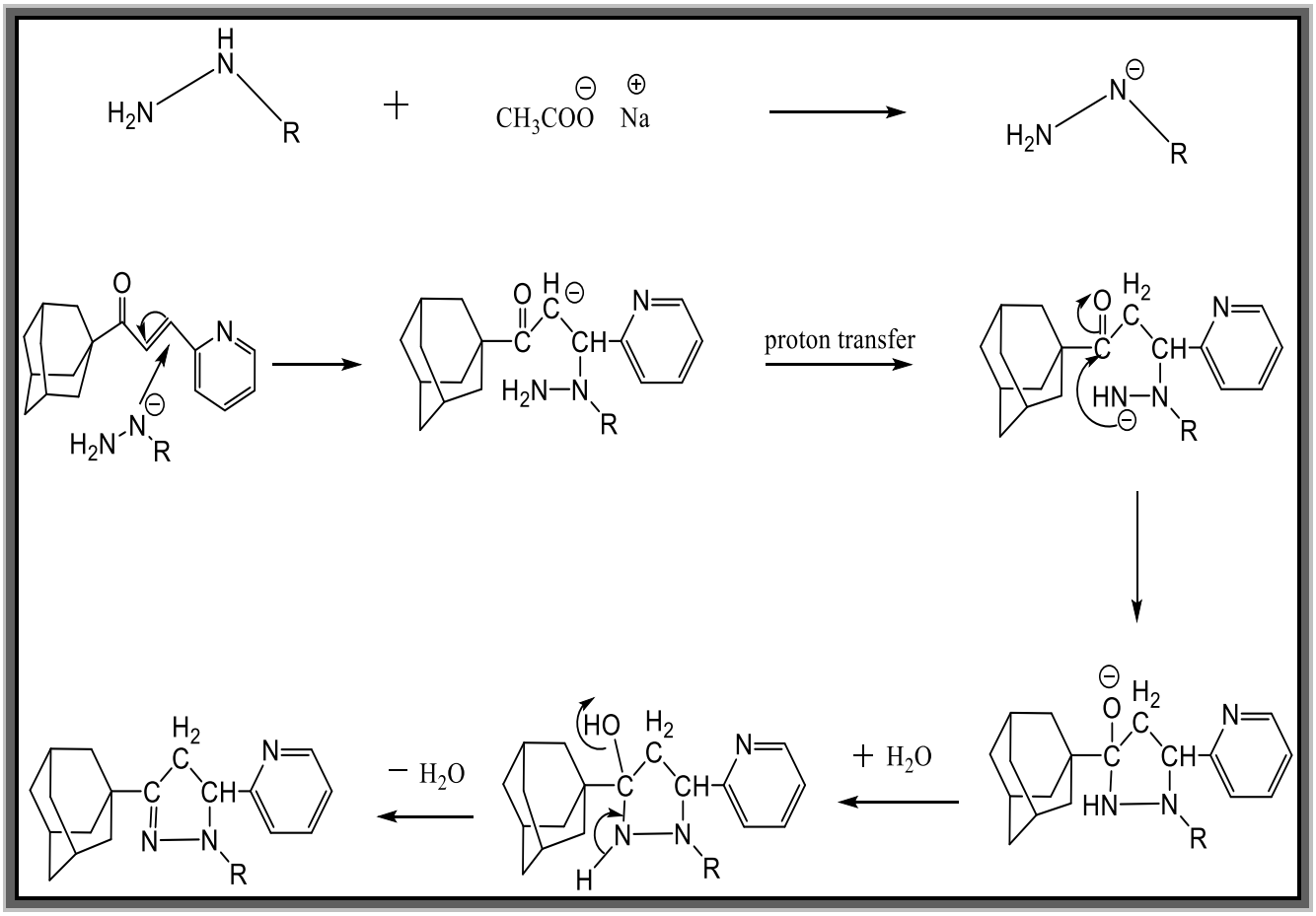
Scheme 3. 4: Mechanism for RS-1 compound.

In new prepared compound, during spectroscopic analysis it's appeared that the RS-1 compound was stayed open chain.

3.4.2 Mechanism for RS 2-5 Compounds

The proposed mechanism for the formation of pyrazoline is depicted in ***(scheme 3.15)***. The reaction proceed by Michael addition of (nucleophile) R-$\bar{N}$-NH_2 to the chalcones (1,4-addition) followed by proton transfer, cyclization via Claisen addition, hydrolysis and spontaneous dehydration (Khazaal & Tomma, 2017; Scheme, 2010) (Patil, Mahajan & Katti, 2010).





Scheme 3. 5: Mechanism for compounds (RS 2-5)

3.5 Evaluation of Antibacterial Activity of RS 1-5 Compounds

The Inhibition Zone Diameters (IZD) results of the synthesized compounds ***RS 1-5*** against gram-negative bacteria species *Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhimurium* and *Escherichia coli,* gram-positive bacteria species such as *Bacillus subtilis* and *Staphylococcus aureus,* are summarized in (***Table-3.4)***. The investigation of the antibacterial screening of the test samples ***RS 1-5*** revealed that all these compounds exhibited different degrees of antibacterial activity in relation to the tested microbial species and showed moderate to weak antibacterial activity against all the organisms (***Fig-3.19)*** show the IZD of two types of bacterial strain .

The results of MIC's determined reveal that the test compounds can act as a good antibacterial agents at higher concentrations, and no inhibition zone at lower concentration (***Fig-3.20)***,(***Fig-3.21)***.

The compound **A** which is the intermediate used for synthesizing the new pyrazoline compounds, also is examined for its antibacterial species and showed good to moderate activity against bacterial strain.

Table-3. 4: IZD of the synthesized compounds RS 1-5 against bacteria species in mm.

	Name of Microorganisms					
Antibiotic	Bacillus subtilis gram(+)	Staphylococcus aureus gram(+)	Pseudomonas aeruginosa gram(-)	Klebsiella pneumonia gram(-)	Salmonella typhimurium gram(-)	Escherichia coli gram(-)
Standard	Meropenem	Ampicillin	Meropenem	Meropenem	Meropenem	Gentamycin
	30	30	25	25	20	20
A	19.5	13.7	12.7	17.7	9.3	11.3
RS-1	8.7	9.2	13.3	15.3	10.3	10.7
RS-2	11.3	11.7	9.7	12.3	9.3	11.3
RS-3	9.3	10.7	8.7	10.3	8.3	11.3
RS-4	13.7	11.7	8.7	13.3	9.3	10.3
RS-5	12.3	10.7	11.7	12.7	10.7	10.7

*Results are expressed as mean of three determinations (n=3)

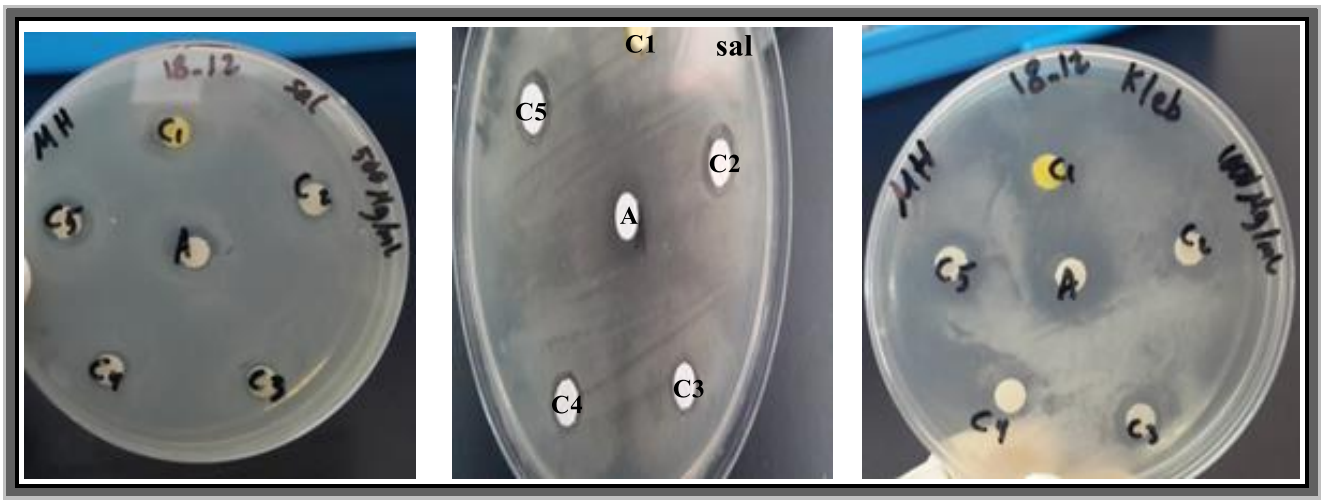


fig-3. 19: Bacterial petri dishes shows the IZD of two types of bacterial strain (*Salmonella* and *Klebsiella*)

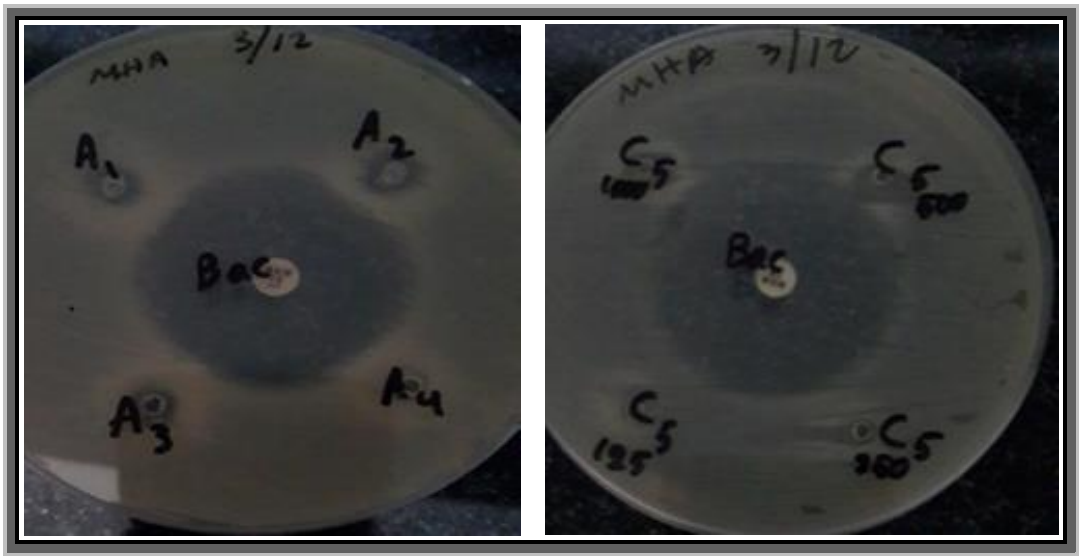


fig-3. 20: Bacterial petri dishes show no inhibition at low concentration (125 µg/ml)

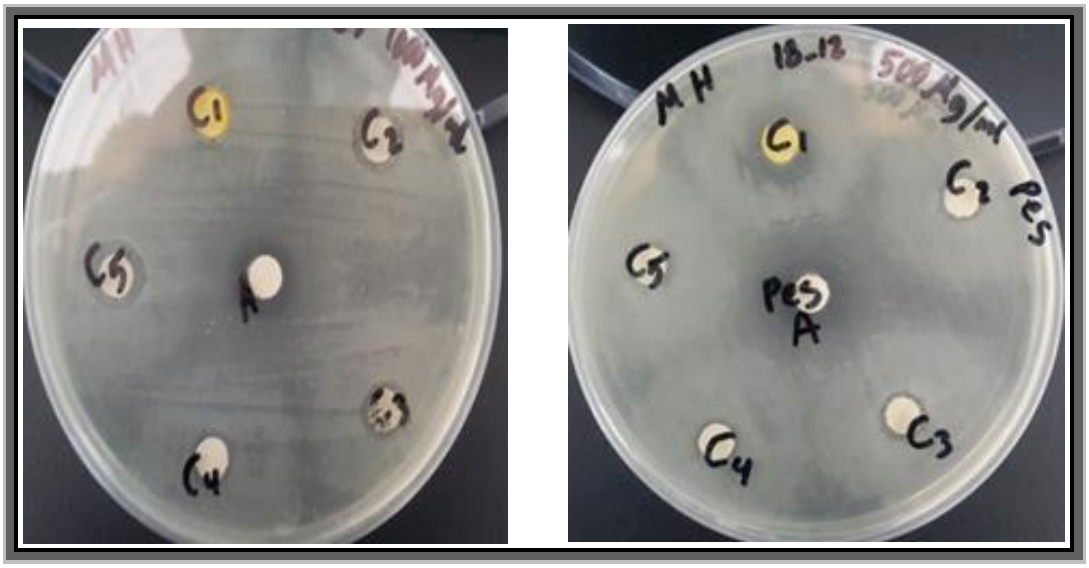


Fig-3. 21: Bacterial petri dishes shows the little changes in IZD when the concentration changes from 1000 µg/ml to 500 µg/ml.

3.6 Evaluation of Antifungal Activity of RS 1-5 Compounds

The experimental results indicated that new compounds ***RS 1-5*** revealed that all compounds significantly reduced the mycelial growth rate of *Fusarium oxysporum* fungus at concentrations (20-160 µg/ml) and clearly had antifungal activity (***Table-3.5)***. The fungal growth reduction induced by the compounds **RS-1, RS-2 , RS-3, RS-4** and **RS-5** at 160 µg/ml were 6%, 31%, 54%, 49%, and 56%, respectively, compared with control. In additions, the reductions were highly negative correlated with highly correlation coefficient (***Table-3.6)***. The compounds **RS-3**, **RS-4**, **RS-5** were highly active against *Fusarium oxysporum,* which may be attributed to the presence of electron donating group, chlorine in ortho position, methoxy in para position and methyl in ortho position of benzene ring, respectively. The test samples **RS-2** has shown moderate activity against *Fusarium oxysporum,* which may be due to no substituents on benzene ring**,** while **RS-1** marked lesser activity against the fungus, which may be due to the presence of electron withdrawing -NO2 groups on the aromatic benzene ring (Jayaroopa, 2013) . The compound **A** which is the intermediate used for synthesis the new pyrazoline compounds, also examined it antifungal species and showed good

activity against *Fusarium oxysporum* fungus (66%). The extent of antifungal activity depended on the concentration and the type of functional groups present in the molecule.

Table-3. 5: Effect of the prepared compounds RS 1-5 against the mycelium growth rate (cm²/day) of the fungus *Fusarium oxysporum* grown on potatoes dextrose agar medium amended with the compounds at concentrations (0-160 µg/ml) and incubated at 25°C.

	Concentration					
Antibiotic	0 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	120 µg/ml	160 µg/ml
A	7.8	4.9	4.5	3.7	3.1	2.5
RS1	7.8	7.6	7.6	7.4	7.4	7.3
RS2	7.8	5.9	5.7	5.6	5.5	5.4
RS3	7.8	4.6	4.3	4	3.9	3.6
RS4	7.8	5.3	4.9	4.8	4.4	4
RS5	7.8	4.5	4.4	4.2	3.8	3.4

*Results are expressed as mean of five determinations (n=5).

The extent of antifungal activity depended on the concentration and the type of functional groups present in the molecule. All test compound reduced significantly the mycelial growth rate MGR of *Fusarium oxysporum* correlated with concentration with highly correlation coefficient, summarised in (***Table-3.6)*** and (***Fig-3.22)***. A close investigation of the in vitro antifungal activity profile of the tri substituted pyrazolines gives a clear picture of the structure activity correlations among the compounds ***RS 1-5*** under study.

Table-3. 6: The Linear regression and correlation coefficient of the mycelial growth rate induced by the prepared compounds RS 1-5 at concentrations 0-160 µg/ml.

compound	Linear regression equation	correlation coefficient (R^2)
A	Y=-0.9343x+7.6867	0.8675
RS-1	Y=-0.0943x+7.8467	0.9242
RS-2	Y=-0.38x+7.3133	0.6151
RS-3	Y=-0.6686x+7.04	0.6454
RS-4	Y=-0.6229x+7.38	0.7461
RS-5	Y=-0.6943x+7.1133	0.6755

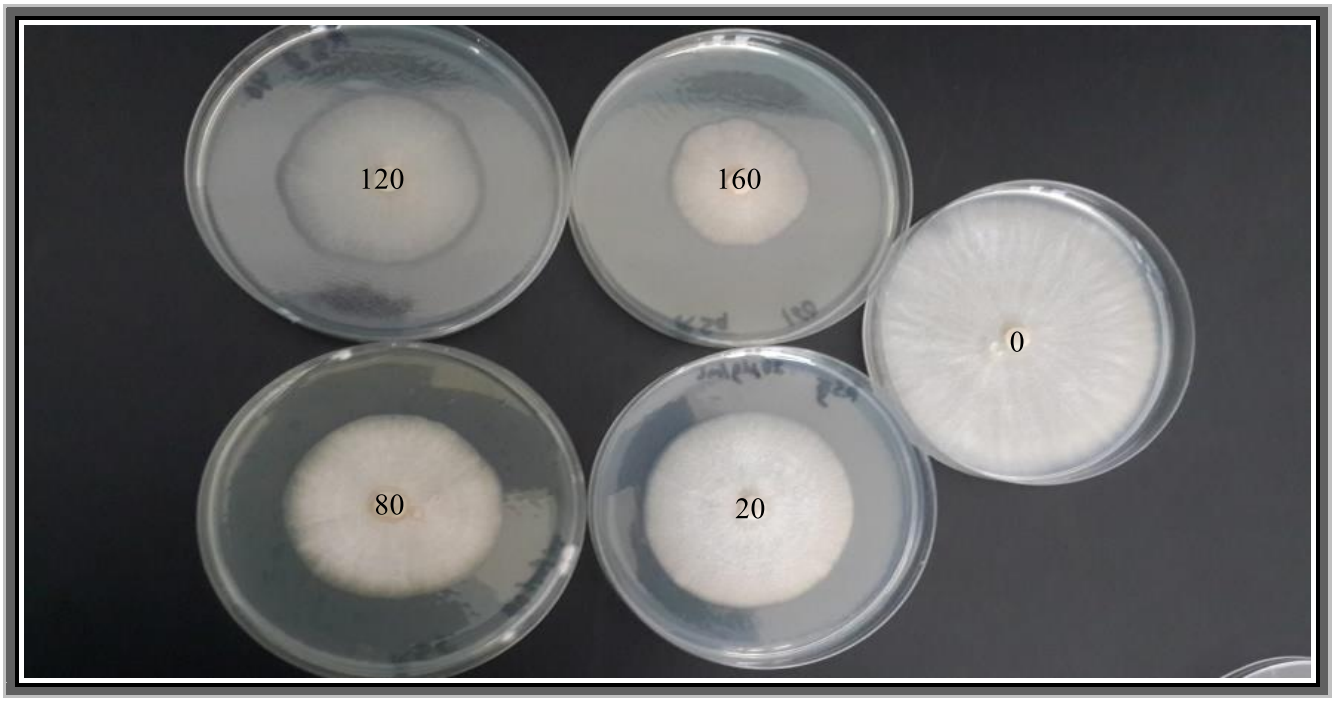


fig-3. 22: Effect of the prepared compound RS-5 against the mycelium growth rate (cm^2/day) of the fungus *Fusarium oxysporum* grown on potatoes dextrose agar medium amended at concentrations (0-160 µg/ml) and incubated at 25°C.

Conclusions and Future directions

4.1 Conclusions

The research work described in this thesis demonstrates the synthesis of new chemical entities of pyrazole/pyrazoline derivatives. The synthesized compounds were characterized by standard spectroscopic techniques. The evaluation of the antimicrobial activity of all new compounds was carried out against fungi and bacteria and proven to have significant to moderate activity. In general; By cyclizing adamantyl chalcones into their corresponding pyrazolines derivatives the anti-microbial activity was shown to turn moderate.

4.2 Future directions

There is a need in drug design strategy to achieve more anti-microbial potency in different bacterial and fungus strain on novel future synthetic heterocyclic compounds. Moreover, there is a need for other ways to test these compounds on various assays for additional therapeutic areas like inflammatory and carcinoma cell lines.

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الملخص

تحظى كيمياء الشالكون بالاهتمام العلمي نظرا لنشاطاتها البيولوجية المتعددة، والشالكون عبارة عن سلسلة مفتوحة من الفلافونويد موجود في النباتات الصالحة للأكل وقد أظهرت هذه المجموعة انشطة دوائية واسعة، ونتيجة لسهولة اجراء تعديلات على هياكلها التركيبية فقد منح ذلك امكانية واسعة لإنتاج مركبات جديدة ذات فاعلية اعلى وسمومية اقل، كما يستخدم الشالكون كمركب وسطي لتحضير المركبات الحلقية غير المتجانسة، وهي مركبات ضرورية للحياة في مجالات متعددة وتمتاز بالنشاط الفسيولوجي والدور الهام في المجالات الدوائية فقدمت مشتقات البيرازولين أنشطة بيولوجية هائلة مثل مضادات الميكروبات، مضادات الالتهابات، مضادات الأورام و مضادات الأكسدة. وفي هذه الدراسة تم اجراء تعديلات على مركب الشالكون من خلال تفاعله مع مشتقات الفينيل هيدرازين وصولا لتحضير خمس مركبات مستحدثة تحوي مجموعة البيرازولين، وقد تم اجراء تحليل طيفي لهذه المركبات باستخدام كل من طيف الرنين النووي المغناطيسي $^{1}HNMR$, $^{13}CNMR$ والأشعة تحت الحمراء *FT-IR*. وتم فحص النشاط البيولوجي لهذه المركبات الجديدة ضد البكتيريا والفطريات وقد اظهرت المركبات الجديدة نشاطاً جيداً إلى معتدل ضد جميع الأنواع الميكروبية المستخدمة للفحص.

جامعة الخليل

كلية العلوم والتكنولوجيا

الدراسات العليا في العلوم الكيميائية

تحضير بعض مركبات البيرازولون ومشتقات البيرزول المستمدة من مشتقات الأدمانتيل شالكون

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قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في الكيمياء بكلية الدراسات العليا والبحوث الأكاديمية في جامعة الخليل، الخليل، فلسطين

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