Studies on Heterocyclic Compounds of Medicinal Interest

A Thesis Submitted in the Fulfillment of the Requirements of the Award of the Degree

Doctor of Philosophy

From

Saurashtra University

By

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Under the Guidance of
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Statement under O.Ph.D.7 of Saurashtra University

The work included in the thesis is done by me under the supervision of Dr. V. H. Shah and the contribution made thereof is my own work.

Date:

Place: Rajkot (Dipti K. Dodiya)

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Certificate

This is to certify that the present work submitted for the Ph. D. degree of Saurashtra University, Rajkot, Gujarat (India) by Ms. Dipti K. Dodiya has been the result of work carried out under my supervision and is a significant contribution in the field of synthetic organic chemistry.

Date:
Place: Rajkot

Dr. Viresh H. Shah
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“It takes a day to make a dream,
But it takes many nights for a seed to become a tree.

Life is a ladder that must be climbed.
But in every stage,

There are many rivers and battles to fight
And our hopes determines our future.

Life is a trip through the wilderness
And everyone must survive for success.

But without a determination
We can never reach our destination.

There are many roads in life,
But choice

Stands between the broad and the narrow.
The world is not only what we see

But what we hear
Life is time and time is tide.

We are making an endless journey
But no ladder is without an end

Problems may fall like rain
But **every seed has its season**.”

Table of Contents

List of Abbreviations

NCEs	New Chemical Entities
R & D	Research & Development
Da	Dalton
HTS	High Throughput Screening
HIV	Human Immunodeficiency Virus
IR	Infrared
^{1}H-NMR	^{1}H- Nuclear Magnetic Resonance spectroscopy
TLC	Thin Layer Chromatography
R_f	Retardation factor
FT-IR	Fourier Transform- Infrared spectroscopy
KBr	Potassium Bromide
GC-MS	Gas Chromatograph- Mass Spectrometry
MHz	Megahertz
m.p.	Melting Point
mL	Milliliter
h.	Hours
DMSO-d_6	Dimethyl sulfoxide-deuteriated
Ms	Mass
Anal. Calcd.	Analytical Calculated
MIC	Minimum Inhibitory Concentration
MTCC	Microbial Type Culture Collection
NCCLS	National Committee for Clinical Laboratory Standards
DMSO	Dimethyl sulfoxide
CDK-1	Cyclin Dependent Kinase-1
PDE4	Phosphodiesterase-4
VEGFR/PDGFR	Vascular Endothelial Growth Factor Receptor-2/ Platelet Derived Growth Factor Receptor
DMF	Dimethylformamide
^{13}C NMR	^{13}C- Nuclear Magnetic Resonance spectroscopy

CS_2CO_3	Cesium carbonate
BF_3	Boron Trifluoride
n-BuLi	n-Butyl lithium
MAOS	Microwave-Assisted Organic Synthesis
MW	Microwave
W	Watt
$POCl_3$	Phosphorus Oxychloride
DNA	Deoxyribo-Nucleic Acid
AchE	Acetylcholinesterase
DHP	Dihydropyridine
NH_4OAc	Ammonium acetate
MWI	Microwave Irradiation
Mins.	Minutes
TMS	Trimethylsilane

General remarks

1. ^{1}H NMR spectra were recorded on Bruker Avance II 400 MHz or Bruker DRX 300 MHz or Varian 400 MHz spectrometer using TMS as an internal reference.

2. Mass spectra were recorded on GC-MS QP-2010 spectrometer.

3. IR spectra were recorded on Schimadzu FT-IR-8400 spectrometer.

4. Elemental analysis was carried out on Vario EL III Carlo Erba 1108.

5. Thin layer chromatography was performed on Silica Gel (Merck 60 F_{254}).

6. The chemicals used for the synthesis of compounds were purchased from Spectrochem, Merck, Thomas-baker and SD fine chemical.

7. Melting Points were taken in open capillary and are uncorrected.

8. Microwave assisted reaction were carried out in QPro-M microwave synthesizer.

9. All the structures are drawn according to ACS Document 1996 style.

Synopsis

A brief summary of the research work to be incorporated in the thesis entitled **"Studies on Heterocyclic Compounds of Medicinal Interest"** can be divided into five chapters which can be summarized as under.

Chapter 1 Introduction

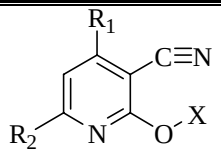
Nowadays, the entire pharmaceutical industry is faced with the challenge of increasing productivity and innovation. The major hurdles are the increasing costs of research and development and a simultaneous stagnating number of New Chemical Entities (NCEs).

Chapter 1 gives a brief introduction for the pressing need of New Chemical Entity (NCE) for pharmaceutical industry. It also describes importance of bicyclic and tricyclic aromatic heterocycles in drug discovery. Concept of "privileged structures" is also explained in brief. Chapter 1 also describes aims and objectives of the proposed research work.

Chapter 2 Studies on Cyanopyridines

Section-I: Synthesis and Biological Evaluation of 6-(substituted-2-chloro quinolin-3-yl)-2-alkoxy-4-aryl-pyridine-3-carbonitriles

Many naturally occurring and synthetic compounds bearing pyridine scaffold possess interesting biological properties. In association with those, pyridine-3-carbonitrile derivatives have been reported to posses a wide spectrum of biological activities such as antitumor, antihypertensive, antimicrobial, antiinflammatory, analgesic and antiviral etc.



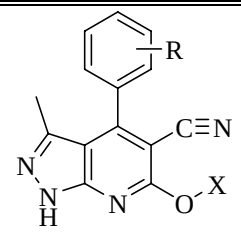
DDK- A-01 to A-20

Where, R_1 = substituted 2-chloroquinolin-3-yl, R_2 = Substituted phenyl
X = Alkyl

In the view of these reports, Section-I of Chapter 2 covers synthesis of new 2-alkoxy-pyridine-3-carbonitrile derivatives in search of better therapeutic agents. The synthesis was accomplished by the condensation of chalcones with malononitrile in presence of corresponding alkoxide anion.

Section-II: Synthesis and Biological Evaluation of 6-alkoxy-3-methyl-4-aryl-1H-pyrazolo[3,4-b]pyridine-5-carbonitriles

Pyrazolopyridines and its hydroderivatives are very interesting pyrazole derivatives with wide-ranging biological activities. A number of pyrazolo[3,4-*b*]pyridines exhibit a wide range of biological activities, including interesting anxiolytic activity, dopamine D_3 receptor antagonist, antiherpetic and antiallergic properties.



DDK- A-21 to A-40

Where R = -H, 3-Cl, 4-Cl, 4-Br, 4-CH_3, 4-OCH_3 etc.; X = Alkyl

Keeping in mind the various biomedical applications of cyanopyridines and pyrazolo[3,4-*b*]pyridines, it was thought worthwhile to incorporate both these scaffolds in a single molecular framework to further assess the biological profile. To achieve this, new pyrazolo[3,4-*b*]pyridine-5-carbonitriles were synthesized by reaction of 3-methyl-1*H*-pyrazol-5(4*H*)-one with arylidene malononitriles in the presence of corresponding alkoxide anion, which is incorporated in the Section-II.

All the compounds were characterized by FT-IR, mass, ^{1}H NMR spectral analysis and elemental analyses. The newly synthesized compounds are subjected to antimicrobial activity.

Chapter 3 Microwave Assisted Synthesis of Heterocycles - An Overview

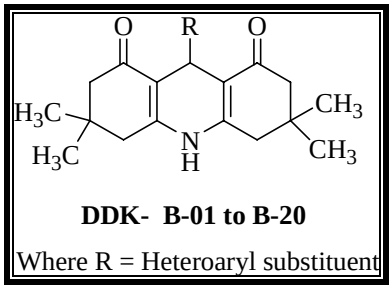
Microwave assisted organic synthesis has become an important tool to medicinal chemists for rapid organic synthesis. A huge number of research papers have appeared over the last decades on the application of microwave technology in organic synthesis. Some of the major advantages of microwave assisted organic synthesis include spectacular decrease in reaction time, improved conversions, clean product formation and wide scope for the development of new reaction conditions.

Chapter-3 briefly discusses the history of microwave assisted organic synthesis and their application in the synthesis of different heterocycles with different heteroatoms.

Chapter 4 Studies on Microwave Assisted Synthesis of Acridines

Acridine derivatives have been found to possess a wide spectrum of biological activities such as antibacterial, antimalarial, anticancer and mutagenic properties, principally connected with their ability to inhibit the enzymes acting on nucleic acids. Acridine derivatives have also been used to synthesize labeled conjugates with medicinals, peptides, proteins, and nucleic acids that exhibit antitumor and DNA-binding properties.

1,8-Dioxo-9-aryl-polyhydroacridines and their derivatives are polyfunctionalized 1,4-dihydropyridine derivatives. In recent years, 1,4-dihydropyridines and their derivatives have attracted strong interest due to a variety of biological activities such as vasodilator, bronchodilator, antiatherosclerotic, antitumor and antidiabetic activity etc.



DDK- B-01 to B-20

Where R = Heteroaryl substituent

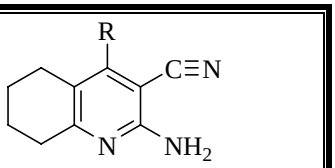
In the view of these observations, synthesis of two new series of 1,8-Dioxo-9-heteroaryl-polyhydroacridines has been undertaken in Chapter 4. The synthesis was effected by one pot, solvent-free condensation of 5,5-dimethyl-1,3-cyclohexane-dione, different heterocyclic aldehydes and ammonium acetate. The structures of all the newly synthesized compounds were elucidated by FT-IR, mass, ^{1}H NMR spectral analysis and elemental analyses. The newly synthesized compounds are subjected to antimicrobial activity.

Chapter 5 Studies on Microwave Assisted Synthesis of Polyhydroquinolines

It is common knowledge that quinolines and their derivatives are very useful compounds because a large number of natural products and drugs contain this heterocyclic unit. They have attracted strong interest due to their useful biological and pharmacological properties, such as antitumor, antiviral, antitubercular, antidiabetic and antibacterial activities.

Section-I: Synthesis and Biological Evaluation of 2-amino-5,6,7,8-tetrahydro-4-heteroaryl-quinoline-3-carbonitriles

In search of better therapeutic agents, two new series of polyhydroquinolines have been synthesized by the microwave-assisted reaction of cyclohexanone with arylidene malononitriles in presence of excess of ammonium acetate, which is included in Section-I.

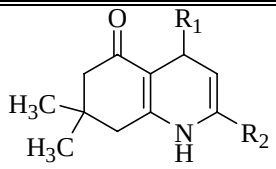


DDK-C-01 to C-20

Where R = Heteroaryl substituent

Section-II: Synthesis and Biological Evaluation of 7,8-dihydro-7,7-dimethyl-2,4-disubstituted-quinolin-5(*1H*,*4H*,*6H*)-ones

In Section-II, synthesis of another two series of substituted 5-oxo-polyhydroquinolines is included. The synthesis was accomplished by the microwave assisted solid state condensation of 5,5-dimethyl-1,3-cyclohexane-dione with different chalcones in presence of excess of ammonium acetate.



DDK-C-21 to C-50

Where R_1 and R_2 = Aryl or heteroaryl substituents

All the compounds were characterized by FT-IR, mass, ^{1}H NMR spectral analysis and elemental analyses. The newly synthesized compounds are subjected to antimicrobial activity.

Chapter 1
General Introduction

1.1 Heterocycles in drug discovery

Nowadays, the entire pharmaceutical industry is faced with the challenge of increasing productivity and innovation. The major hurdles are the increasing costs of research and development and a simultaneous stagnating number of new chemical entities (NCEs).

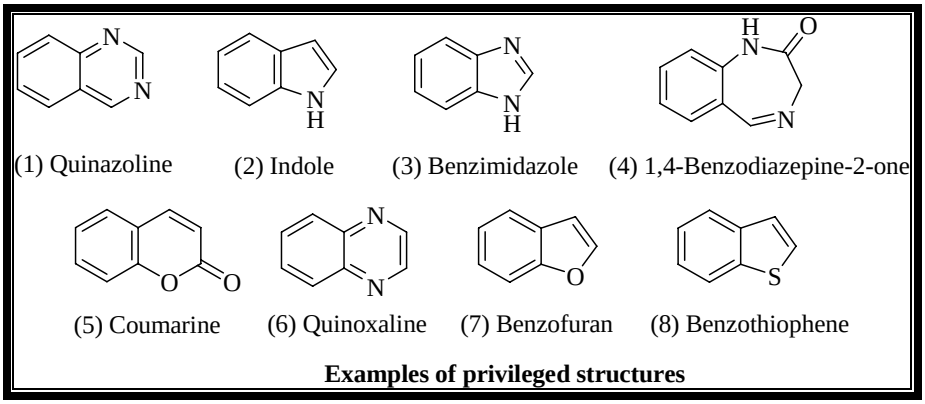
The cause of this innovation deficit is definitively not the biology. Decoding of the human genome has led to a wealth of drug targets. With more than 20,000 human genes, the assumption is that at least 1,000 are significantly involved in the emergence and course of disease. Furthermore, because each of these genes is linked to the function of between five and ten proteins, the conclusion is that there might be 5,000-10,000 targets for new drugs [1]. Despite the successful introduction of protein therapeutics and the promise of gene therapy, major pharmaceutical companies are still focused on the discovery and development of low-molecular weight compounds. Hence, the challenge is to select the most drugable targets and to find the corresponding drug-like molecules, substances that not only interact with the target, but also have specific pharmacokinetic and toxicological properties, that allow them to be developed as a drug.

Medicinal chemistry as a scientific discipline has introduced several new techniques over the last few years in order to speed up the drug discovery process, such as combinatorial chemistry, microwave-assisted organic synthesis, and high-throughput purification [2]. Despite this steady increase in R & D, the number of new chemical entities (NCEs) reaching the market has actually decreased dramatically.

It seems clear that selecting the appropriate molecules to synthesize is one of the most troublesome questions. It has been estimated that the number of possible molecules with a molecular weight of less than 500 atomic mass unit (Da) is 10^{200}, of which only

10^{60} may possess drug-like properties. The proportion of these drug-like molecules synthesized to date has been estimated as one part in 10^{57}, or roughly the ratio of the mass of one proton to the mass of the sun! The issue is therefore the selection of new molecules from this vast universe that have the potential to be biologically active [3].

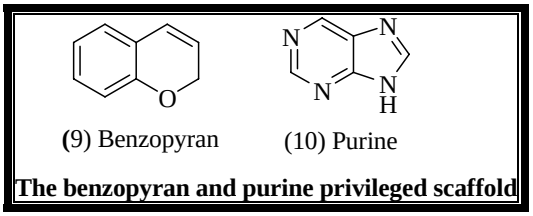
In order to start a new drug discovery project and to find biologically active compounds, different options are available. Hits can be obtained *via* a virtual screening approach or can be copied from scientific or patent literature. Very often, drug discovery projects start with a high-throughput screening campaign of commercially available compound libraries against the target of interest. It became clear in recent years that combinatorial libraries are not diverse enough. In this context, the concept of privileged structure has drawn remarkable attention. Privileged structures, as firstly formulated by Evans et al. [4], are structural motifs common to several drug and lead compounds, and endowed with versatile binding properties. Therefore, in principle, libraries derived from such scaffolds may provide promising compounds in diverse therapeutic areas [5, 6]. Notably, the likely drug-like properties of privileged structures and substructures might produce more drug-like compound libraries and leads [7].



Examples of privileged structures

Well-known examples of privileged substructures include benzodiazepines (4), coumarins (5), quinoxalines (6), benzofurans (7) and benzothiophenes (8) [6]. In order to improve the hit rate in HTS campaigns, privileged structures provide an ideal source of lead compounds. A single library based upon privileged substructures can lead to active compounds in variety of biological assays. Several research groups have utilized these structures in such a manner. For example, Nicolau K. C. et al.

constructed a library based on the benzopyran (9) privileged scaffold [8], whereas Schultz P. G. et al. made use of the purine (10) scaffold [9].



(9) Benzopyran (10) Purine

The benzopyran and purine privileged scaffold

1.2 Nomenclature of the fused ring system

As the following chapters deal with the synthesis of bicyclic fused ring systems, its nomenclature is herewith shortly reviewed. The nomenclature follows the following rules:

(1) The individual components are named without any application of fused ring system.

(2) The parent component is represented in the fusion name by citing it last in the name. The parent component is the one with highest priority according to the following criteria:

(a) a heterocyclic component containing the heteroatom occurring earliest in the order: N, F, Cl, Br, I, O, S, Se, Te, P, As, Sb, Bi, Si, Ge, Sn, Pb, B, Hg.

(b) a component containing the larger ring.

(c) a component containing the greater number of heteroatoms.

(d) a component containing the greater variety of heteroatoms.

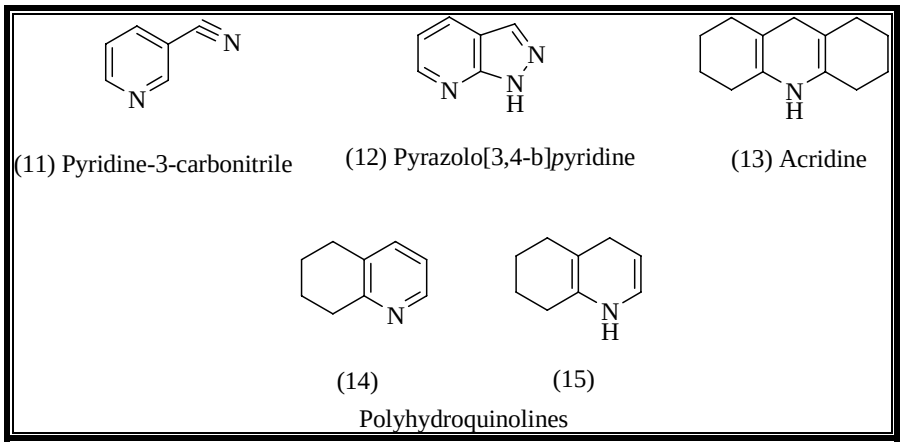
(3) The attached component is then added as a prefix to the parent component. In the name of the prefix, the terminal 'e' is changed to 'o'.

(4) The bonds of the parent component are indicated by a, b, c…starting with the bond normally occupying the 1,2 positions. The atoms of the attached component are numbered as usual, following the order of numbers in the original heterocycle.

(5) The numbering of the final condensed heterocycle is carried out independently, starting at an atom adjacent to a bridged-head atom, whereby heteroatoms receive the smallest possible number.

1.3 Objectives

Our interest in the synthesis and biological evaluation of heterocyclic bi/tricycles and the fact that some of these compounds viz. pyridine-3-carbonitriles (11), pyrazolo[3,4-*b*]pyridine-5-carbonitriles (12), acridines (13) and polyhydroquinolines (14 & 15) are not frequently used in the synthesis of compound libraries, prompted us to elaborate this type of chemistry and to synthesize four different heterocyclic scaffolds, which are privileged scaffolds or their derivatives with wide spectrum of biological activity.



1.4 References and notes

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Chapter 2
Studies on cyanopyridines

2.0 Introduction

The pyridine skeleton is of great importance to chemists as well as to biologists as it is found in a large variety of naturally occurring compounds and also in clinically useful molecules having diverse biological activities. Cyanopyridines comprise a very interesting class of compounds because of their significant and versatile biological and pharmacological activities. Cyanopyridines are important intermediates in the pharmaceutical industry for the synthesis of nicotinamide, nicotinic acid and isonicotinic acid etc [1]. The importance of cyanopyridines in organic synthesis has increased over the past few decades because they are among the most versatile organic synthetic intermediates [2]. Many fused cyanopyridines have drawn attention due to their wide spectrum biological activities [3].

In view of these observations and with a view to further explore the pharmacological profile of this class of compounds, the present study includes synthesis of novel 3-cyanopyridines (pyridine-3-carbonitriles) and fused cyanopyridines viz. pyrazolo[3,4-*b*]pyridine-5-carbonitriles. The study is divided in two sections:

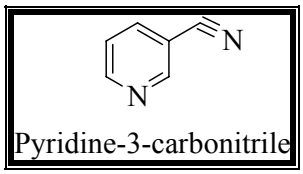
2.1: Synthesis and biological evaluation of 6-aryl-4-(substituted-2-chloro-quinolin-3-yl)-2-alkoxy-pyridine-3-carbonitriles

2.2: Synthesis and biological evaluation of 6-alkoxy-3-methyl-4-aryl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitriles

2.1: Synthesis and biological evaluation of 6-aryl-4-(substituted-2-chloro quinolin-3-yl)-2-alkoxy-pyridine-3-carbonitriles

2.1.1 Introduction

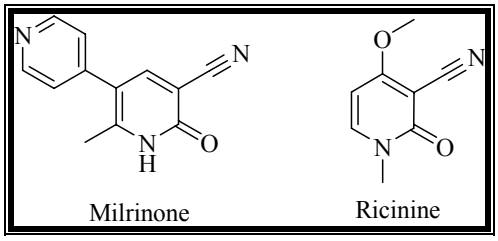
The pyridine motif is among the most common *N*-heteroaromatics incorporated into the structure of various therapeutic agents. Many naturally occurring and synthetic compounds bearing pyridine scaffold possess interesting biological properties. In association with those, 3-cyanopyridine or pyridine-3-carbonitrile derivatives draw a special attention for their wide spectrum biological activities along with their importance and utility as intermediates in preparing variety of heterocyclic compounds [1].



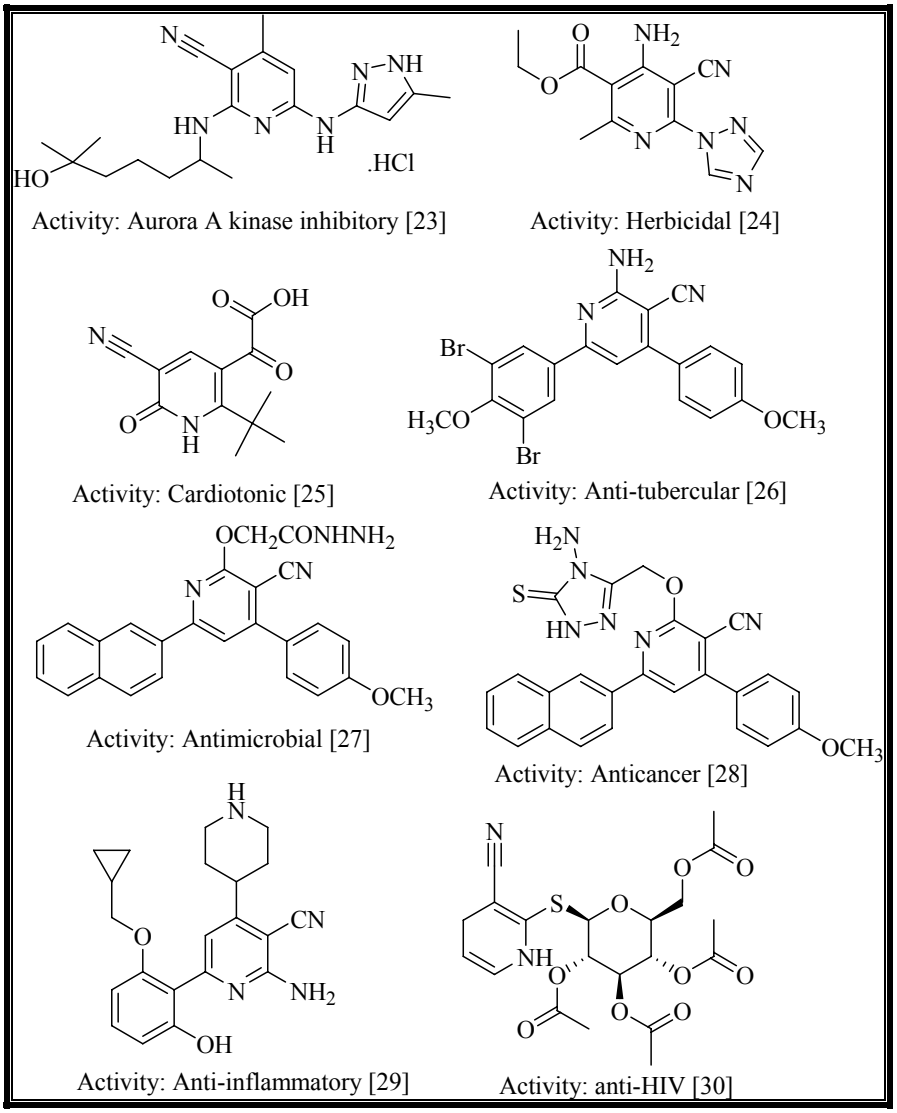
Pyridine-3-carbonitrile

It has been demonstrated that molecules containing 3-cyanopyridine moiety may be able to work as ligands towards transition-metal ions [4], new drugs [5], and significant intermediates for the synthesis of important vitamins [6] such as nicotinic acids [7] and nicotinamides [8].

The pharmacological and physiological activity of 3-cyanopyridines has attracted much attention in recent years with the synthesis and the study of the non-glycosidic cardiotonic agent milrinone [9, 10], as well as with a number of 3-cyanopyridine derivatives which proved to be active against the herpes virus and the human immunodeficiency virus [11, 12]. The 3-cyanopyridin-2-one nucleus is also the structural basis of the alkaloid ricinine-the first known alkaloid containing a cyano-group.



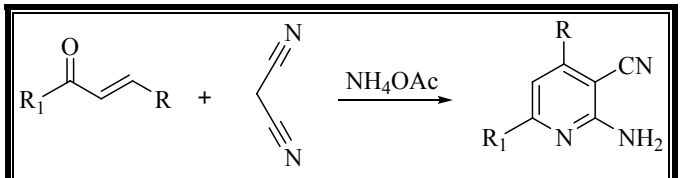
3-cyanopyridines with different alkyl and aryl/heteroaryl groups were found to have anti-tubercular [13], antimicrobial [14], anti-cancer [15, 16], A_{2A} adenosine receptor antagonists [17], antihypertensive [18, 19], anti-histaminic [20], anti-inflammatory, analgesic & antipyretic properties [21] as well as 1KK-β inhibitor properties [22]. Some examples of published derivatives of 3-cyanopyridines with their biological activities are as following.



2.1.2 Reported synthetic strategies

2.1.2.1. From α,β-unsaturated synthons

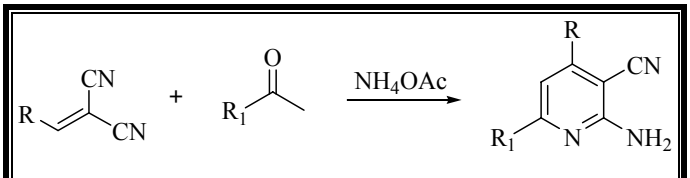
Large number of publications describe the synthesis of 3-cyanopyridines from α,β-unsaturated synthons. The Michael-type condensation of α,β-unsaturated ketones or 1,3-diaryl-prop-2-en-1-ones with malononitrile and ammonium acetate is probably the most reported strategy for the synthesis of 4,6-diaryl/heteroaryl-2-amino-3-cyano pyridines [26, 36-38] (Scheme 2.1.1). The reaction is generally carried out in alcohol in presence of excess of ammonium acetate. Recently, Sarda et al. have reported the reaction using ionic liquid ethylammonium nitrate [39].



R, R_1 = aryl or heteroaryl

Scheme 2.1.1

Reaction of arylidene malononitrile with alkyl and aryl ketones in presence of ammonium acetate is reported to yield 6-alkyl/aryl substituted 2-amino-3-cyano pyridines [40, 41] (Scheme 2.1.2).

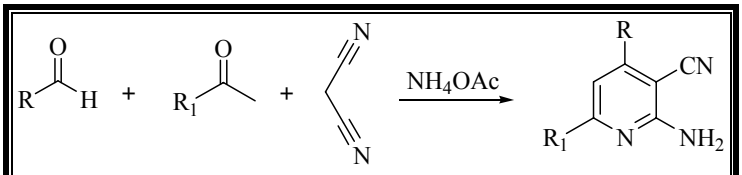


R = aryl or heteroaryl, R_1 = alkyl or aryl

Scheme 2.1.2

Number of multi-component approaches for the synthesis of 2-amino-3-cyano pyridines has also been reported involving one pot reaction of different aldehydes, ketones, malononitrile and ammonium acetate [22, 29, 42-44]. Heravi et al. have reported the green one-pot synthesis of 2-amino-3-cyano pyridines from 3,4-dimethoxyaceto-phenone, different aromatic aldehydes, malonitrile and ammonium acetate using hetero-

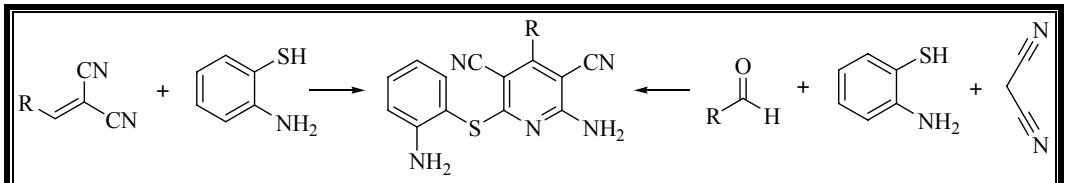
polyacid as heterogeneous and recyclable catalyst [45] (Scheme 2.1.3).



R, R_1 = aryl or heteroaryl

Scheme 2.1.3

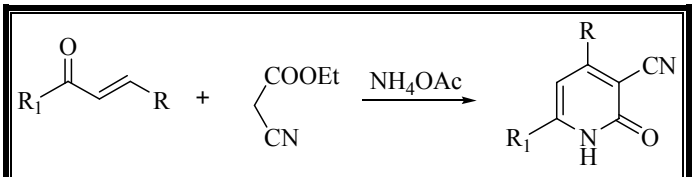
Reaction of arylidene malononitriles with 2-amino-thiophenol yielded 2-amino-3,5-dicyano-6-substitutedthioxo-pyridines [46]. Recently, multi-component reactions involving aldehydes, malononitrile and 2-aminothiophenol using boric acid [47] as well as potassium fluoride on alumina ($KF\text{-}Al_2O_3$) [48] as green catalysts have been described (Scheme 2.1.4).



R = aryl or heteroaryl

Scheme 2.1.4

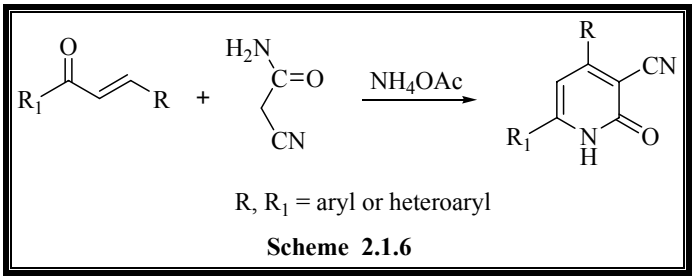
Condensation of ethyl cyanoacetate with α,β-unsaturated ketones in presence of excess ammonium acetate afforded 3-cyanopyridin-2-ones [31, 49-51] (Scheme 2.1.5). Also, a green chemistry approach describing reaction of α,β-unsaturated ketones with ethyl cyanoacetate using samarium iodide as catalyst has been reported recently [52].



R, R_1 = aryl or heteroaryl

Scheme 2.1.5

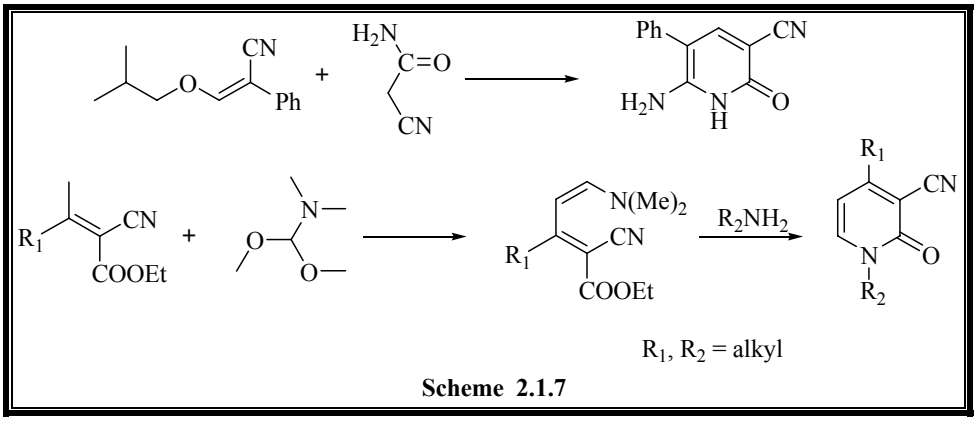
Barat reported first that condensation of cyanoacetamide with α,β-unsaturated ketones also affords 3-cyanopyridin-2-ones [53]. Number of reports following this approach have been reported till date [54-56] (Scheme 2.1.6).



R, R_1 = aryl or heteroaryl

Scheme 2.1.6

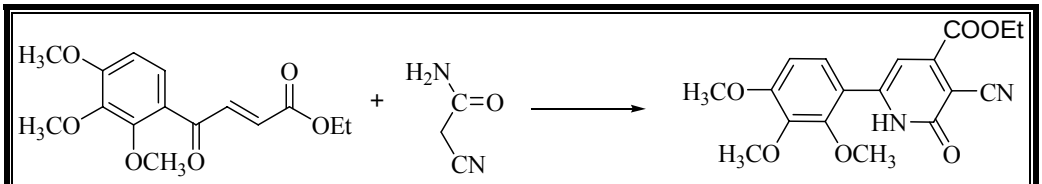
Chase et al. have synthesized 6-amino-5-phenyl-3-cyanopyridin-2-one by the reaction of 3-isobutoxy-2-phenylacrylonitrile with cyanoacetamide [57] (Scheme 2.1.7). Recently, Melikyan et al. have reported synthesis of novel N-substituted-3-cyanopyridin-2-ones from ylidenecyanoacetic acid ethyl esters in two steps [58] (Scheme 2.1.7).



R_1, R_2 = alkyl

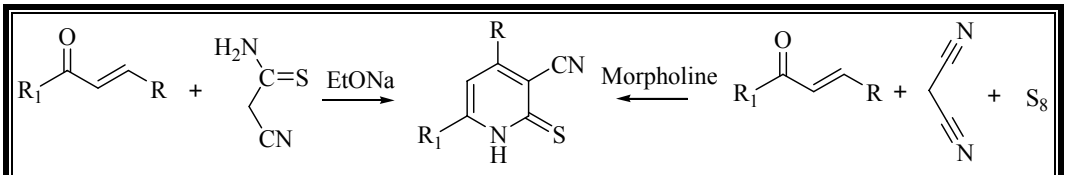
Scheme 2.1.7

Reaction of ethyl-(2,3,4-trimethoxybenzoyl)-pyruvate with cyanoacetamide in ethanol in presence of piperidine gave 4-carbehtoxy-6-(2,3,4-trimethoxybenzyl)-3 cyanopyridin-2-one [59] (Scheme 2.1.8).



Scheme 2.1.8

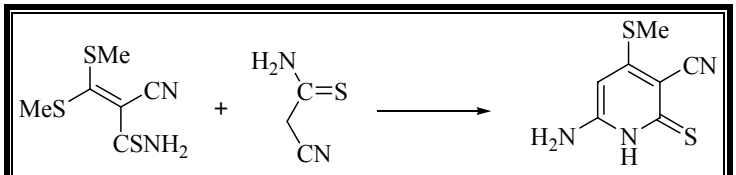
Chalcones react with cyanothioacetamide in the presence of sodium ethoxide to afford 3-cyano-pyridin-2-thiones [60, 61]. A one pot approach has also been reported to afford the 3-cyano-pyridin-2-thiones involving reaction of chalcones, malononitrile and elemental sulfur [62] (Scheme 2.1.9).



R, R_1 = aryl or heteroaryl

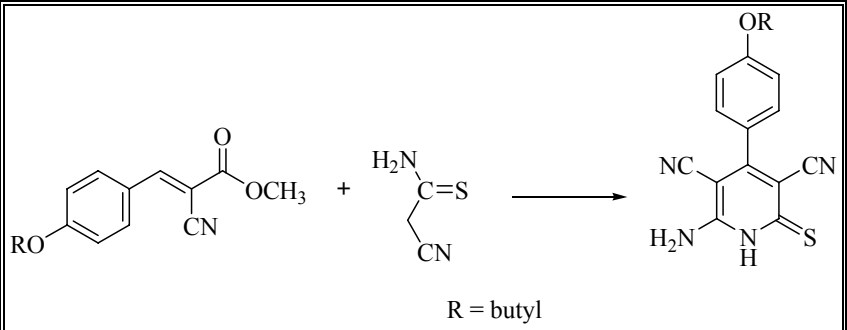
Scheme 2.1.9

The 6-amino-4-methylthio-3-cyano-2(1*H*)-pyridinethione was prepared by the reaction of 1,1-dimethylthio-1-thiocarbamoyl-2-cyanoethylene with cyanoacetamide [63] (Scheme 2.1.10).



Scheme 2.1.10

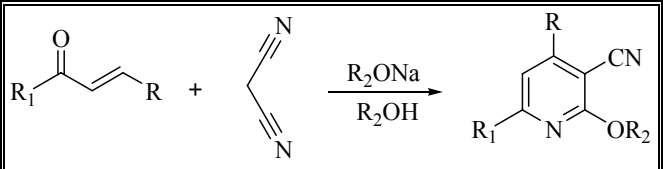
The reaction of 4-butoxybenzalcyanoacetic ester with cyanothioacetamide yielded 6-amino-4-(4-butoxyphenyl)-3,5-dicyano-2(1*H*)-pyridinethione [64] (Scheme 2.1.11).



R = butyl

Scheme 2.1.11

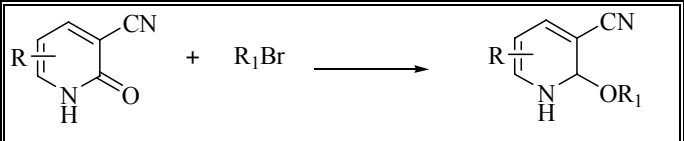
Synthesis of 2-alkoxy-3-cyanopyridines is generally achieved by the reaction of chalcones with malononitrile in corresponding sodium alkoxide [65-68] (Scheme 2.1.12).



R, R_1 = aryl or heteroaryl

Scheme 2.1.12

Another frequently used approach for the synthesis of 2-alkoxy-3-cyano-pyridines is the O-alkylation of 3-cyanopyridin-2-ones by the reaction with appropriate alkyl /aryl halide [69, 70] (Scheme 2.1.13).

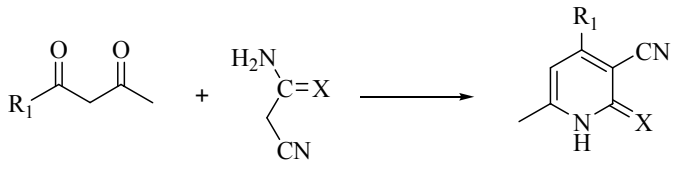


R = substituent/substituents on 4, 5 and/or 6 position of pyridine
R_1 = alkyl or aryl

Scheme 2.1.13

2.1.2.2. From 1,3-dicarbonyl synthons

Literature survey revealed many reports on synthesis of 3-cyanopyridin-2-one and 3-cyanopyridin-2-thione by reaction of 1,3-dicarbonyl compounds with cyanoacetamide [71-74] and cyanothioacetamide [75-77] respectively (Scheme 2.1.14).

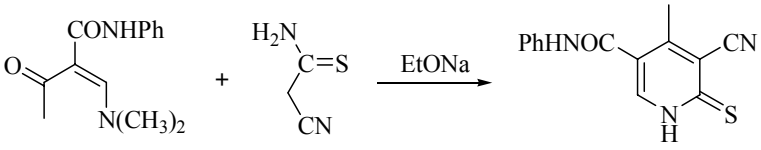


R_1 = H [69, 70], R_1 = -$COOCH_3$ [71], R_1 = heteroaryl [72], ; X = O [69-72], S [73-75]
R_1 = benzoyl [73], R_1 = CH_3, -OC_2H_5 [74], R_1 = -OC_2H_5 [75]

Scheme 2.1.14

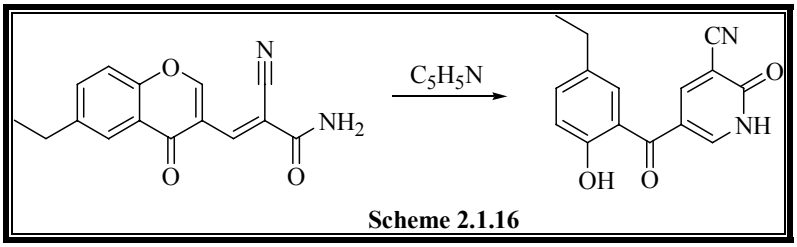
2.1.2.3. Miscellaneous

Enaminones have been attractive starting materials for chemists for the synthesis of diverse functionalized 3-cyanopyridines [78-83]. Abu-Elmaati has reported reaction of enaminone 1-(*N*-*p*chlorophenyl)-2-(*N*-dimethylaminomethino)-3-oxobutanamide with cyanothioacetamide in ethanol/sodium ethoxide to yield 3-cyano-4-methylpyridin-2(1*H*)one [80] (Scheme 2.1.15).



Scheme 2.1.15

Literature survey also revealed a few ring transformation reactions of 4-amino-1H-1,5-benzodiazepine-3-carbonitrile [84] and substituted uracil [85] yielding 3-cyanopyridines. Nohara et al. have reported that heating 2-cyano-3-(6-ethyl-4-oxo-4H-1-benzopyran-3-yl)acrylamide in pyridine yields 3-cyano-5-(5-ethyl-2-hydroxybenzoyl)-2(1*H*)-pyridones [86] (Scheme 2.1.16).



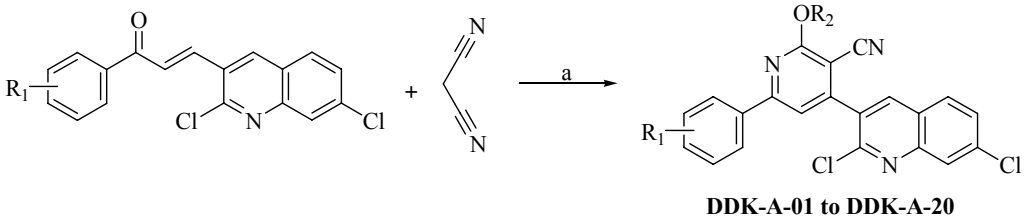
Scheme 2.1.16

2.1.3 Current work

The chemistry of pyridine and its derivatives has been studied for over a century due to their diverse biological activities. 3-cyanopyridine or pyridine-3-carbonitrile derivatives draw a special attention for their wide spectrum biological activities viz. anti-tubercular, anti-cancer, cardiovascular, anti-histaminic, anti-inflammatory, analgesic and antipyretic properties along with their importance and utility as intermediates in preparing variety of heterocyclic compounds.

Keeping in mind various biomedical applications and with a view to further assess the pharmacological profile of this class of compounds, two novel series of pyridine-3-carbonitriles (**DDK-A-01 to DDK-A-20**) have been synthesized. The synthesis of pyridine-3-carbonitriles (**DDK-A-01 to DDK-A-10**) and (**DDK-A-11 to DDK-A-20**) was achieved by Michael addition of α,β-unsaturated ketones (chalcones) to malononitrile in sodium methoxide/methanol and sodium ethoxide/ethanol systems resepectively. 3-(2,7-dichloroquinolin-3-yl)-1-(aryl)-prop-2-en-1-ones (chalcones) were prepared by the reaction of 2,7-dichloroquinoline-3-carbaldehyde with different substituted acetophenones s using 40% sodium hydroxide as a catalyst [87]. The products were characterized by FT-IR, mass, ^{1}H NMR spectroscopy and elemental analyses. The newly synthesized compounds were subjected to antimicrobial activity.

2.1.4 Reaction scheme



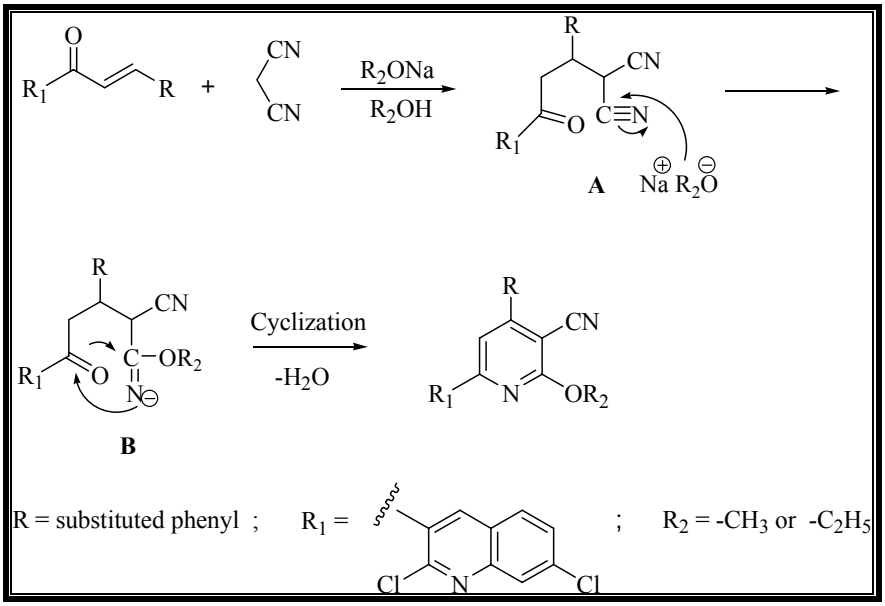
DDK-A-01 to DDK-A-20

Reagents and conditions: (a) R_2ONa, R_2OH, reflux, 6-8 h.

Code	R_1	R_2	M.F.	M.W.	M.P. °C	Yield %	R_{f1}	R_{f2}
DDK-A-01	H	CH_3	$C_{22}H_{13}Cl_2N_3O$	406	215-217	69	0.47	0.68
DDK-A-02	3-NO_2	CH_3	$C_{22}H_{12}Cl_2N_4O_3$	451	233-235	62	0.48	0.71
DDK-A-03	4-NO_2	CH_3	$C_{22}H_{12}Cl_2N_4O_3$	451	247-249	60	0.53	0.72
DDK-A-04	4-Cl	CH_3	$C_{22}H_{12}Cl_3N_3O$	439	211-213	70	0.54	0.74
DDK-A-05	2-OH	CH_3	$C_{22}H_{13}Cl_2N_3O_2$	422	189-191	65	0.47	0.69
DDK-A-06	4-OH	CH_3	$C_{22}H_{13}Cl_2N_3O_2$	422	195-197	73	0.52	0.70
DDK-A-07	2-OCH_3	CH_3	$C_{23}H_{15}Cl_2N_3O_2$	436	198-200	65	0.55	0.76
DDK-A-08	4-OCH_3	CH_3	$C_{23}H_{15}Cl_2N_3O_2$	436	206-208	75	0.51	0.66
DDK-A-09	4-F	CH_3	$C_{22}H_{12}Cl_2FN_3O$	424	265-267	78	0.56	0.69
DDK-A-10	4-Br	CH_3	$C_{22}H_{12}BrN_3O$	485	228-231	62	0.49	0.73
DDK-A-11	H	C_2H_5	$C_{23}H_{15}Cl_2N_3O$	420	128-130	68	0.53	0.61
DDK-A-12	3-NO_2	C_2H_5	$C_{23}H_{14}Cl_2N_4O_3$	465	241-243	74	0.58	0.67
DDK-A-13	4-NO_2	C_2H_5	$C_{23}H_{14}Cl_2N_4O_3$	465	178-180	70	0.49	0.58
DDK-A-14	4-Cl	C_2H_5	$C_{23}H_{14}Cl_3N_3O$	454	239-241	80	0.53	0.60
DDK-A-15	2-OH	C_2H_5	$C_{23}H_{15}Cl_2N_3O_2$	436	256-258	69	0.59	0.70
DDK-A-16	4-OH	C_2H_5	$C_{23}H_{15}Cl_2N_3O_2$	436	263-265	56	0.51	0.59
DDK-A-17	2-OCH_3	C_2H_5	$C_{24}H_{17}Cl_2N_3O_2$	450	229-231	63	0.54	0.61
DDK-A-18	4-OCH_3	C_2H_5	$C_{24}H_{17}Cl_2N_3O_2$	450	234-236	68	0.56	0.63
DDK-A-19	4-F	C_2H_5	$C_{23}H_{14}Cl_2FN_3O$	438	173-175	52	0.48	0.57
DDK-A-20	4-Br	C_2H_5	$C_{22}H_{12}BrN_3O$	499	238-240	50	0.58	0.68

TLC Solvent system R_{f1}: Hexane: Ethyl acetate – 6:4,
TLC Solvent system R_{f2}: Chloroform:Methanol – 9.5:0.5.

2.1.5 Mechanism



The reaction proceeds through Michael addition of α,β-unsaturated ketones to the malononitrile to afford adduct **A** which undergoes a nucleophilic attack by alkoxide anion followed by cyclization and subsequent dehydration of the cyclized product leads to the 2-alkoxycyanopyridines as suggested by Radwan et al. [68]

2.1.6 Experimental

2.1.6.1 Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a Bruker Avance II 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

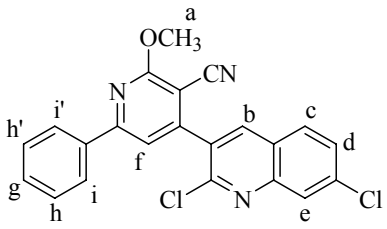
2.1.6.2 Synthesis of 3-(2,7-dichloroquinolin-3-yl)-1-(aryl)-prop-2-en-1-ones

Synthesis of 3-(2,7-dichloroquinolin-3-yl)-1-(aryl)-prop-2-en-1-ones was achieved using previously published method [87].

2.1.6.3 General procedure for the synthesis of 4-(2,7-dichloroquinolin-3-yl)-2-methoxy-6-(aryl)-pyridine-3-carbonitriles (DDK A-01 to DDK-A-10)

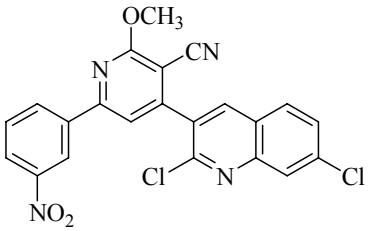
3-(2,7-dichloroquinolin-3-yl)-1-(aryl)-prop-2-en-1-one (0.01 mol) was added to a freshly prepared sodium methoxide solution (0.015 mol of sodium in 15 mL of methanol). Malononitrile (0.01 mol) was then added to this solution. The resulting mixture was heated under reflux for 6-8 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the separated solid was collected by filtration, washed with cold methanol and crystallized from ethanol.

2.1.6.3.1 4-(2,7-dichloroquinolin-3-yl)-2-methoxy-6-phenylpyridine-3-carbonitrile (DDK-A-01)



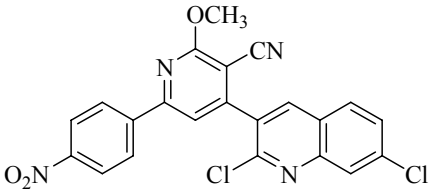
Yield: 69%; m.p. 215-217 ºC; IR (cm^{-1}): 3022 (C-H stretching of aromatic ring), 2951 (C-H asymmetrical stretching of CH_3 group), 2864 (C-H symmetrical stretching of CH_3 group), 2218 (C≡N stretching of nitrile group), 1616, 1585 & 1548 (C=C stretching of aromatic ring), 1462 (C-H asymmetrical deformation of CH_3 group), 1359 (C-H symmetrical deformation of CH_3 group), 1261 (C-O-C asymmetrical stretching of OCH_3 group), 1074 (C-O-C symmetrical stretching OCH_3 group), 1020 (C-H in plane bending for aromatic ring), 769 (C-Cl stretching), 704 & 769 (C-H out of plane bending for mono-substituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 4.03 (s, 3H, H_a), 7.95 (s, 1H, H_b), 7.67-7.71 (d, 1H, H_d), 7.83-7.85 (m, 2H, $H_{c,\ f}$), 8.14-8.15 (d, 1H, H_e), 7.42-7.47 (m, 3H, $H_{g,\ h,\ h'}$), 8.03-8.05 (m, 2H, $H_{i,\ i'}$); MS: *m/z* 406; Anal. Calcd. for $C_{22}H_{13}Cl_2N_3O$: C, 65.04; H, 3.23; N, 10.34. Found: C, 64.06; H, 3.16; N, 10.27.

2.1.6.3.2 4-(2,7-dichloroquinolin-3-yl)-2-methoxy-6-(3-nitrophenyl)pyridine-3-carbonitrile (DDK-A-02)



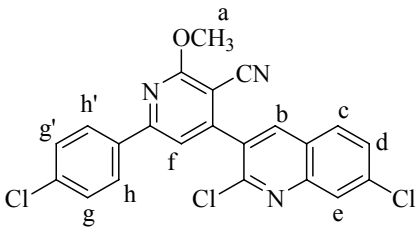
Yield: 62%; m.p. 233-235 ºC; MS: *m/z* 451; Anal. Calcd. for $C_{22}H_{12}Cl_2N_4O_3$: C, 58.55; H, 2.68; N, 12.42. Found: C, 58.46; H, 2.61; N, 12.33%.

2.1.6.3.3 4-(2,7-dichloroquinolin-3-yl)-2-methoxy-6-(4-nitrophenyl)pyridine-3-carbonitrile (DDK-A-03)



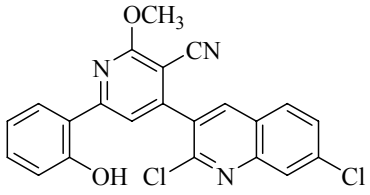
Yield: 60%; m.p. 247-249 ºC; MS: *m/z* 451; Anal. Calcd. for $C_{22}H_{12}Cl_2N_4O_3$: C, 58.55; H, 2.68; N, 12.42. Found: C, 58.47; H, 2.61; N, 12.34%.

2.1.6.3.4 6-(4-chlorophenyl)-4-(2,7-dichloroquinolin-3-yl)-2-methoxypyridine-3-carbonitrile (DDK-A-04)



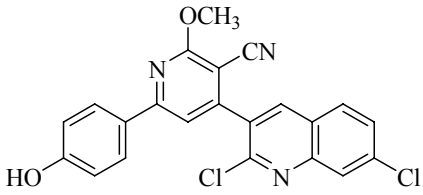
Yield: 70%; m.p. 211-213 ºC; IR (cm^{-1}): 3043 (C-H stretching of aromatic ring), 2922 (C-H asymmetrical stretching of CH_3 group), 2852 (C-H symmetrical stretching of CH_3 group), 2218 (C≡N stretching of nitrile group), 1546, 1506 & 1456 (C=C stretching of aromatic ring), 1402 (C-H asymmetrical deformation of CH_3 group), 1348 (C-H symmetrical deformation of CH_3 group), 1257 (C-O-C asymmetrical stretching of OCH_3 group), 1089 (C-O-C symmetrical stretching OCH_3 group), 1010 (C-H in plane bending for aromatic ring), 725 (C-Cl stretching), 821 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 4.02 (s, 3H, H_a), 7.96 (s, 1H, H_b), 7.88-7.89 (d, 1H, H_c), 7.43-7.44 (m, 1H, H_d), 8.28-8.29 (d, 1H, H_e), 7.66 (s, 1H, H_f), 7.33-7.35 (d, 2H, $H_{g, g'}$, J = 8.36 Hz), 8.17-8.19 (d, 2H, $H_{h, h'}$, J = 8.32 Hz),; MS: *m/z* 439; Anal. Calcd. for $C_{22}H_{12}Cl_3N_3O$: C, 59.96; H, 2.74; N, 9.53. Found: C, 59.89; H, 2.66; N, 9.43%.

2.1.6.3.5 4-(2,7-dichloroquinolin-3-yl)-6-(2-hydroxyphenyl)-2-methoxypyridine-3-carbonitrile (DDK-A-05)



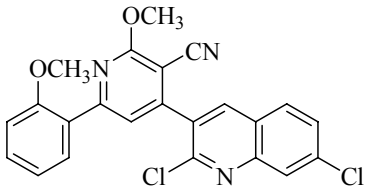
Yield: 65%; m.p. 189-191 ºC; MS: *m/z* 422; Anal. Calcd. for $C_{22}H_{13}Cl_2N_3O_2$: C, 62.58; H, 3.10; N, 9.95. Found: C, 62.50; H, 3.01; N, 9.87%.

2.1.6.3.6 4-(2,7-dichloroquinolin-3-yl)-6-(4-hydroxyphenyl)-2-methoxypyridine-3-carbonitrile (DDK-A-06)



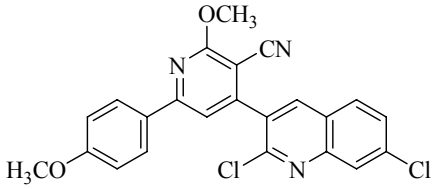
Yield: 73%; m.p. 195-197 ºC; MS: *m/z* 422; Anal. Calcd. for $C_{22}H_{13}Cl_2N_3O_2$: C, 62.58; H, 3.10; N, 9.95. Found: C, 62.48; H, 3.02; N, 9.87%.

2.1.6.3.7 4-(2,7-dichloroquinolin-3-yl)-2-methoxy-6-(2-methoxyphenyl)pyridine-3-carbonitrile (DDK-A-07)



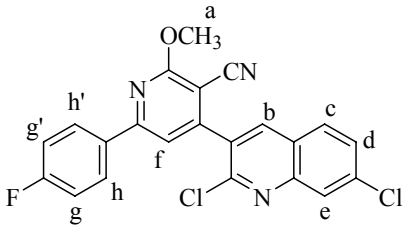
Yield: 65%; m.p. 198-200 ºC; MS: *m/z* 436; Anal. Calcd. for $C_{23}H_{15}Cl_2N_3O_2$: C, 63.32; H, 3.47; N, 9.63. Found: C, 63.24; H, 3.39; N, 9.53%.

2.1.6.3.8 4-(2,7-dichloroquinolin-3-yl)-2-methoxy-6-(4-methoxyphenyl)pyridine-3-carbonitrile (DDK-A-08)



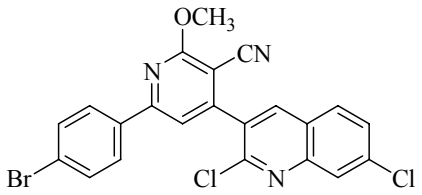
Yield: 75%; mp 254-256 ºC; MS: *m/z* 436; Anal. Calcd. for $C_{23}H_{15}Cl_2N_3O_2$: C, 63.32; H, 3.47; N, 9.63. Found: C, 63.25; H, 3.38; N, 9.54%.

2.1.6.3.9 4-(2,7-dichloroquinolin-3-yl)-6-(4-fluorophenyl)-2-methoxypyridine-3-carbonitrile (DDK-A-09)



Yield: 78%; m.p. 265-267 ºC; IR (cm^{-1}): 3057 (C-H stretching of aromatic ring), 2999 (C-H asymmetrical stretching of CH_3 group), 2899 (C-H symmetrical stretching of CH_3 group), 2216 (C≡N stretching of nitrile group), 1579 & 1558, (C=C stretching of aromatic ring), 1465 (C-H asymmetrical deformation of CH_3 group), 1352 (C-H symmetrical deformation of CH_3 group), 1255 (C-O-C asymmetrical stretching of OCH_3 group), 1022 (C-O-C symmetrical stretching of OCH_3 group), 987 (C-H in plane bending for aromatic ring), 725 (C-Cl stretching), 839 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 3.98 (s, 3H, H_a), 7.96 (s, 1H, H_b), 7.73-7.78 (m, 2H, $H_{c, f}$), 7.40-7.44 (m, 1H, H_d), 8.16-8.18 (d, 1H, H_e), 7.18-7.23 (m, 2H, $H_{g, g'}$), 8.25-8.28 (t, 2H, $H_{h, h'}$); MS: *m/z* 424; Anal. Calcd. for $C_{22}H_{12}Cl_2FN_3O$: C, 62.28; H, 2.85; N, 9.90. Found: C, 62.19; H, 2.78; N, 9.83%.

2.1.6.3.10 6-(4-bromophenyl)-4-(2,7-dichloroquinolin-3-yl)-2-methoxypyridine-3-carbonitrile (DDK-A-10)

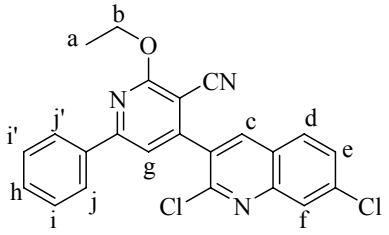


Yield: 62%; m.p. 228-231 ºC; MS: *m/z* 485; Anal. Calcd. for $C_{22}H_{12}BrN_3O$: C, 54.46; H, 2.49; N, 8.66. Found: C, 54.37; H, 2.40; N, 8.58%.

2.1.6.4 General procedure for the synthesis of 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(aryl)pyridine-3-carbonitriles (DDK-A-11 to DDK-A-20)

3-(2,7-dichloroquinolin-3-yl)-1-(aryl)-prop-2-en-1-one (0.01 mol) was added to a freshly prepared sodium ethoxide solution (0.015 mol of sodium in 15 mL of ethanol). Malononitrile (0.01 mol) was then added to this solution. The resulting mixture was heated under reflux for 6-8 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the separated solid was collected by filtration, washed with cold methanol and crystallized from ethanol.

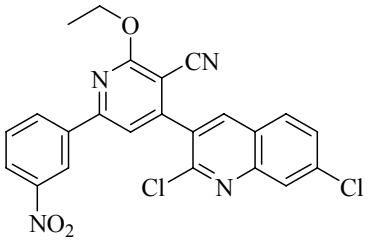
2.1.6.4.1 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-phenylpyridine-3-carbonitrile (DDK-A-11)



Yield: 67%; m.p. 209-211 ºC; IR (cm^{-1}): 3068 (C-H stretching of aromatic ring), 2956 (C-H asymmetrical stretching of CH_3 and CH_2 group), 2872 (C-H symmetrical stretching of CH_3 and CH_2 group), 2214 (C≡N stretching of nitrile group), 1529 & 1481 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of CH_3 group), 1348 (C-H symmetrical deformation of CH_3 group), 1257 (C-O-C asymmetrical stretching of OC_2H_5 group), 1074 (C-O-C symmetrical stretching OC_2H_5 group), 1016 (C-H in plane bending for aromatic ring), 738 (C-Cl stretching), 690 & 738 (C-H out of plane bending for mono-substituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 4.71-4.73 (t, 3H, H_a), 1.53-1.56 (q, 2H, H_b), 7.97 (s, 1H, H_c), 7.68-7.70 (d, 1H, H_d), 7.85-7.91(m, 2H, $H_{e, g}$); 8.29-8.30 (d, 1H, H_f), 7.49-7.53 (m,

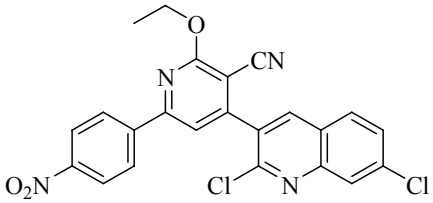
3H, $H_{h, i, i'}$), 8.14-8.17 (m, 2H, $H_{j, j'}$); MS: *m/z* 420; Anal. Calcd. for $C_{23}H_{15}Cl_2N_3O$: C, 65.73; H, 3.60; N, 10.00. Found: C, 65.66; H, 3.51; N, 09.94%.

2.1.6.4.2 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(3-nitrophenyl)pyridine-3-carbonitrile (DDK-A-12)



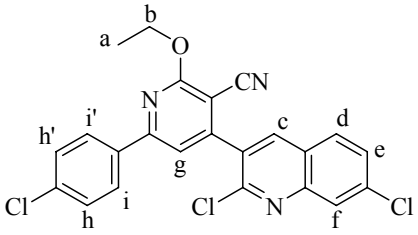
Yield: 62%; m.p. 242-244 ºC; MS: *m/z* 465; Anal. Calcd. for $C_{23}H_{14}Cl_2N_4O_3$: C, 59.37; H, 3.03; N, 12.04;. Found: C, 59.27; H, 2.94; N, 11.95%.

2.1.6.4.3 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(4-nitrophenyl)pyridine-3-carbonitrile (DDK-A-13)



Yield: 70%; m.p. 178-180 ºC; MS: *m/z* 465; Anal. Calcd. for $C_{23}H_{14}Cl_2N_4O_3$: C, 59.37; H, 3.03; N, 12.04;. Found: C, 59.28; H, 2.94; N, 11.96%.

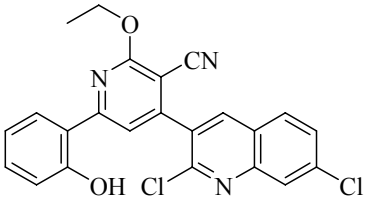
2.1.6.4.4 6-(4-chlorophenyl)-4-(2,7-dichloroquinolin-3-yl)-2-ethoxypyridine-3-carbonitrile (DDK-A-14)



Yield: 71%; m.p. 233-235 ºC; IR (cm^{-1}): 3048 (C-H stretching of aromatic ring), 2991 (C-H asymmetrical stretching of CH_3 and CH_2 group), 2822 (C-H symmetrical stretching of CH_3 and CH_2 group), 2220 (C≡N stretching of nitrile group), 1616 & 1581 (C=C stretching of aromatic ring), 1467 (C-H asymmetrical deformation of CH_3 group), 1340 (C-H symmetrical deformation of CH_3 group), 1255 (C-O-C asymmetrical stretching of OC_2H_5 group), 1109 (C-O-C symmetrical stretching OC_2H_5 group), 1024 (C-H in plane bending for aromatic ring), 761 (C-Cl stretching), 835 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 4.66-4.70 (t, 3H, H_a), 1.50-1.54 (q, 2H, H_b), 7.96 (s, 1H, H_c), 7.44-7.47 (m, 1H, H_d), 7.88-7.90 (m, 1H, H_e), 8.28-8.29 (d, 1H, H_f), 7.66 (s, 1H, H_g), 7.22-7.25 (d, 2H, $H_{h, h'}$, J = 8.60 Hz), 8.18-8.20 (d, 2H, $H_{i, i'}$, J = 8.28 Hz); MS: *m/z*

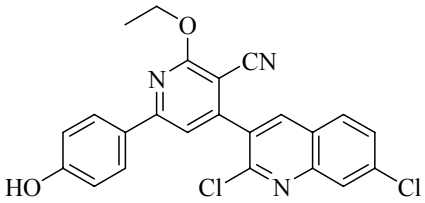
454; Anal. Calcd. for $C_{23}H_{14}Cl_3N_3O$: C, 60.75; H, 3.10; N, 9.24. Found: C, 60.69; H, 3.03; N, 9.17%.

2.1.6.4.5 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(2-hydroxyphenyl)pyridine-3-carbonitrile (DDK-A-15)



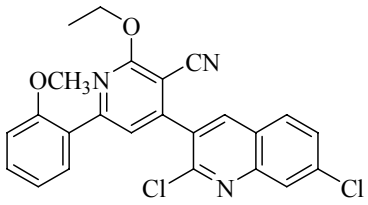
Yield: 61%; m.p. 169-171 ºC; MS: *m/z* 436; Anal. Calcd. for $C_{23}H_{15}Cl_2N_3O_2$: C, 63.32; H, 3.47; N, 9.63. Found: C, 63.23; H, 3.39; N, 9.55%.

2.1.6.4.6 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(4-hydroxyphenyl)pyridine-3-carbonitrile (DDK-A-16)



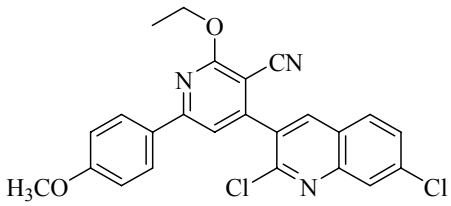
Yield: 62%; m.p. 183-185 ºC; MS: *m/z* 436; Anal. Calcd. for $C_{23}H_{15}Cl_2N_3O_2$: C, 63.32; H, 3.47; N, 9.63. Found: C, 63.25; H, 3.38; N, 9.56%.

2.1.6.4.7 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(2-methoxyphenyl)pyridine-3-carbonitrile (DDK-A-17)



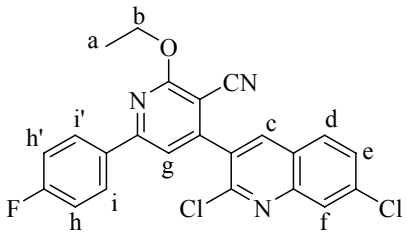
Yield: 63%; m.p. 211-213 ºC; MS: *m/z* 450; Anal. Calcd. for $C_{24}H_{17}Cl_2N_3O_2$: C, 64.01; H, 3.81; N, 9.33; Found: C, 63.93; H, 3.75; N, 9.27%.

2.1.6.4.8 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(4-methoxyphenyl)pyridine-3-carbonitrile (DDK-A-18)



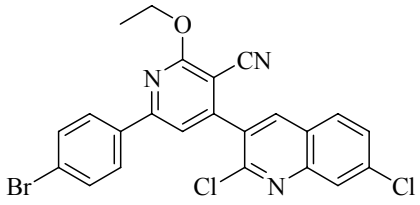
Yield: 77%; m.p. 268-272 ºC; MS: *m/z* 450; Anal. Calcd. for $C_{24}H_{17}Cl_2N_3O_2$: C, 64.01; H, 3.81; N, 9.33; Found: C, 63.93; H, 3.74; N, 9.25%.

2.1.6.4.9 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(4-fluorophenyl)pyridine-3-carbonitrile (DDK-A-19)



Yield: 78%; m.p. 265-267 ºC; IR (cm^{-1}): 3041 (C-H stretching of aromatic ring), 2991 (C-H asymmetrical stretching of CH_3 group), 2947 (C-H asymmetrical stretching of CH_2 group), 2854 (C-H symmetrical stretching of CH_3 and CH_2 group), 2214 (C≡N stretching of nitrile group), 1585, 1556, 1504 (C=C stretching of aromatic ring), 1467 (C-H asymmetrical deformation of CH_3 group), 1435 (C-H asymmetrical deformation of CH_3 group), 1348 (C-H symmetrical deformation of CH_3 group), 1238 (C-O-C asymmetrical stretching of OC_2H_5 group), 1076 (C-O-C symmetrical stretching OC_2H_5 group), 1020 (C-H in plane bending for aromatic ring), 748 (C-Cl stretching), 839 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 4.66-4.69 (t, 3H, H_a), 1.49-1.53 (q, 2H, H_b), 8.07 (s, 1H, H_c), 7.44-7.47 (m, 1H, H_e), 8.20-8.23 (m, 1H, $H_{i,\,i}$), 8.34-8.35 (d, 1H, H_f), 7.72 (s, 1H, H_g), 7.22-7.27 (t, 2H, $H_{h,\,h'}$), 7.84-7.93 (m, 2H, $H_{d,g}$); MS: *m/z* 438; Anal. Calcd. for $C_{23}H_{14}Cl_2FN_3O$: C, 63.03; H, 3.22; N, 9.59. Found: C, 62.95; H, 3.15; N, 9.51%.

2.1.6.4.10 6-(4-bromophenyl)-4-(2,7-dichloroquinolin-3-yl)-2-methoxypyridine-3-carbonitrile (DDK-A-20)



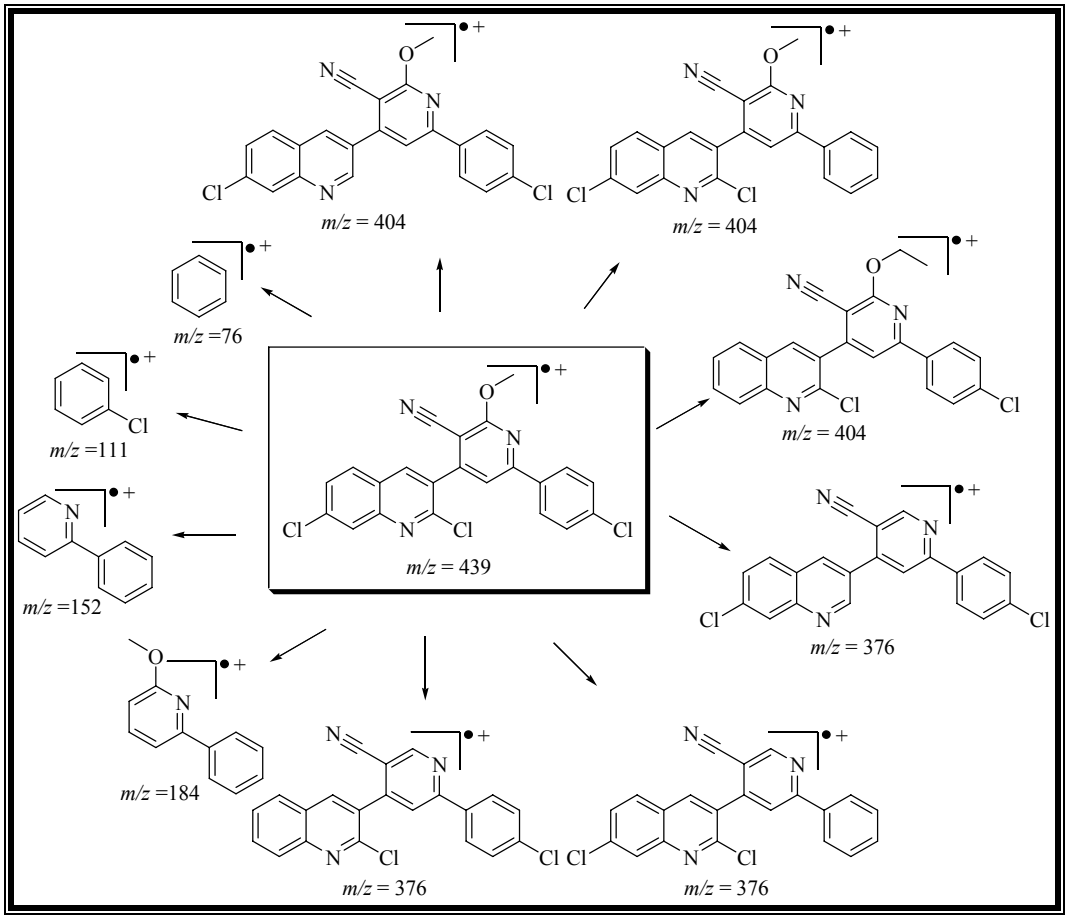
Yield: 66%; m.p. 226-228 ºC; MS: *m/z* 499; Anal. Calcd. for $C_{22}H_{12}BrN_3O$: C, 55.34; H, 2.83; N, 8.42. Found: C, 55.27; H, 2.76; N, 8.36%.

2.1.7 Spectral discussion

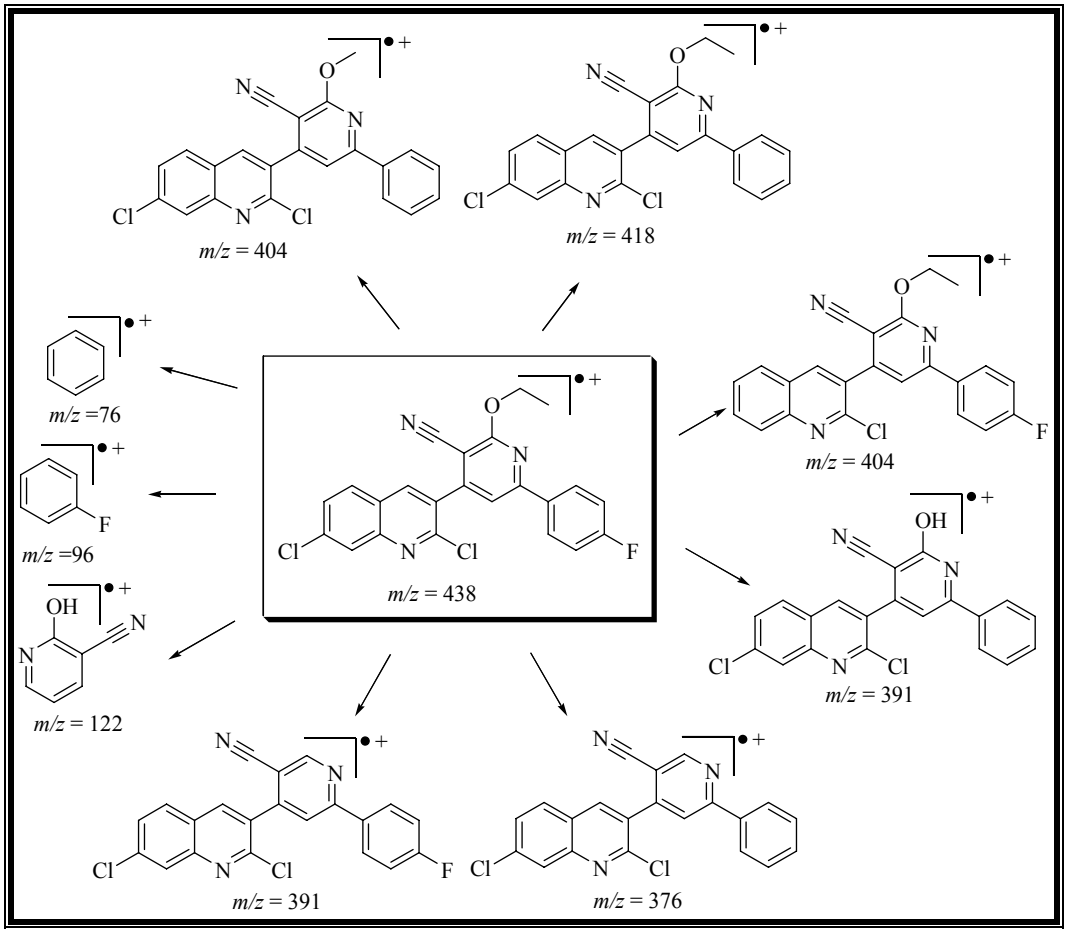
2.1.7.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of respective compound. Mass fragmentation pattern for a representative compound of each series is depicted below.

2.1.7.1.1 Mass fragmentation pattern for DDK-A-04



2.1.7.1.2 Mass fragmentation pattern for DDK-A-19



2.1.7.2 IR spectral study

IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For 3-cyanopyridines (**DDK-A-01 to DDK-A-20),** a characteristic band of nitrile group was observed in the range of 2214-2220 cm^{-1}. Confirmatory bands of C-O-C asymmetrical stretching at 1238-1261 cm^{-1} and C-O-C symmetrical stretching at 1022-1109 cm^{-1} were observed for methoxy and ethoxy groups.

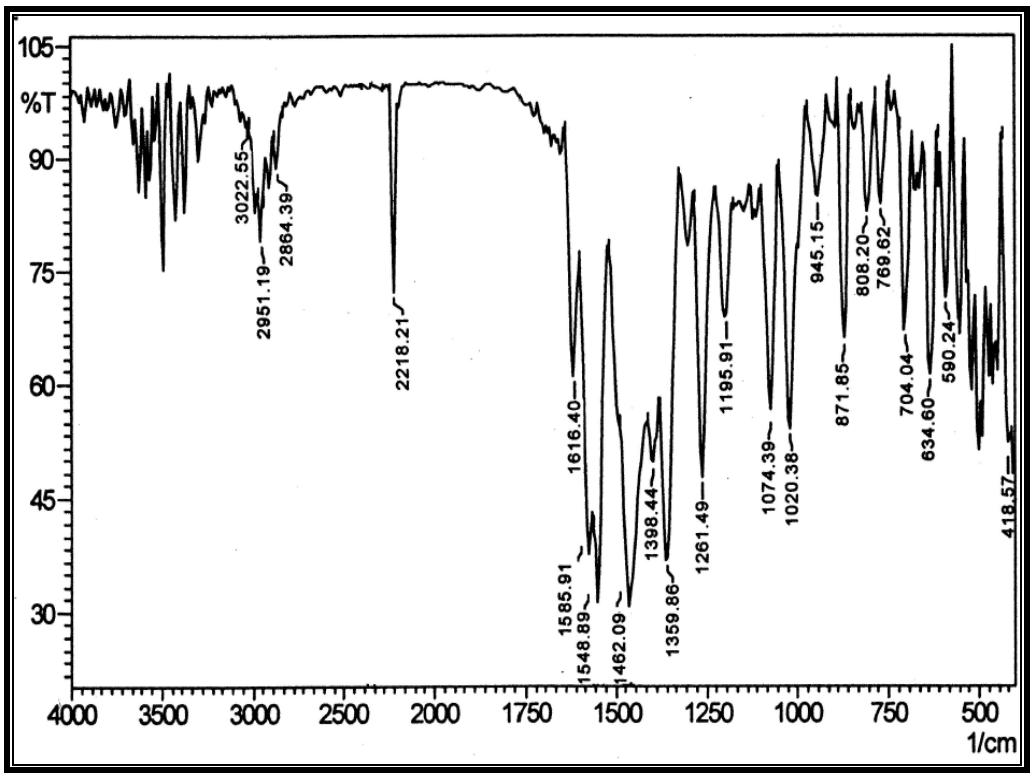
2.1.7.3 ^{1}H NMR spectral study

^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

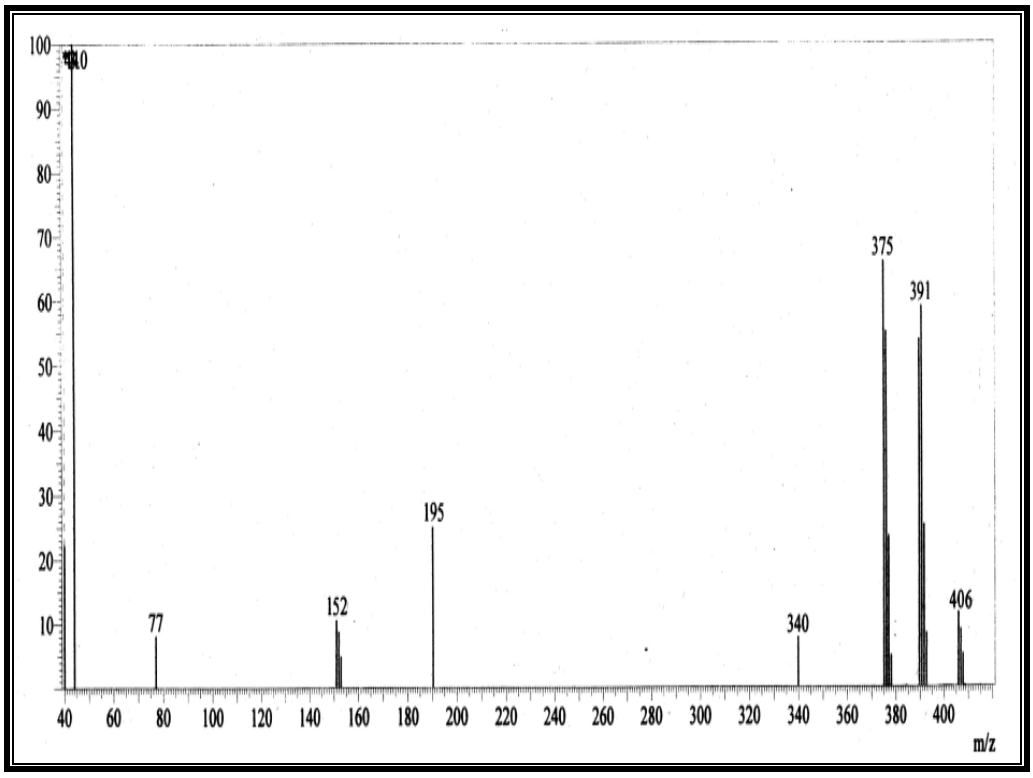
For 3-cyanopyridines (**DDK-A-01 to DDK-A-10**), characteristic singlet was observed for methoxy group at 4.02-4.03 δ ppm. The aromatic ring protons were observed at 7.18-8.28 δ ppm and J value were found to be in accordance with substitution pattern on phenyl ring.

While, for 3-cyanopyridines (**DDK-A-11 to DDK-A-20**), characteristic triplet-quartet pattern was observed for ethoxy group. A signal as triplet at 1.49-1.56 δ ppm corresponding to three methyl (O-CH_2-C$\underline{H}_3$) protons and a quartet at 4.66-4.73 δ ppm corresponding to two methylene (O-C$\underline{H}_2$-CH_3) protons was observed confirming the formation of 2-ethoxy-3-cyanopyridines. The aromatic ring protons were observed at 7.22-8.35 δ ppm and J value were found to be in accordance with substitution pattern on phenyl ring.

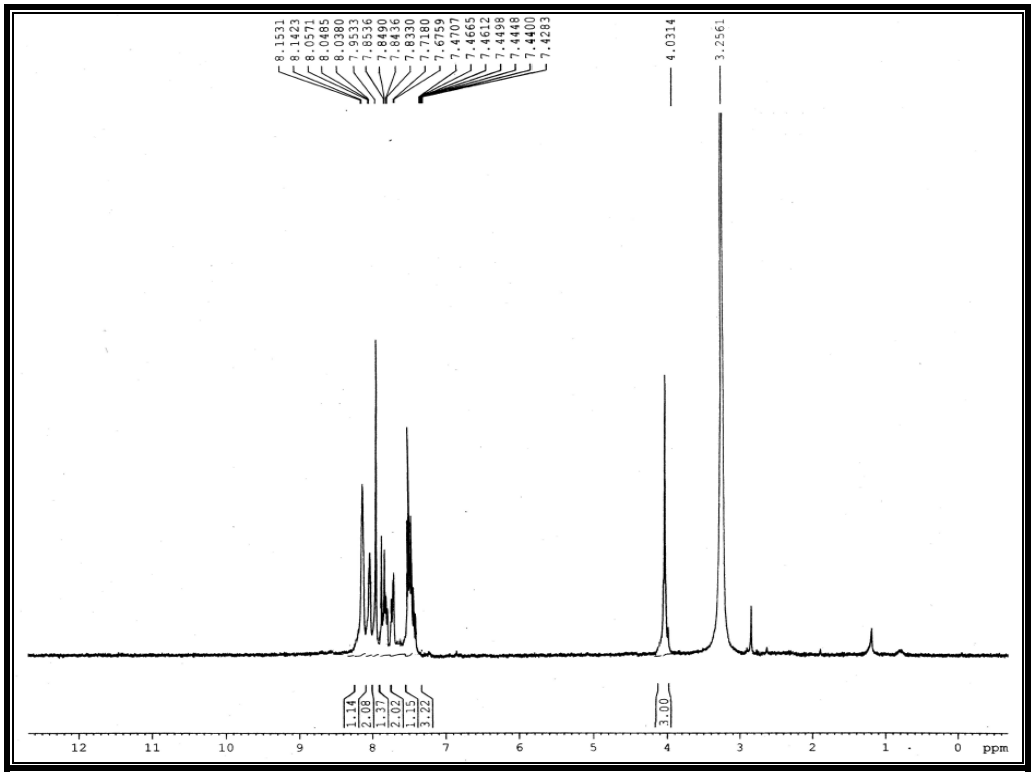
IR spectrum of DDK-A-01



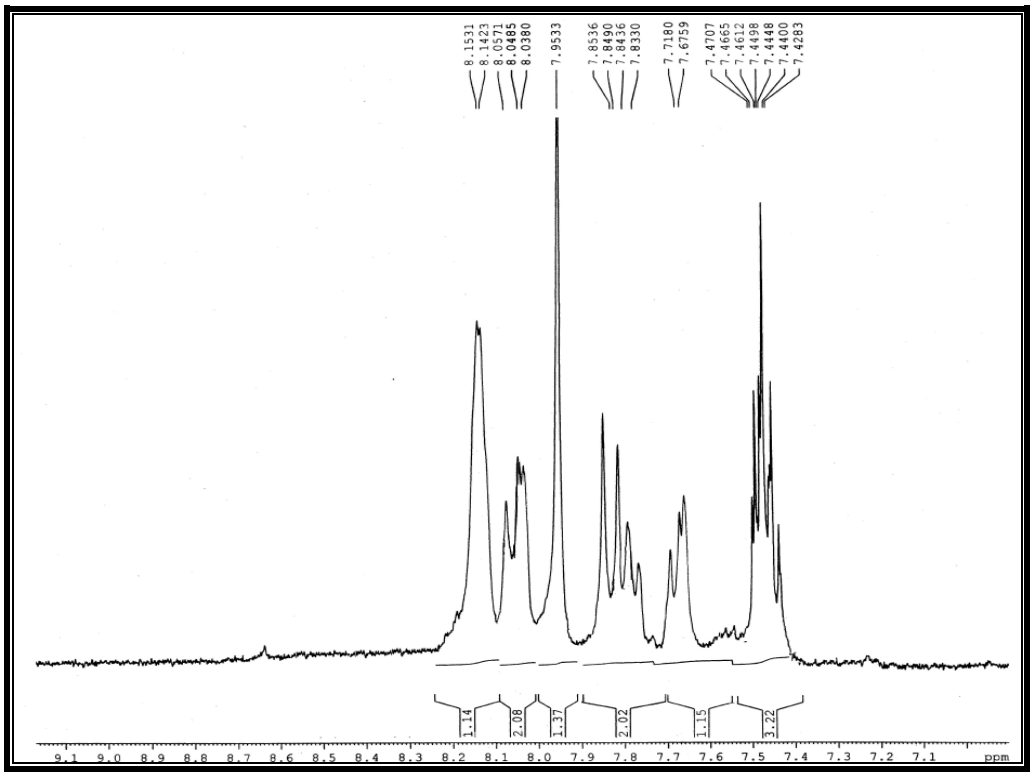
Mass spectrum of DDK-A-01



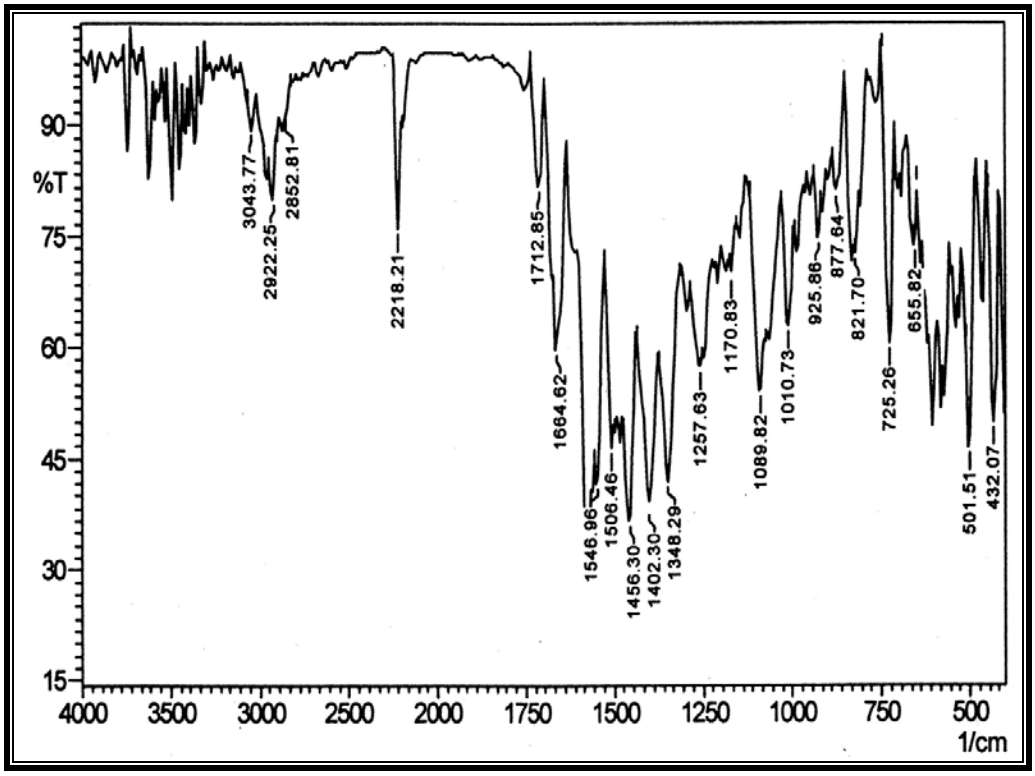
^{1}H NMR spectrum of DDK-A-01



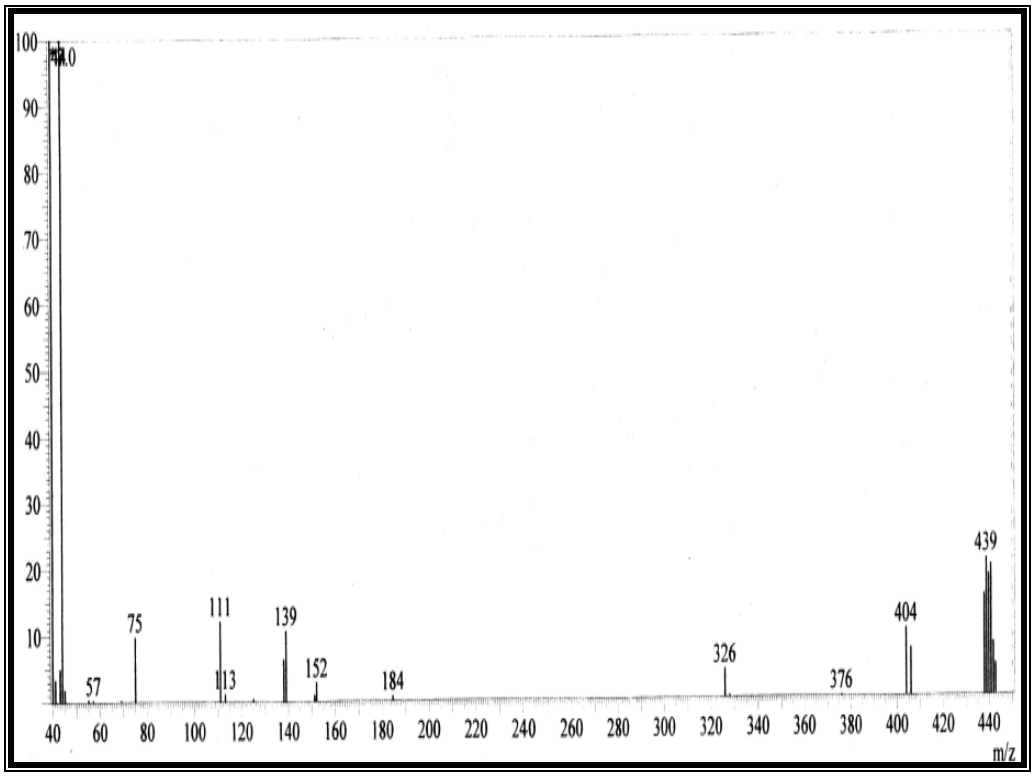
Expanded ^{1}H NMR spectrum of DDK-A-01



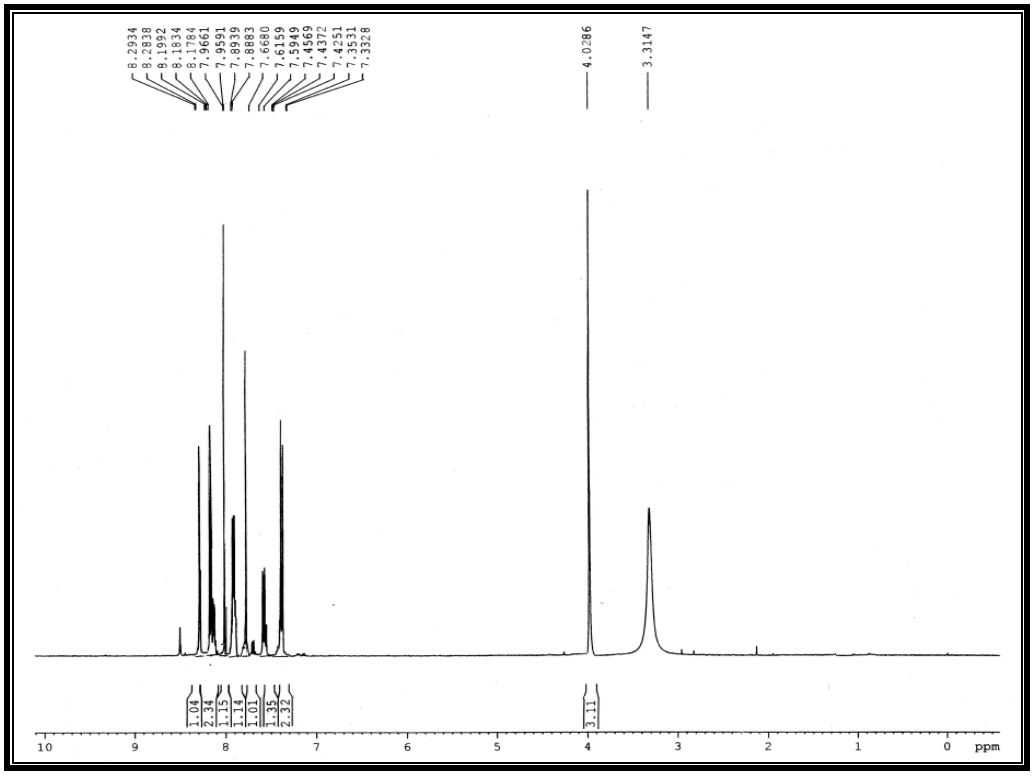
IR spectrum of DDK-A-04



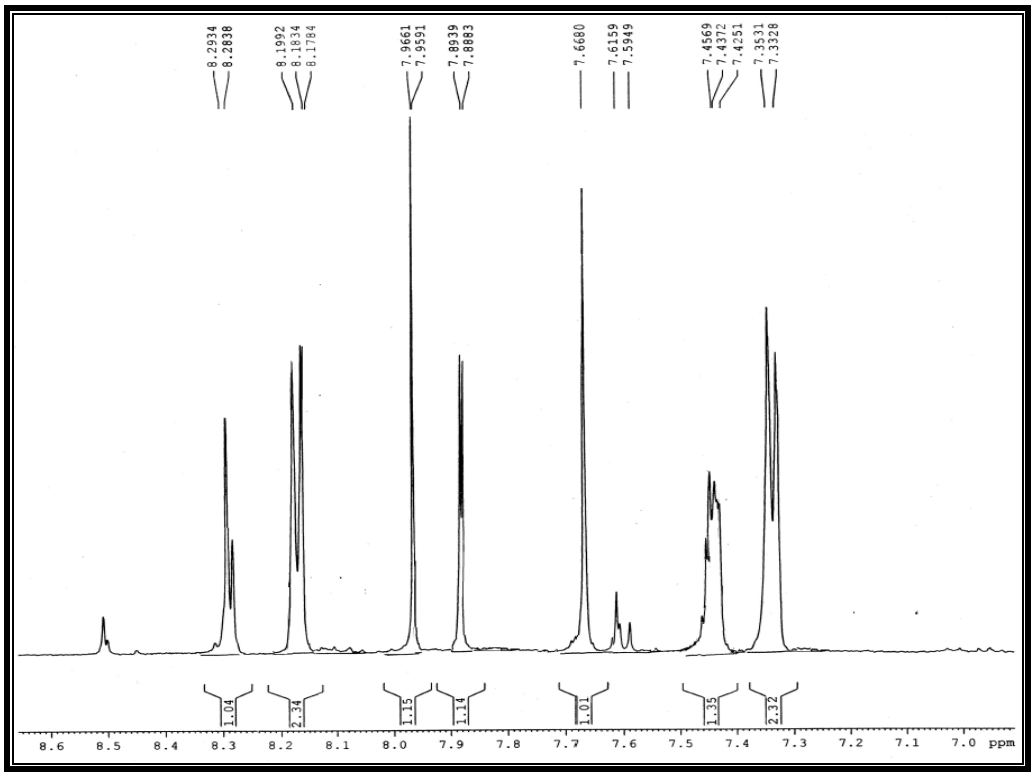
Mass spectrum of DDK-A-04



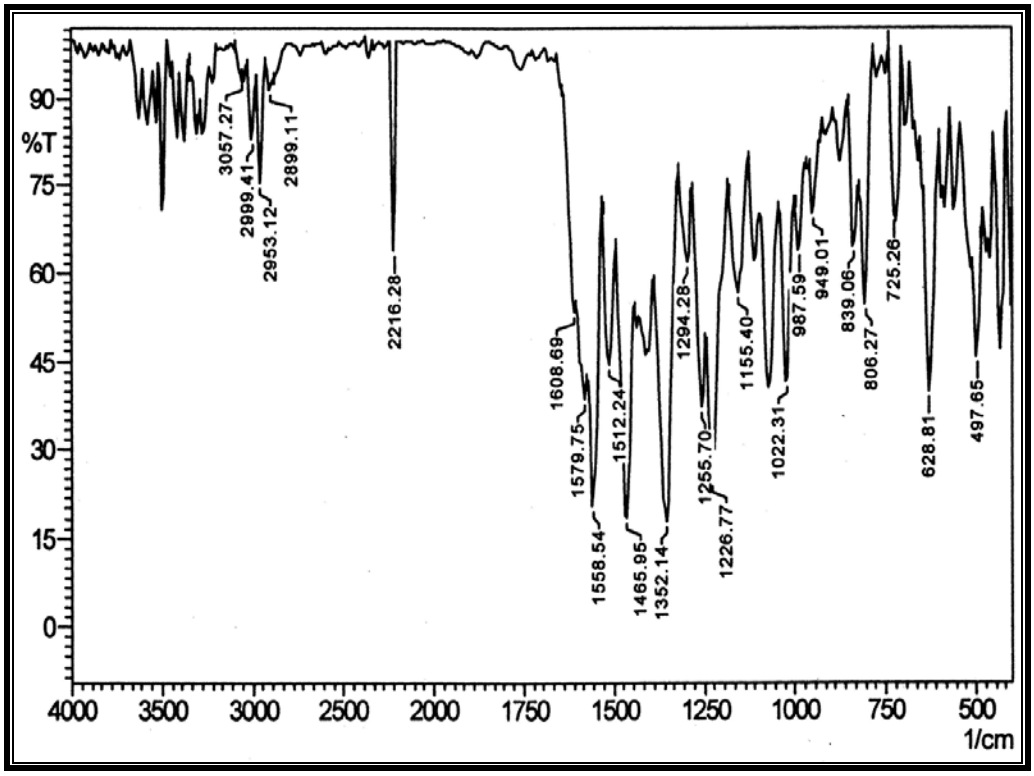
^{1}H NMR spectrum of DDK-A-04



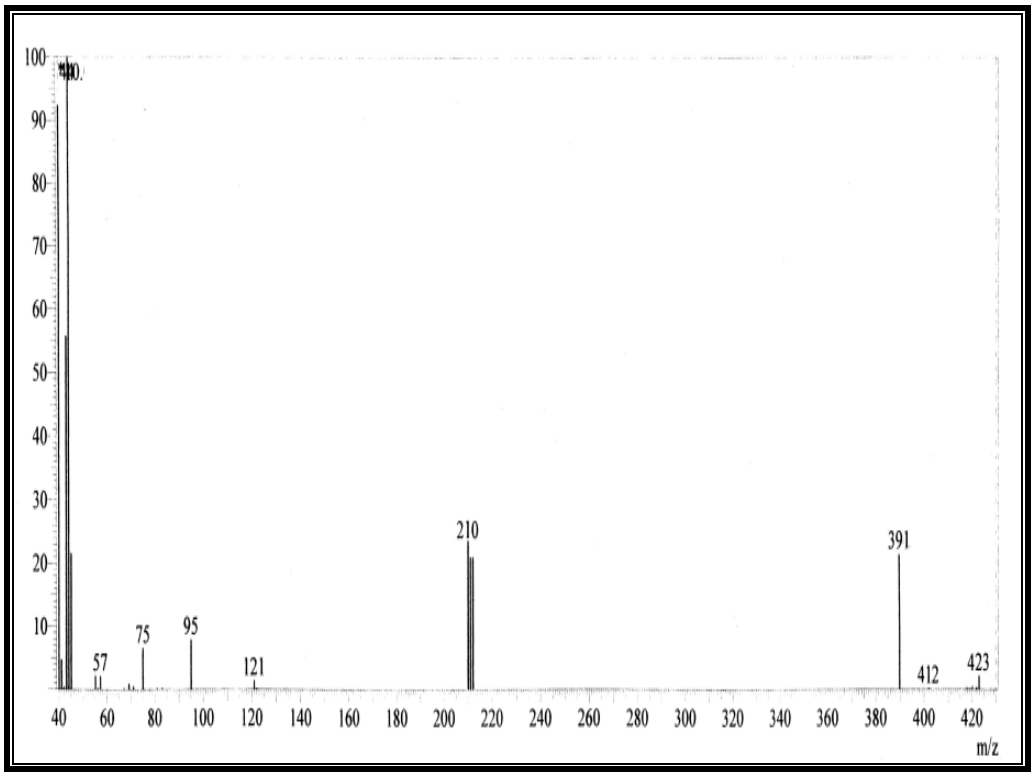
Expanded ^{1}H NMR spectrum of DDK-A-04



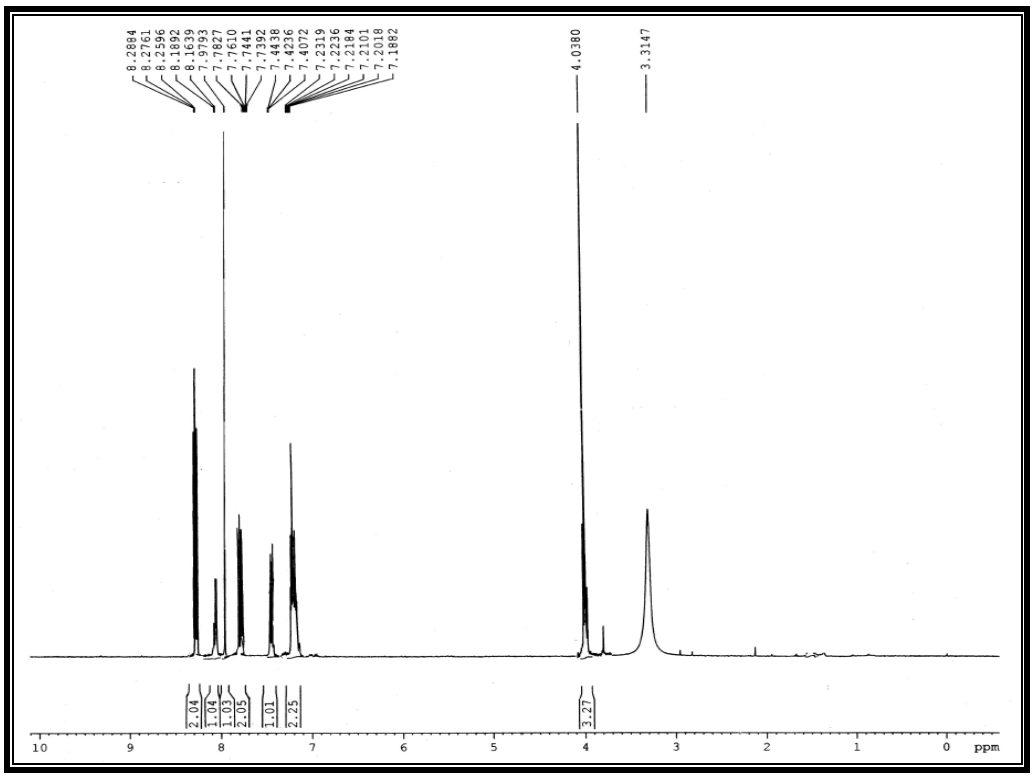
IR spectrum of DDK-A-09



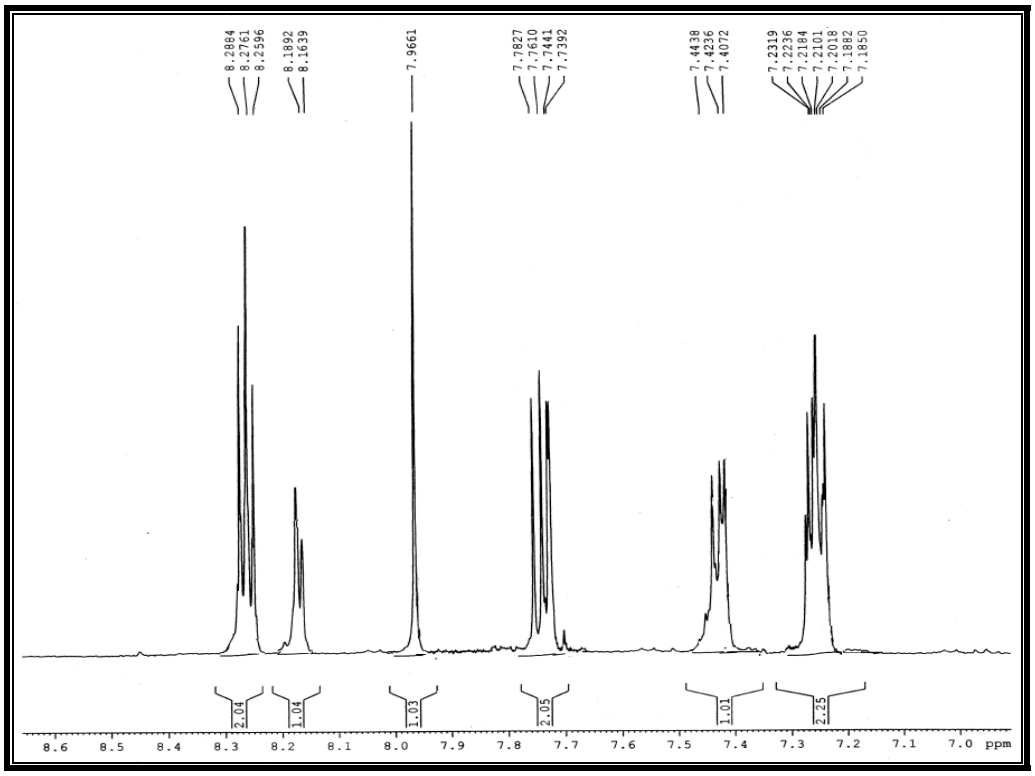
Mass spectrum of DDK-A-09



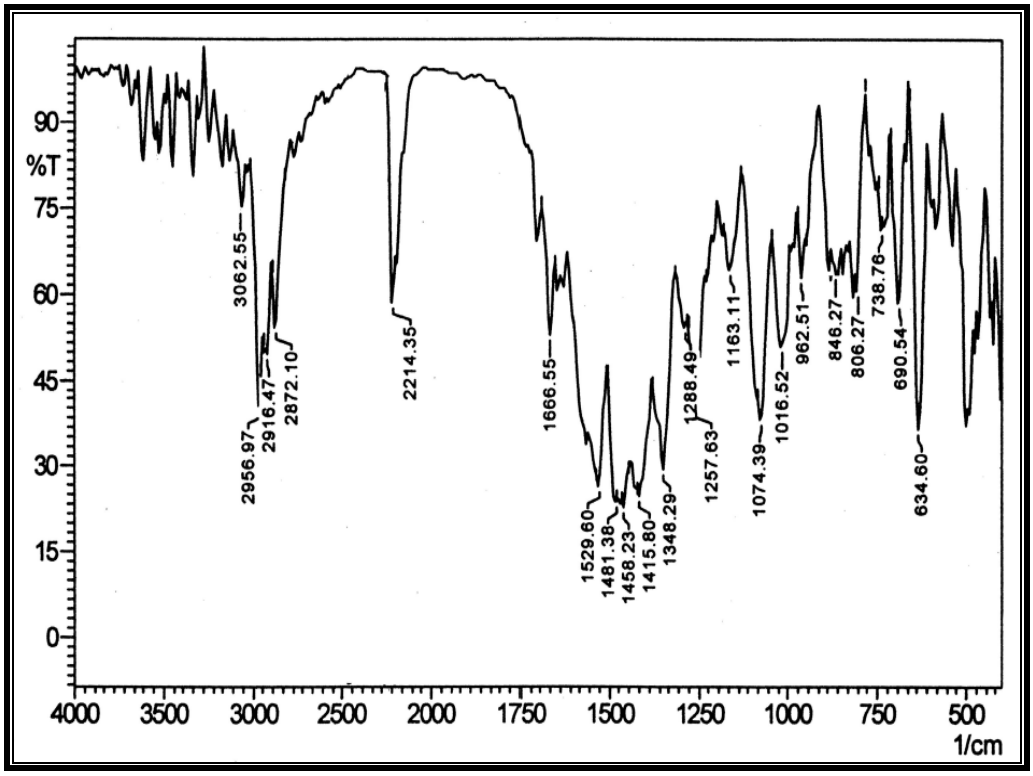
^{1}H NMR spectrum of DDK-A-09



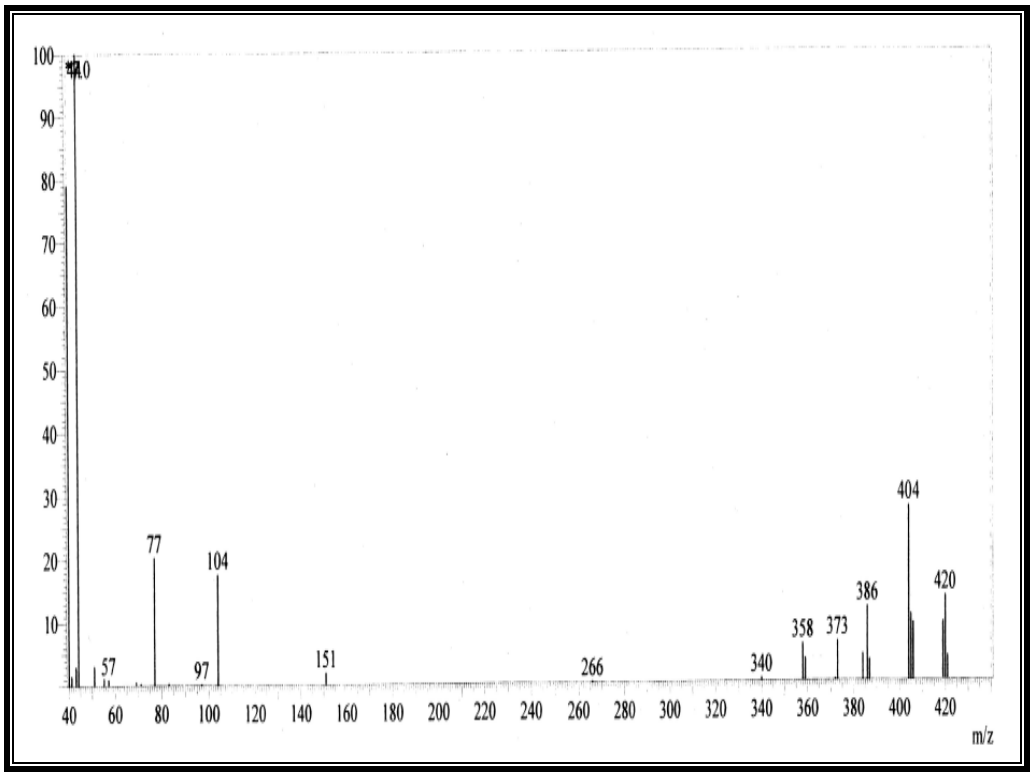
Expanded ^{1}H NMR spectrum of DDK-A-09



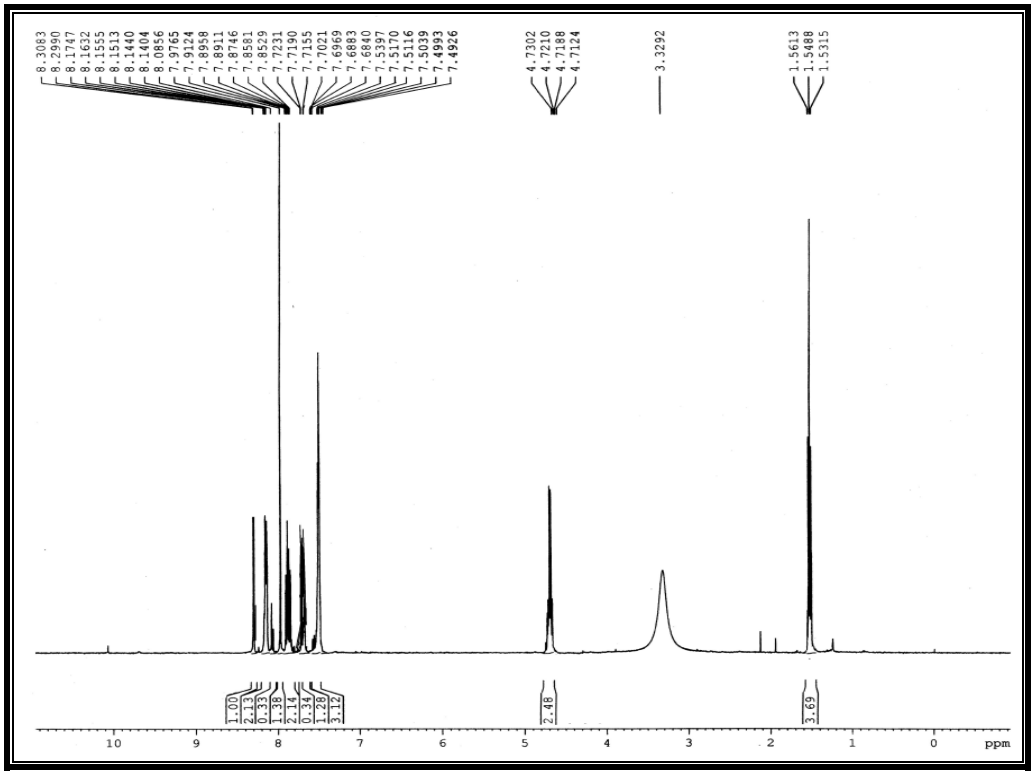
IR spectrum of DDK-A-11



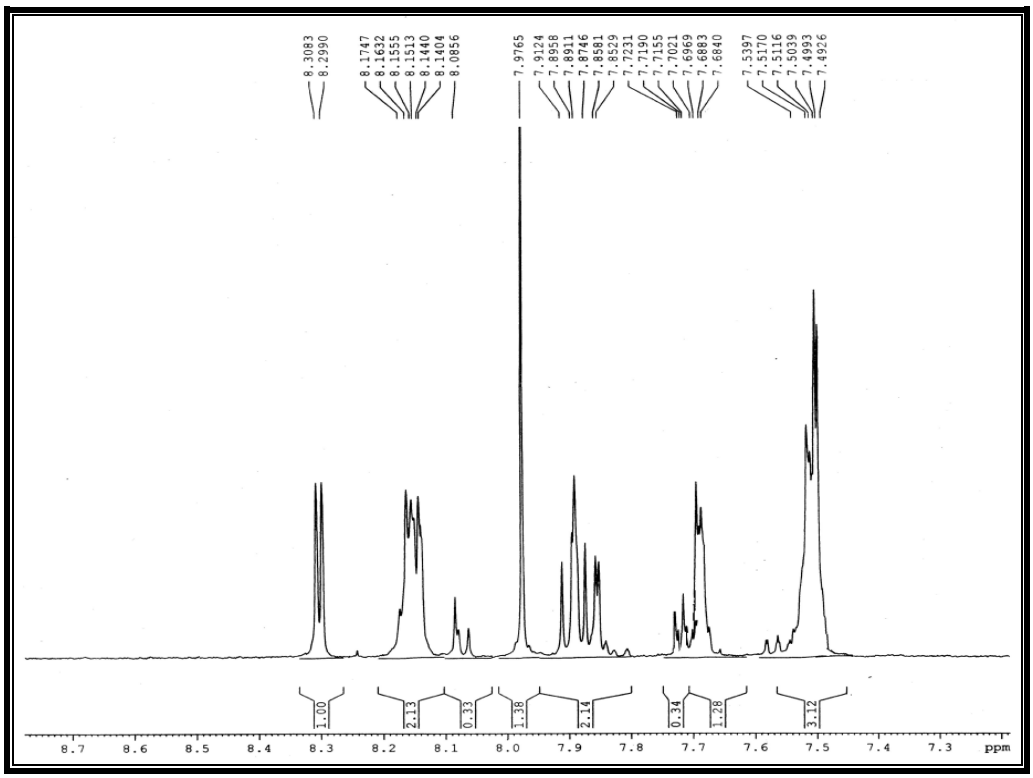
Mass spectrum of DDK-A-11



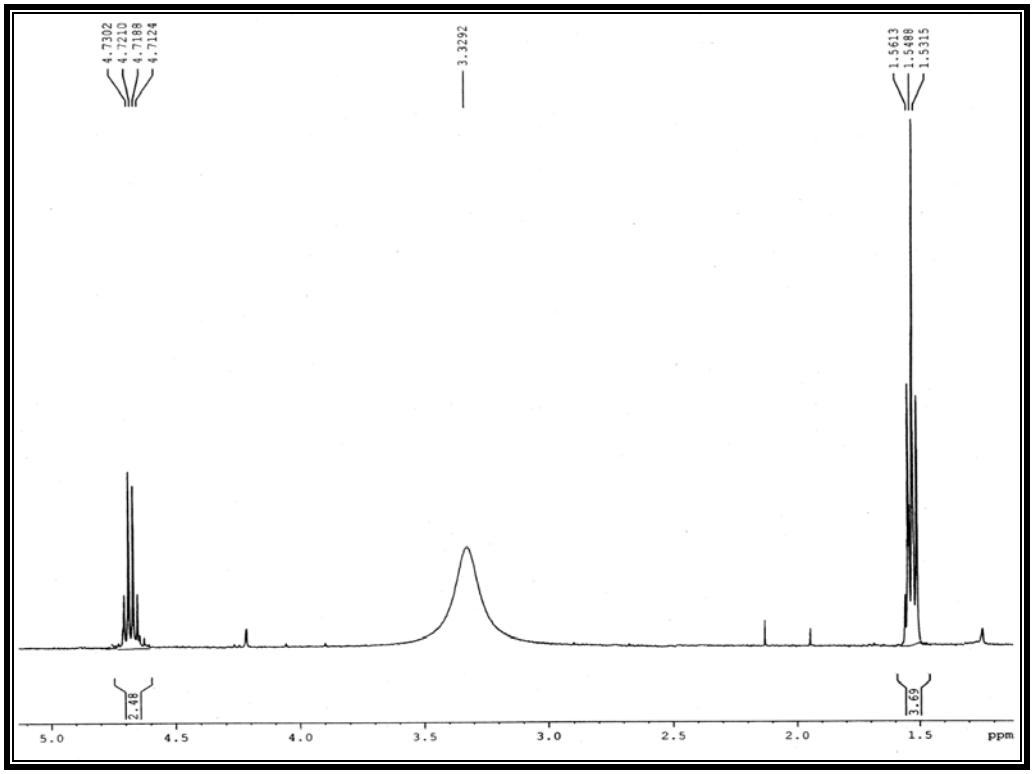
^{1}H NMR spectrum of DDK-A-11



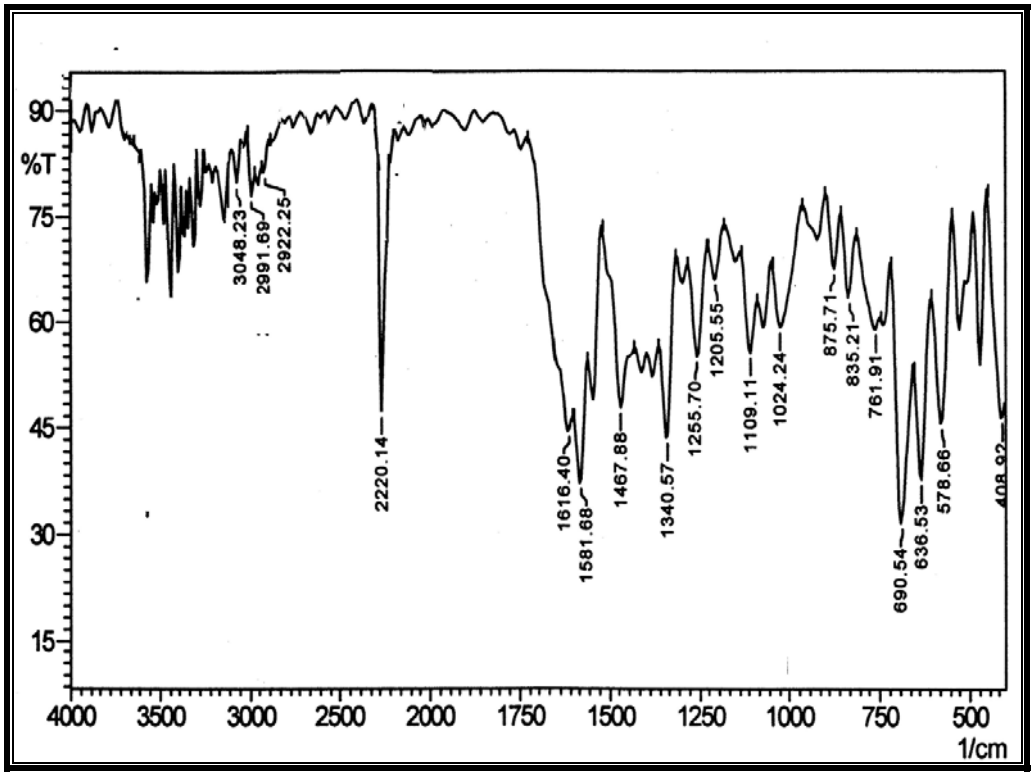
Expanded ^{1}H NMR spectrum of DDK-A-11



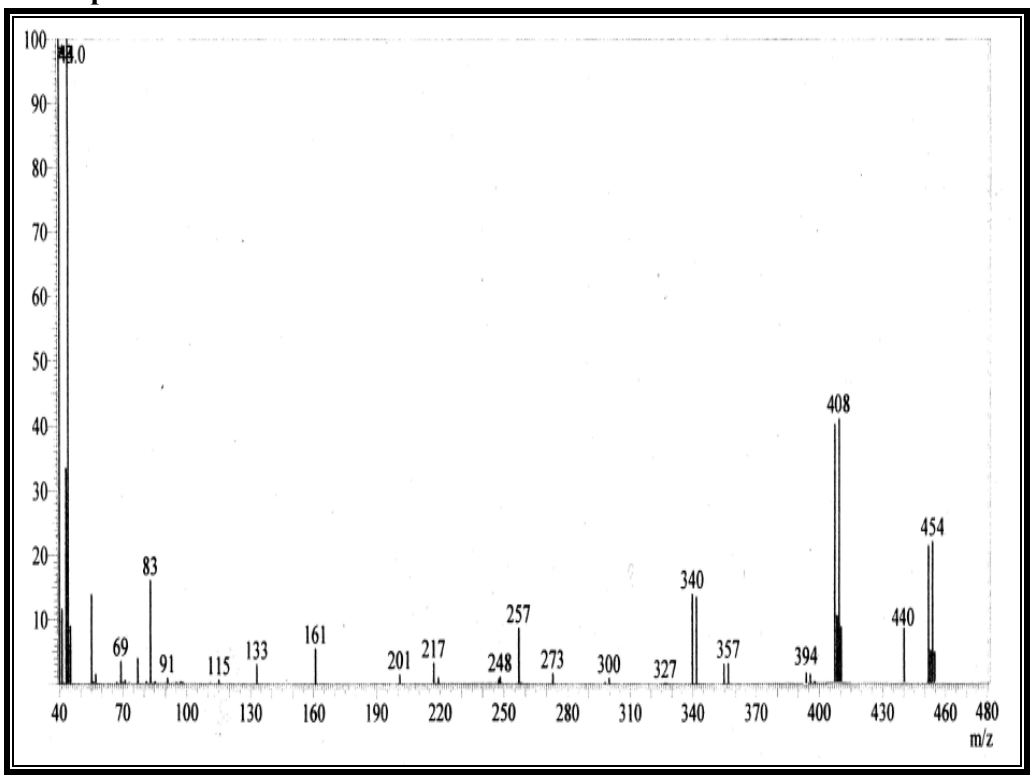
Expanded ^{1}H NMR spectrum of DDK-A-11



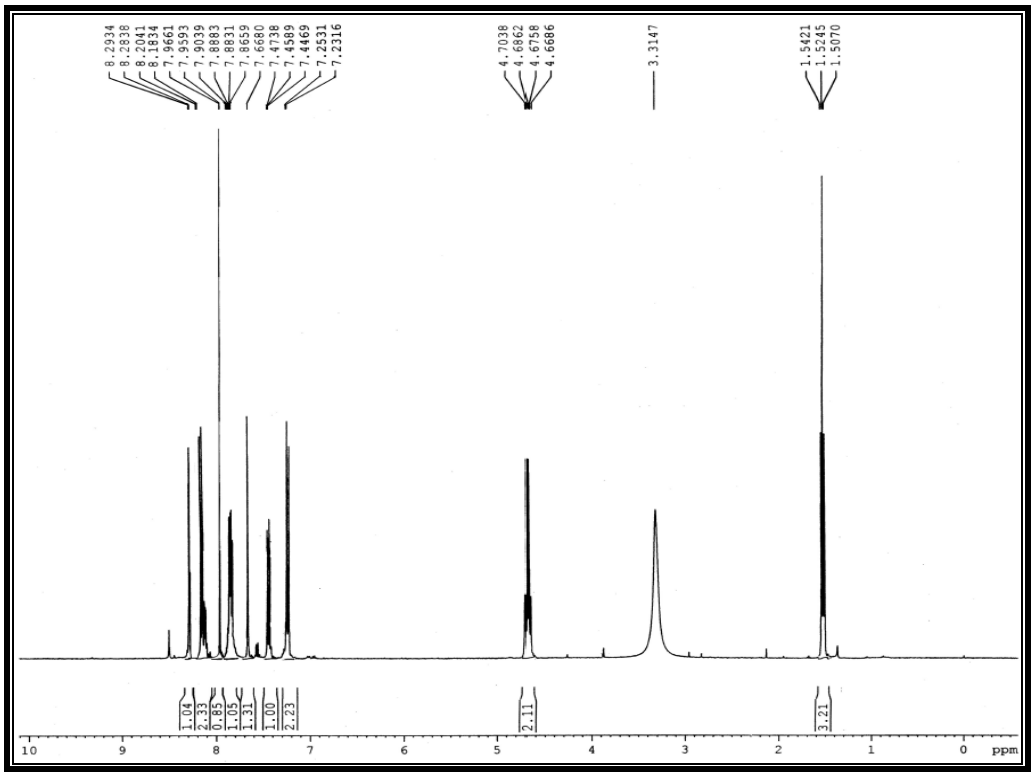
IR spectrum of DDK-A-14



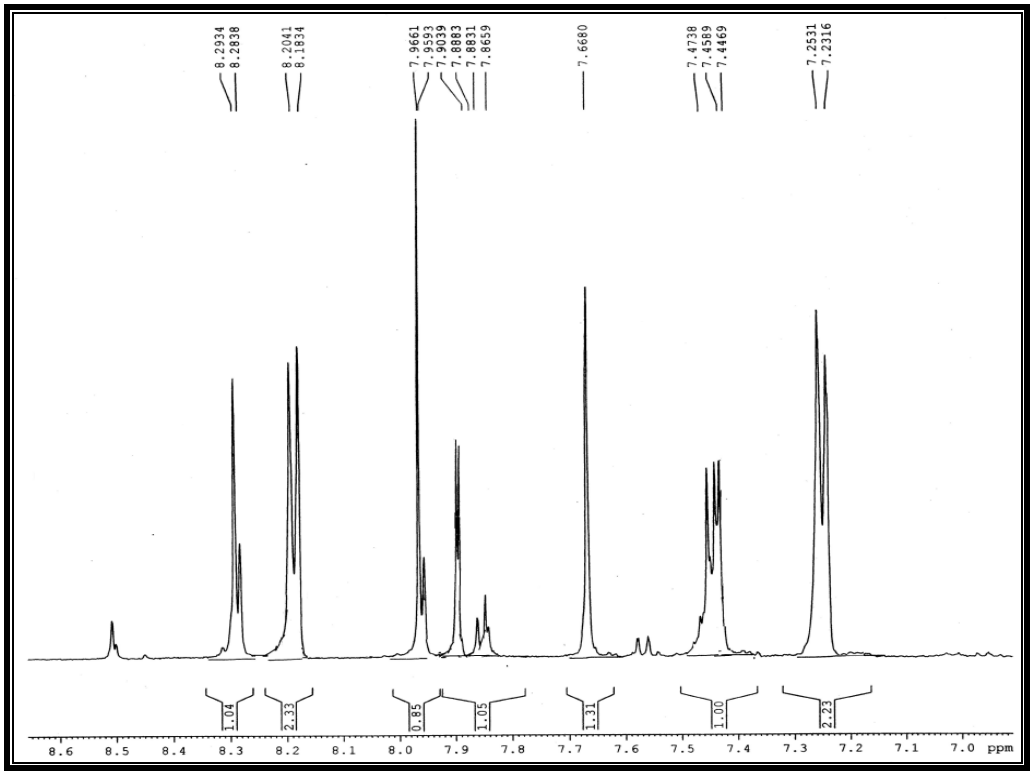
Mass spectrum of DDK-A-14



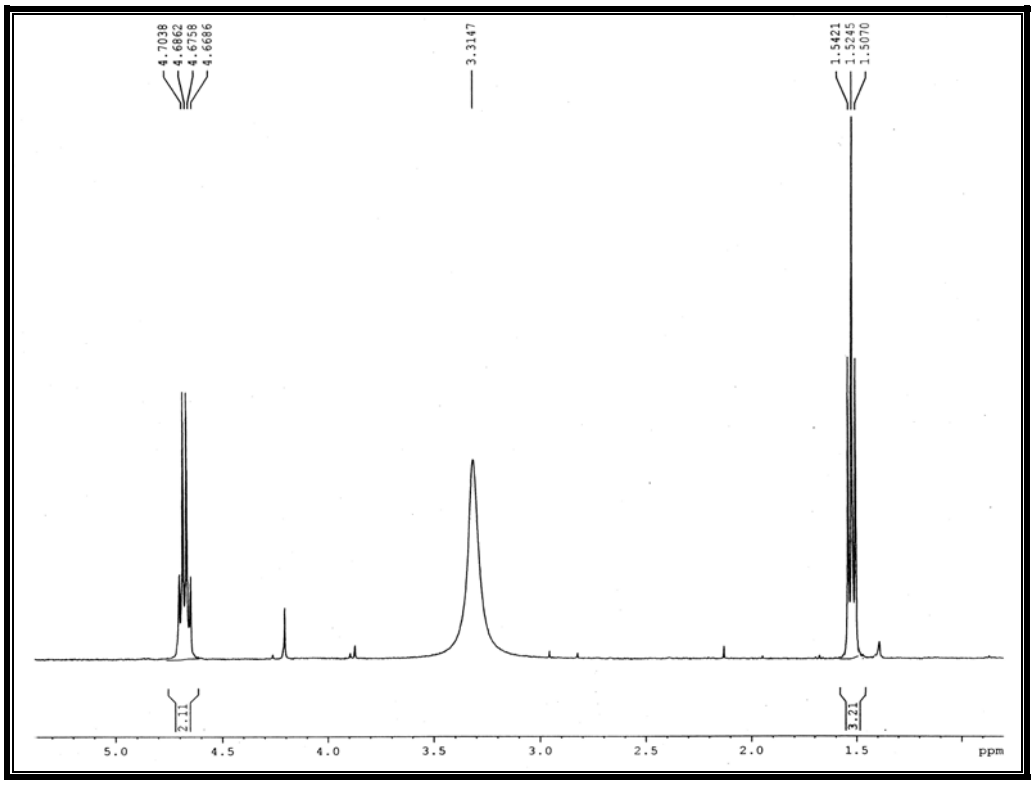
^{1}H NMR spectrum of DDK-A-14



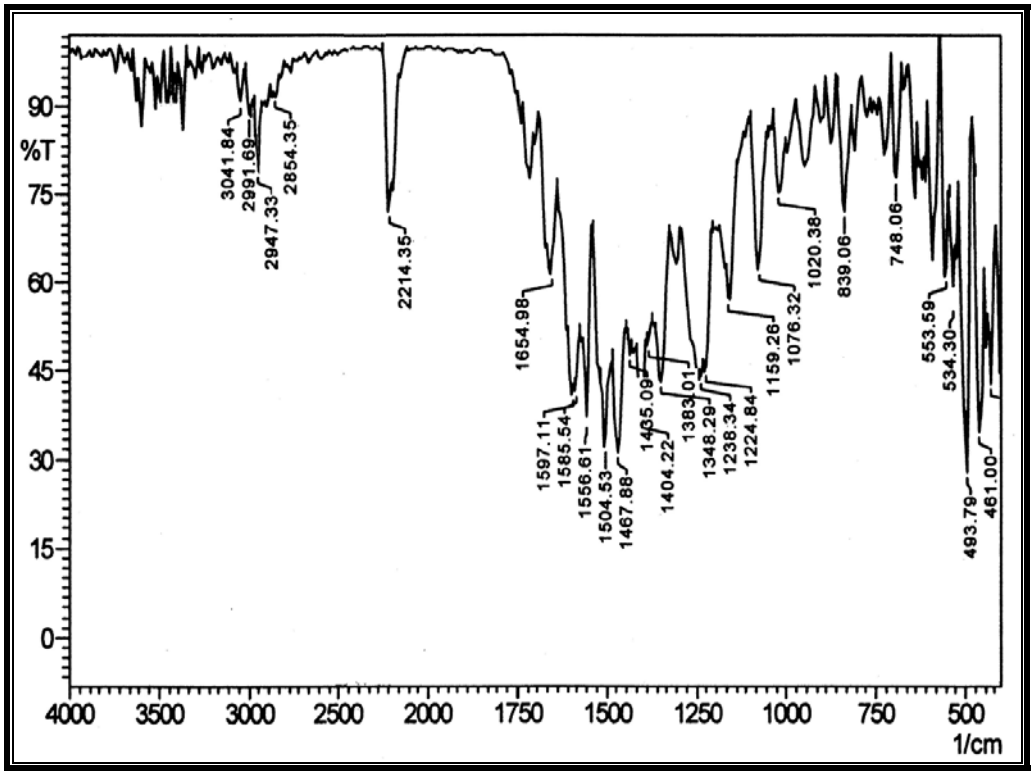
Expanded ^{1}H NMR spectrum of DDK-A-14



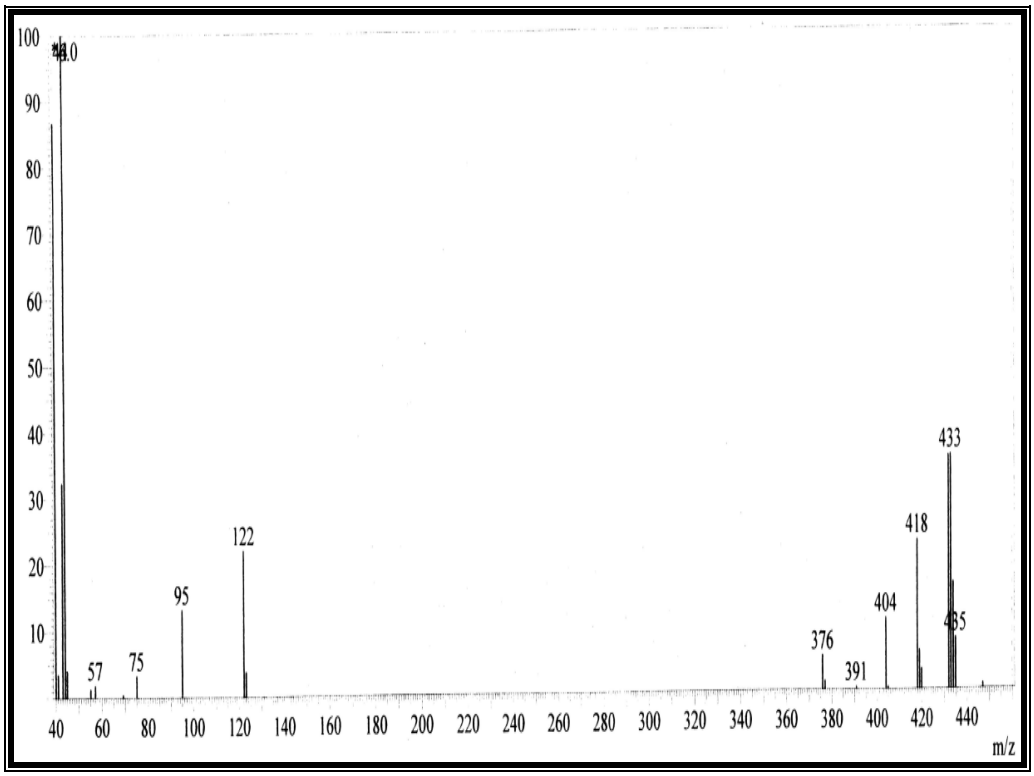
Expanded ^{1}H NMR spectrum of DDK-A-14



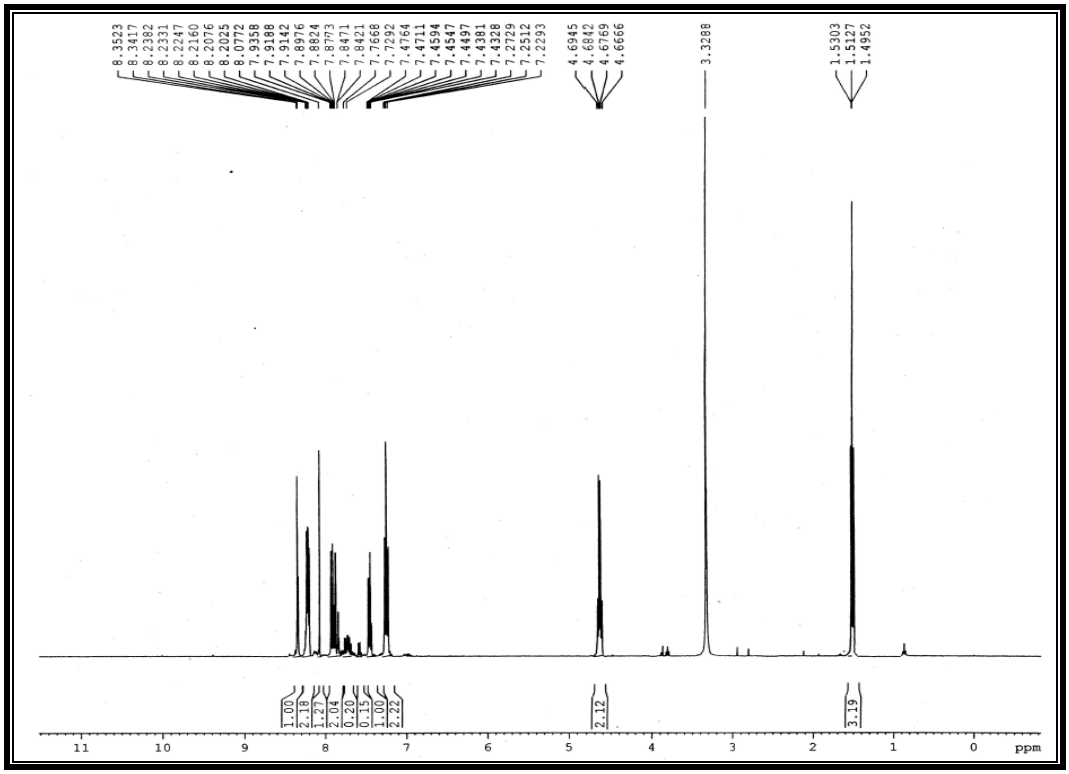
IR spectrum of DDK-A-19



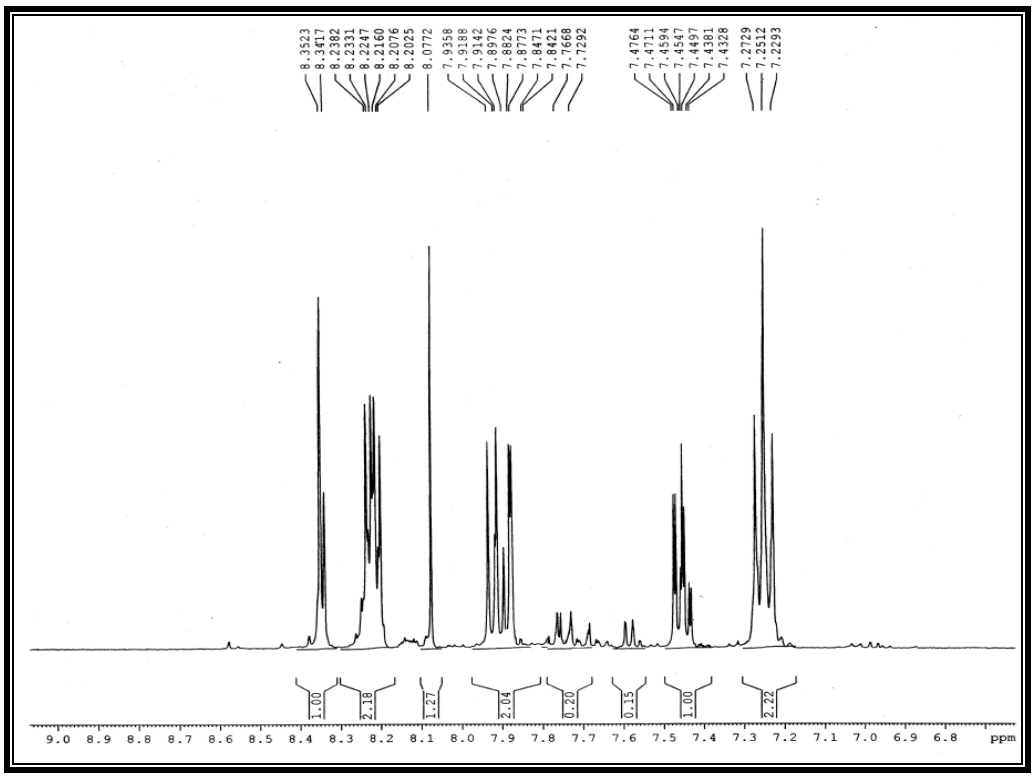
Mass spectrum of DDK-A-19



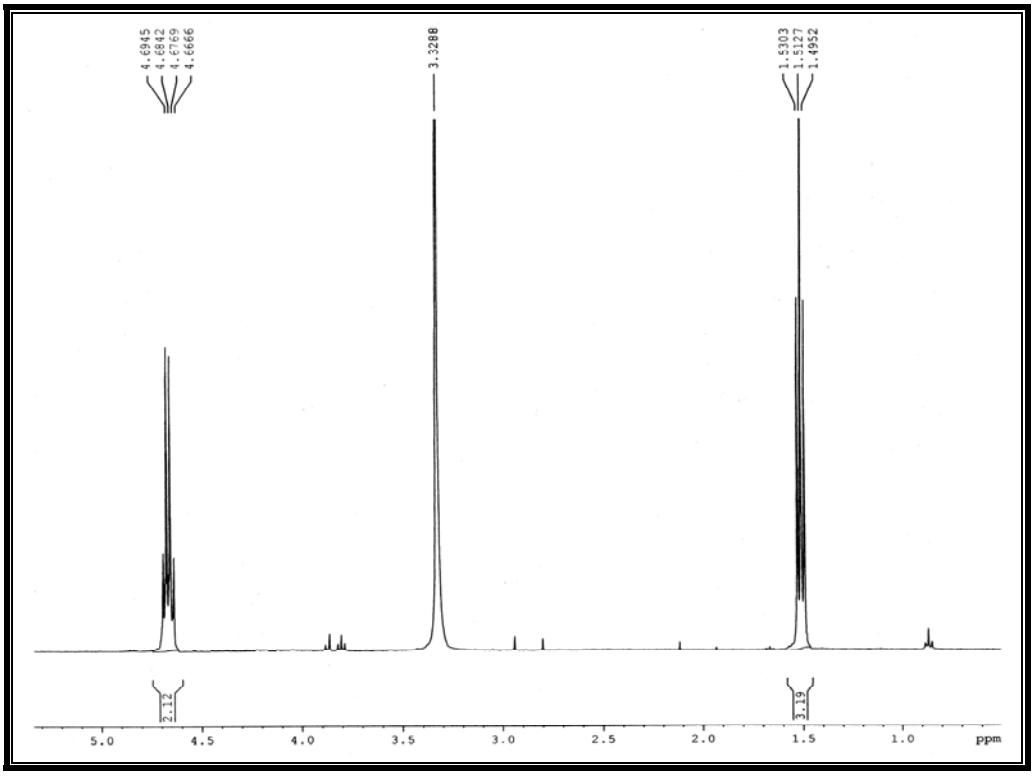
^{1}H NMR spectrum of DDK-A-19



Expanded ^{1}H NMR spectrum of DDK-A-19



Expanded ^{1}H NMR spectrum of DDK-A-19



2.1.8 Biological evaluation

2.1.8.1 Antimicrobial evaluation

All of the synthesized compounds (**DDK-A-01 to DDK-A-20**) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [88] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin, and Griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using microdilution broth method according to NCCLS standards [88(a)]. Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations. In primary screening 1000 μg mL^{-1}, 500 μg mL^{-1} and 250 μg mL^{-1} concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution at 125 μg mL^{-1}, 62.5 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1}, and 6.250 μg mL^{-1} concentration against all microorganisms. The tubes were inoculated with 10^8 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table 1.

Table 1. Antibacterial and antifungal activity of synthesized compounds DDK-A-01to DDK-A-20

Code	Minimum inhibition concentration (μg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	***S. p.***	***E.c.***	***P.a.***	***C. a.***	***A. n.***	***A.c.***
DDK-A-01	500	500	500	500	500	500	>1000
DDK-A-02	1000	1000	1000	1000	1000	500	500
DDK-A-03	>1000	500	250	500	1000	250	250
DDK-A-04	100	62.5	125	100	500	500	500
DDK-A-05	1000	1000	1000	1000	1000	>1000	500
DDK-A-06	25	100	200	100	1000	500	500
DDK-A-07	125	200	500	250	500	500	250
DDK-A-08	100	125	100	100	1000	500	500
DDK-A-09	50	100	25	100	>1000	500	1000
DDK-A-10	25	50	100	50	500	500	1000
DDK-A-11	1000	500	100	1000	>1000	500	>1000
DDK-A-12	500	>1000	500	100	500	250	1000
DDK-A-13	500	500	500	1000	>1000	500	125
DDK-A-14	125	100	50	250	250	1000	500
DDK-A-15	500	>1000	500	500	500	500	1000
DDK-A-16	50	125	100	200	>1000	500	>1000
DDK-A-17	125	100	250	125	500	500	>1000
DDK-A-18	125	250	125	50	500	1000	500
DDK-A-19	100	125	100	250	500	1000	>1000
DDK-A-20	50	125	250	100	100	1000	500
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

2.1.8.2 Antimycobacterial, anticancer and antiviral evaluation

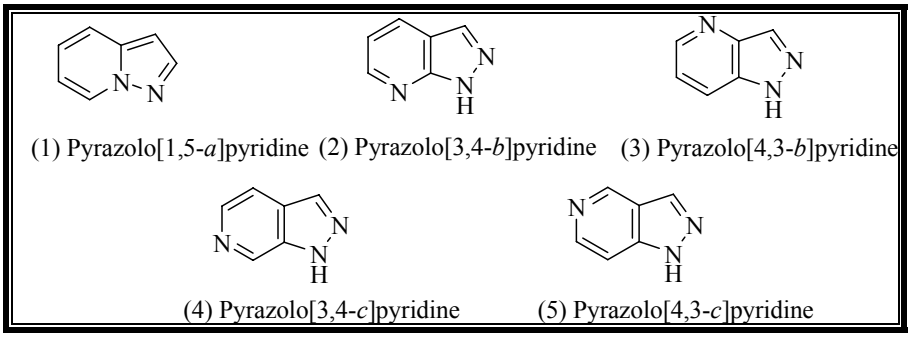
Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds (**DDK-A-01** to **DDK-A-20**) is currently under investigation and results are awaited.

2.2: Synthesis and biological evaluation of 6-alkoxy-3-methyl-4-aryl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitriles

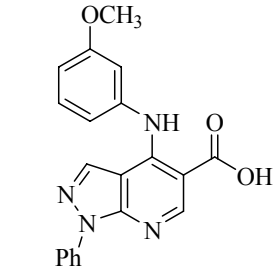
2.2.1 Introduction

Fused pyridines continue to attract considerable attention of researchers in different countries because of their great practical usefulness, primarily, due to a very wide spectrum of their biological activities. Pyrazolopyridines occupy a special position among these compounds. Along with some other pyridine systems containing an annelated five membered heteroaromatic ring, pyrazolopyridines are isosters of bioactive indoles or indazoles [89, 90] and have been reported to possess useful properties as antimetabolites in the biochemical synthesis of purine [91].

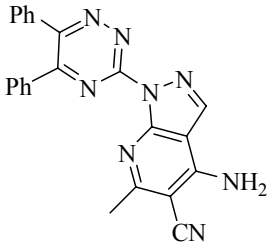
When pyrazole and pyridine rings are fused together, five isomeric pyrazolopyridines arise from such fusion corresponding to the five possible types of annulations of pyrazole to the pyridine ring as shown below.



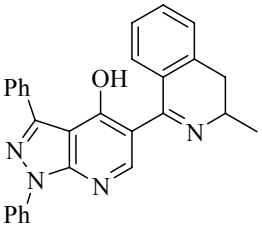
Pyrazolo[3,4-*b*]pyridines have been reported to possess wide variety of biological activities. Analgesic [92], anxiolytic [93], hypnotic [94], xanthine oxidase inhibitory [95, 96], antiviral [97], anti-HIV [98], Corticotropin-Releasing Factor (CRF) antagonist [99, 100], antidiabetic [101], antiarrhythmic [102], antitumor [103] activities have been reported for certain pyrazolopyridine derivatives. Some examples of published derivatives of pyrazolo[3,4-*b*]pyridines with their biological activities are as follows.



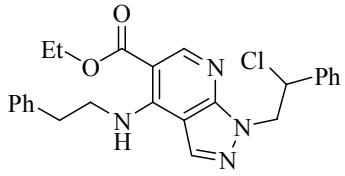
Activity: Antibacterial [104]



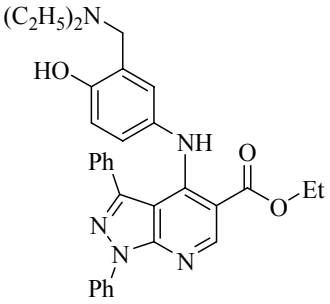
Activity: Antimicrobial [105]



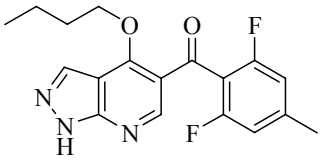
Activity: Antifilarial [106]



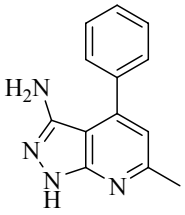
Activity: A_1 Adenosine Receptor inhibitory [107]



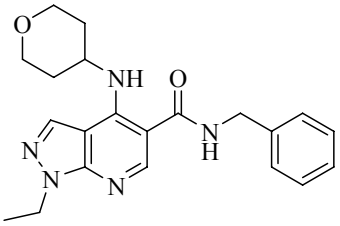
Activity: Antileishmanial [108]



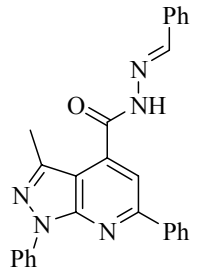
Activity: CDK-1 inhibitory [109]



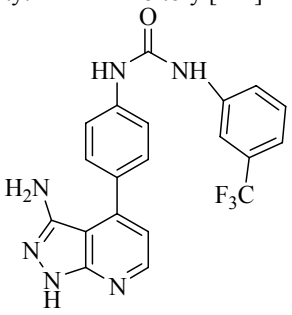
Activity: Antiinflammatory [110]



Activity: PDE4 inhibitory [111]



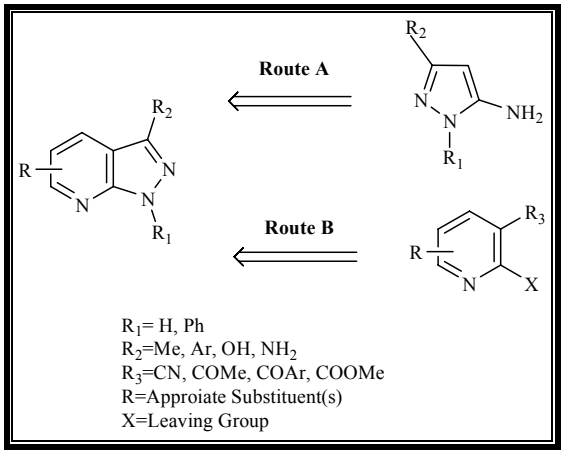
Activity: Antiparasitic [112]



Activity: VEGFR/PDGFR kinase inhibitory [113]

2.2.2 Reported synthetic strategies

Synthetic approaches towards pyrazolo[3,4-*b*]pyridines can be divided conceptually into two main groups: (a) appropriately substituted pyrazoles onto which a pyridine ring is annelated, and (b) suitably substituted pyridines onto which a pyrazole ring is annelated as summarized in the figure below.



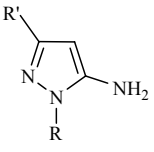
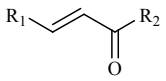
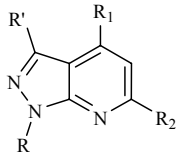
2.2.2. 1. ANNELATION OF PYRIDINE RING ONTO PYRAZOLE

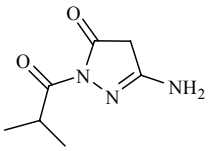
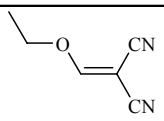
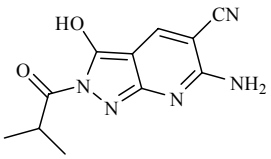
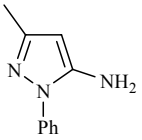
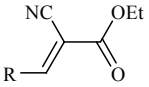
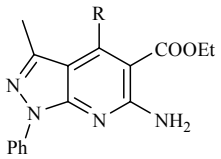
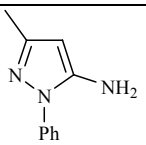
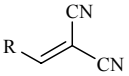
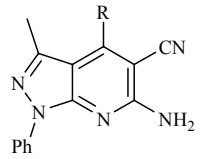
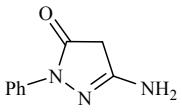
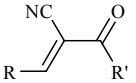
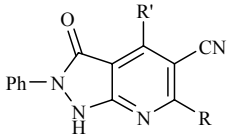
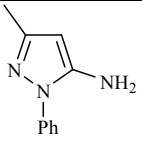
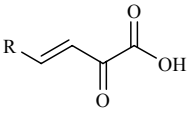
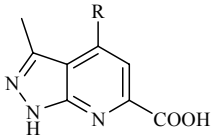
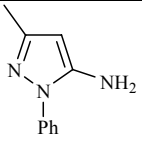
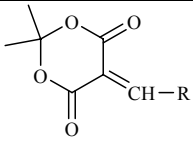
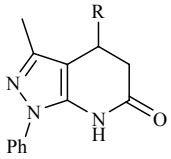
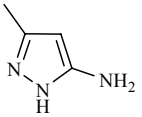
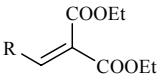
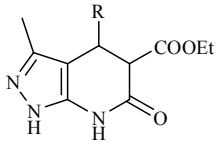
2.2.2.1.1 From 3(5)-Aminopyrazoles

2.2.2.1.1.1 Use of α, β- unsaturated reagents (as bifunctional synthons)

By far the most pyrazolo[3,4-*b*]pyridine synthesis are condensations of aminopyrazole with α, β- unsaturated synthons. Reactions of aminopyrazole as 1,3-binucleophiles with variety of such α, β- unsaturated synthons have been reported proving this method highly useful for the construction of pyrazolo[3,4-*b*]pyridine core with versatile substituents.

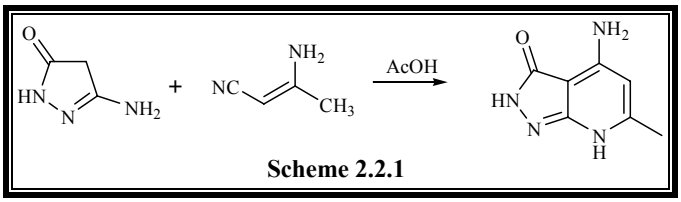
Examples of pyrazolo[3,4-*b*]pyridine synthesis published in the relevant period are listed in Table below, arranged according to the synthons used.

Entry	Aminopyrazole	α, β- unsaturated synthon	Pyrazolo[3,4-*b*]pyridine	Ref.
1				[114]

2				[115]
3				[116]
4				[116]
5				[117]
6				[118]
7				[118]
8				[120]

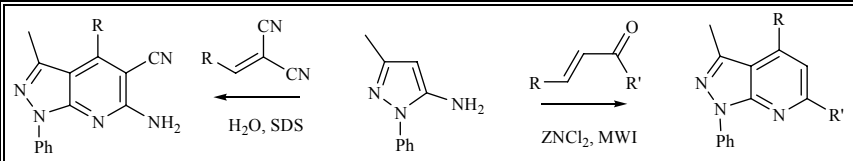
[a]R and R' means (substituted) aryl or heteroaryl substituent.

An interesting variation in this type of reactions is the use of β-aminocrotononitrile or its derivatives resulting in generation of bioactive substituted dihydropyrazolo[3,4-*b*]pyridines with 4-amino and 6-methyl substituents available for further synthetic explorations [121] (Scheme 2.2.1).



Scheme 2.2.1

Generally, the cyclizations are accomplished in acidic medium [121, 122] or in presence of base catalysts like triethyl amine or piperidine [116]. New green chemistry approaches recently described involve solvent-free reaction under microwave irradiation with or without catalyst [123, 124] as well as reactions in aqueous media [125], which are environmentally benign and afford high yields (Scheme 2.2.2).



R = 4-OMeC$_6$H$_4$, 4-ClC$_6$H$_4$, 4-BrC$_6$H$_4$, 4-NO$_2$C$_6$H$_4$, 4-MeC$_6$H$_4$, 4-FC$_6$H$_4$, 3-ClC$_6$H$_4$, 3, 4-Cl$_2$C$_6$H$_4$

a R = 4-BrC$_6$H$_4$, R' = 2-ClC$_6$H$_4$; **b** R = 4-BrC$_6$H$_4$, R' = 4-MeC$_6$H$_4$; **c** R = 4-FC$_6$H$_4$, R' = 4-MeC$_6$H$_4$; **d** R = 3,4-Cl$_2$C$_6$H$_4$, R' = 4-OMeC$_6$H$_4$; **e** R = 3-NO$_2$C$_6$H$_4$, R' = 4-OMeC$_6$H$_4$; **f** R = 4-ClC$_6$H$_4$, R' =2-OMeC$_6$H$_4$ **g** R = 4-OMeC$_6$H$_4$, R' = 4-MeC$_6$H$_4$ **h** R = 4-ClC$_6$H$_4$, R' = 4-FC$_6$H$_4$ **i** R = 4-OMeC$_6$H$_4$, R' = 2-ClC$_6$H$_4$ **j** R = 4-ClC$_6$H$_4$, R' = 2,4-Cl$_2$C$_6$H$_4$

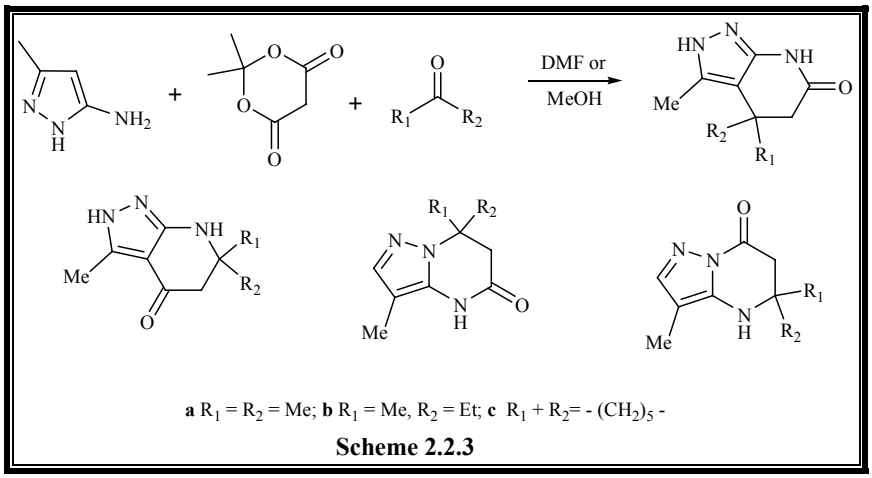
Scheme 2.2.2

Several efficient multi-component reaction (MCR) approaches have been reported attractively replacing the use of α, β- unsaturated synthons with their synthetic precursors. MCR approaches avoid the synthesis of α, β- unsaturated synthons, thereby reduce the reaction steps. The MCRs generally involve the one-pot reaction of aminopyrazole with aldehyde and 1,3-bifunctional reagents such as pyruvic acid [118], malononitrile [126], ethyl cyanoacetate [127] etc.

Unraveling site selectivity in these additions is not an easy task unless an acyclic intermediate can be isolated or the same reaction products can be synthesized by alternate routes. In several cases, modern NMR techniques studies were used to corroborate the structures of reaction products.

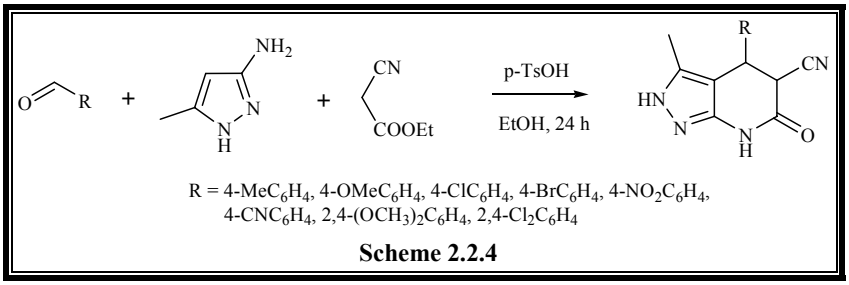
Lipson et al. reported that on boiling 3-methyl-5-aminopyrazole with an equimolar amount of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) and ketones, both in methanol and in DMF, only the corresponding pyrazolo[3,4-*b*]pyridin-6-ones were formed and other possible isomeric products were not formed [128]. The structures of compounds were established ^{1}H NMR spectra signals and *Nuclear Overhauser Experiment* (NOE) (Scheme 2.2.3).

Rahmati investigated a one-pot, three-component condensation reaction of 3-amino-5-methylpyrazole, aldehyde, ethyl cyanoacetate under reflux conditions in ethanol using p-toluenesulfonic acid (p-TsOH) as the catalyst [129] (Scheme 2.2.4). The ^{1}H NMR spectra of the products indicated the formation of two diastereoisomers (cis and trans).



a $R_1 = R_2 = Me$; **b** $R_1 = Me$, $R_2 = Et$; **c** $R_1 + R_2 = -(CH_2)_5-$

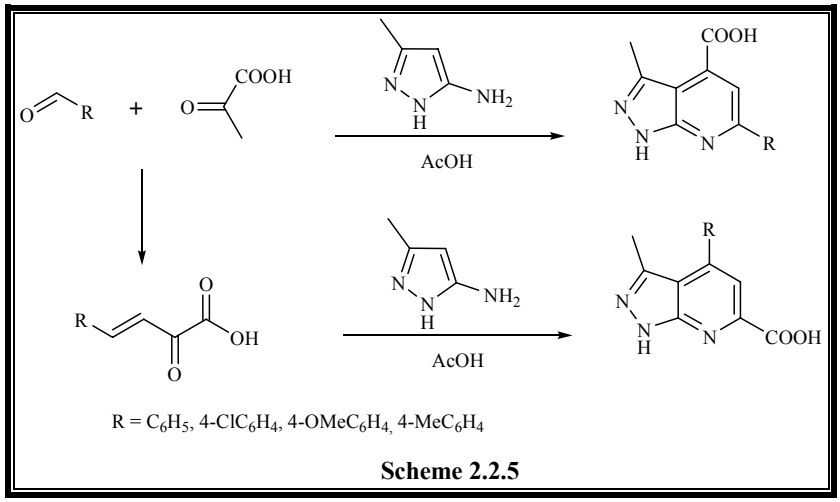
Scheme 2.2.3



R = 4-MeC_6H_4, 4-$OMeC_6H_4$, 4-ClC_6H_4, 4-BrC_6H_4, 4-$NO_2C_6H_4$, 4-CNC_6H_4, 2,4-$(OCH_3)_2C_6H_4$, 2,4-$Cl_2C_6H_4$

Scheme 2.2.4

In some cases, a choice of multicomponent or linear protocol allows obtaining different heterocycles. For instance, MCR involving 5-aminopyrazole, aldehyde and pyruvic acid afforded positional isomer of product obtained from sequence pathway via preliminary synthesis of arylidenpyruvic acids [118] (Scheme 2.2.5).



R = C_6H_5, 4-ClC_6H_4, 4-$OMeC_6H_4$, 4-MeC_6H_4

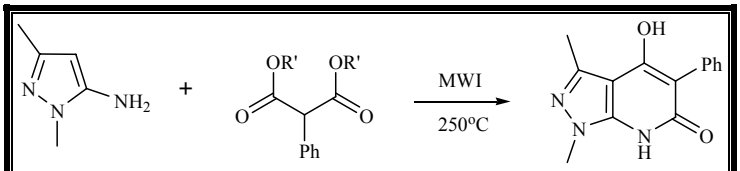
Scheme 2.2.5

Different products of the three-component and linear treatments in the case of 5-aminopyrazole can be the evidence that this MCR follows independent pathway without *in situ* formation of α, β- unsaturated compound.

2.2.2.1.1.2 Use of 1,3-dicarbonyl reagents (1,3-dielectrophiles) and other C-H acids

Cyclocondensations of aminopyrazoles with 1,3-dielectrophiles are extensively used for preparation of bicyclic nitrogen heterocycles viz. pyrazolo[1,5-*a*]pyrimidines and pyrazolo[3,4-*b*]pyridines. N1-unsubstituted 3(5)- aminopyrazoles usually react with 1,3-dicarbonyl compounds providing pyrazolo[1,5-*a*]pyrimidines [130-132]. The reaction of N1-substituted 5-aminopyrazoles with 1,3-dielectrophiles forms substituted pyrazolo[3,4-*b*]pyridines; as bifunctional reagents 1,3-ketoesters [133-136] as well as symmetric 1,3-diketones [135] are generally employed. The reaction is generally accomplished using acetic acid as a solvent [133], but cyclizations using hydrochloric acid in ethanol [134] or using zinc chloride and hydrochloric acid in ethanol [135] are also documented.

Microwave-assisted reaction of 1,3-dimethyl-5-amino-pyrazole with activated aryl malonates is reported by Rivkin et al. [137] (Scheme 2.2.6). Reaction of 5-aminopyrazole with aryl 1,3-ketoester is also documented [133].



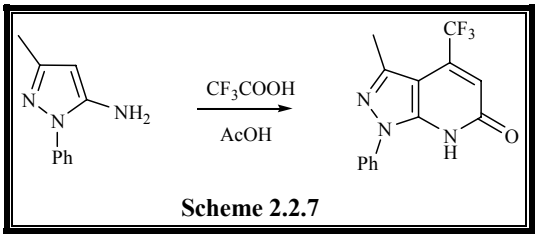
a R' = Et; **b** R' = 2,4,6-Cl-C_6H_2

Scheme 2.2.6

Literature survey also revealed few examples involving use of trifluoromethyl containing 1,3-ketoesters [135, 138] and symmetric 1,3-diketones [134], which exclusively lead to formation of a single pyrazolo[3,4-*b*]pyridine isomer as a product. Volochnyuk et al. have reported the synthesis of pyrazolo[3,4-*b*]pyridine using trifluoroacetic acid [139] (Scheme 2.2.7**)**.

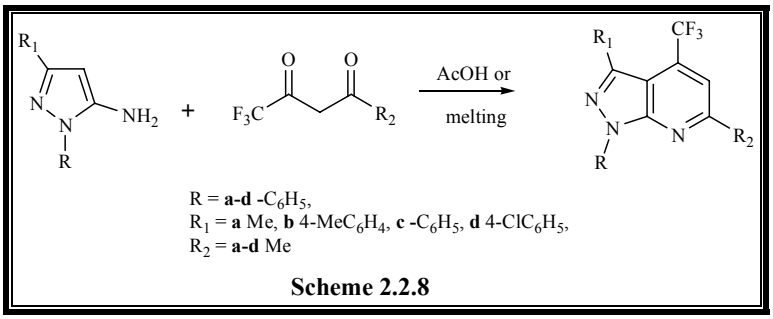
However, the application of unsymmetrical 1,3-diketones to the synthesis of pyrazolo[3,4-*b*]pyridines is poorly documented evidently because two regioisomeric

products may form in the reaction. The problem of regiodirection of the reaction is still urgent for the molecules of 3(5)-aminopyrazoles and unsymmetrical 1,3-dielectrophiles.



Scheme 2.2.7

Emelina et al. investigated the direction of reaction between N1-substituted aminopyrazoles and trifluoromethyl-containing unsymmetrical 1,3-diketones [140]. The study established characteristic spectral distinctions of individual regioisomers using ^{1}H and ^{13}C NMR spectroscopy. It was proved that the regioisomer was formed having trifluoromethyl group attached at the C-4 (Scheme 2.2.8).



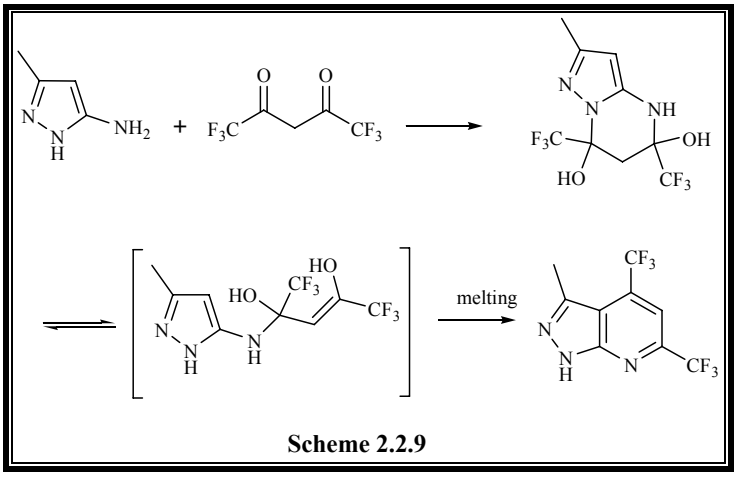
R = **a-d** -C_6H_5,
R_1 = **a** Me, **b** 4-MeC_6H_4, **c** -C_6H_5, **d** 4-ClC_6H_5,
R_2 = **a-d** Me

Scheme 2.2.8

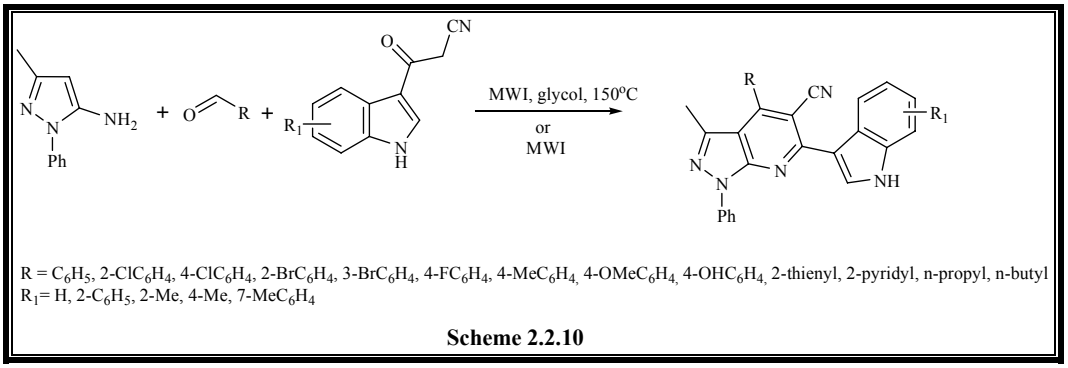
The same research group further published the studies on regiodirection of the reaction of N1-unsubstituted 5-amino-3-methyl-pyrazole with hexafluoroacetyl-acetone under different reaction conditions [141]. They established the structure of intermediate compound leading to the formation of pyrazolo[3,4-*b*]pyridine by 2D NMR by means of homo and heteronuclear correlation procedures: DQF-COSY, J-COSY, NOESY, and HSQC without decoupling from ^{13}C (Scheme 2.2.9).

Three component reaction of 5-aminopyrazole with aldehydes and ethyl acetoaceate under microwave irradiation is recently reported [116]. As CH-acids in the MCRs with 5-aminopyrazoles and aldehydes, aroylacetonitriles have been efficiently employed affording 4,6-diaryl-5-cyanopyrazolo[3,4-*b*]pyridines. Zhu et

al.have reported microwave-assisted synthesis of 3-methyl-5-aminopyrazole with 3-cyanoacetyl indole and aldehydes in glycol as a reaction medium [142]. The same reaction was effected regioselectively by Quiroga et al. using solvent-free approach under microwave irradiation [117] (Scheme 2.2.10). A solvent-free green chemistry approach was adopted in one pot cyclocondensation of 5-amino-3-aryl-1H-phenylpyrazole, p-substituted benzoylacetonitriles, and some aldehydes using ammonium acetate as a reaction medium [143] as well as under neat conditions for 5-amino-3-pyrazolone, aldehydes and aroylacetonitriles [144].



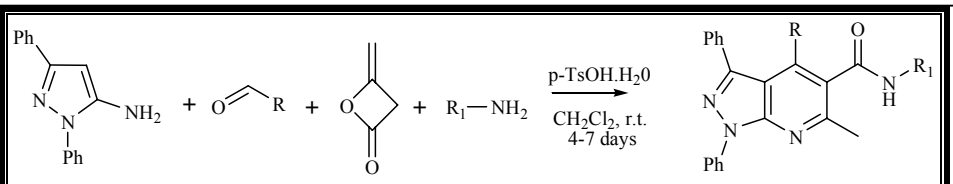
Scheme 2.2.9



R = C_6H_5, 2-ClC_6H_4, 4-ClC_6H_4, 2-BrC_6H_4, 3-BrC_6H_4, 4-FC_6H_4, 4-MeC_6H_4, 4-$OMeC_6H_4$, 4-OHC_6H_4, 2-thienyl, 2-pyridyl, n-propyl, n-butyl
R_1= H, 2-C_6H_5, 2-Me, 4-Me, 7-MeC_6H_4

Scheme 2.2.10

Shaabani et al. have reported novel four-component reaction of aryl/alkyl amines, diketene, aldehydes and 1,3-diphenyl-5-aminopyrazole in the presence of p-toluene sulfonyl chloride as a catalyst in dry dichloromethane at ambient temperature [145] (Scheme 2.2.11).

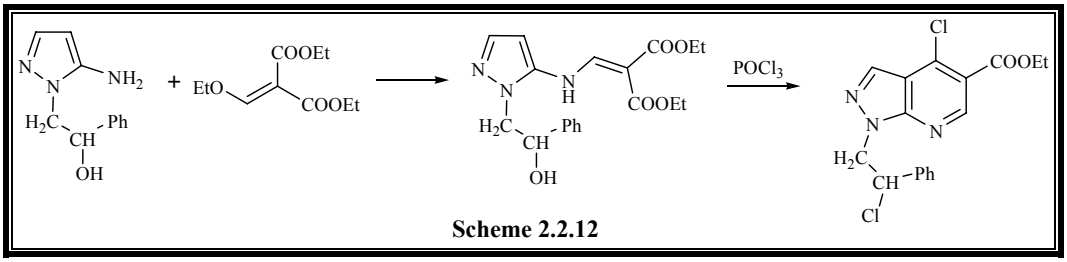


R = C_6H_5, 4-FC_6H_4, 4-ClC_6H_4, 4-BrC_6H_4, 4-MeC_6H_4, 3-ClC_6H_4
R_1= -C_6H_5, 4-MeC_6H_4, n-Pr

Scheme 2.2.11

2.2.2.1.1.3 Gould-Jacobs reaction

The classical Gould-Jacobs reaction for quinoline synthesis involves reaction between aniline and diethyl ethoxymethylene malonate. This reaction is widely documented in recent literature for the synthesis of 4-hydroxy- or 4-chloro-pyrazolo[3,4-*b*]pyridine-5-carboxylates using 5-aminopyrazole as a substitute for aniline in the classical Gould-Jacobs method [109, 146-149]. The first step of the reaction involves the addition of diethyl ethoxymethylene malonate to 5-aminopyrazole and the reaction is effected in solvents like ethanol [148, 149], or under solvent-free conditions at higher temperatures (120-130^{o}C) [109, 147]. The adduct is further cyclized in diphenyl ether at 240^o C to give 4-hydroxy-pyrazolo[3,4-*b*]pyridine-5-carboxylate [109] or in phosphorus oxychloride to give the 4-chloro-pyrazolo[3,4-*b*]pyridine-5-carboxylates [146-149]. The scope of the reaction is extended by employing novel N1-substituted 5-aminopyrazoles [109, 146] (Scheme 2.2.12).



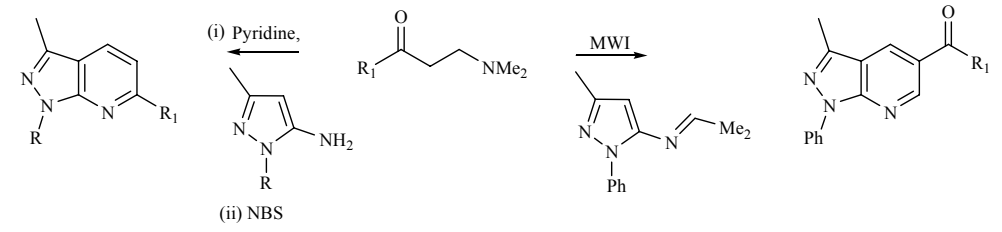
Scheme 2.2.12

2.2.2.1.1.4 Reactions with β-dimethyl-aminopropiophenones

Quiroga et al. have reported an interesting synthesis from 5-aminopyrazoles and β-dimethyl aminopropiophenones yielding 6-aryl-4,5-dihydropyrazolo[3,4-*b*]pyridines, which are aromatized upon treatment with N-bromo succinimide (NBS) to give [150] (Scheme 2.2.13).

Also of interest is the microwave promoted synthesis of 5-aroyl-pyrazolo[3,4-

b]pyridines by hetero-Diels-Alder reaction of N-[(dimethylamino)methylene]-3-methyl-1-phenyl-5-pyrazolamine with β-dimethyl aminopropiophenones in dry media in just one step [151] (Scheme 2.2.13).



50 R = **a** -C_6H_5, **b** -C_6H_5, **c** 4-ClC_6H_4, **d** 4-ClC_6H_4, **e** 2-ClC_6H_4, **f** 4-$NO_2C_6H_4$
R_1 = **a** 4-ClC_6H_4, **b** 4-$NO_2C_6H_4$, **c** 4-ClC_6H_4, **d** 4-$NO_2C_6H_4$, **e** 4-$NO_2C_6H_4$, **f** 4-ClC_6H_4

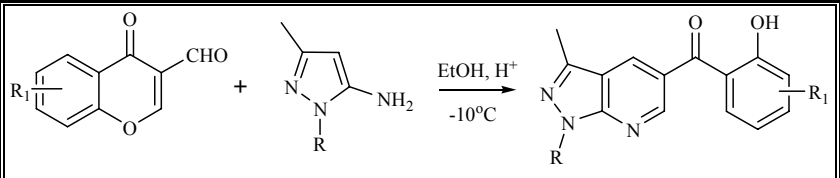
52 R_1 = 4-ClC_6H_4, 4-BrC_6H_4, 4-$NO_2C_6H_4$

Scheme 2.2.13

2.2.2.1.1.5 Ring opening reactions

Recently, ring opening reactions of variety of reagents with aminopyrazoles have drawn considerable attention for the complexity-generating synthesis of pyrazolo[3,4-b]pyridines baring versatile substituents.

Synthesis of pyrazolo[3,4-*b*]pyridine derivatives, which contain synthetically useful hydroxyl and carbonyl groups, was achieved from aminopyrazole and 3-formyl chromone via opening of the γ-pyrone ring of 3-formyl chromone [152] (Scheme 2.2.14). Similar reaction was efficiently achieved from 3-formyl chromone and aminopyrazole in presence of chlorotrimethylsilane in DMF [153]. Reaction of 6-methyl-4-oxo-4H-[1]-benzopyran-3-carboxaldehyde with 5-amino-3-methyl-1-phenylpyrazole is also reported to furnish 5-(2-hydroxy-5-methylbenzoyl)-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine [154].

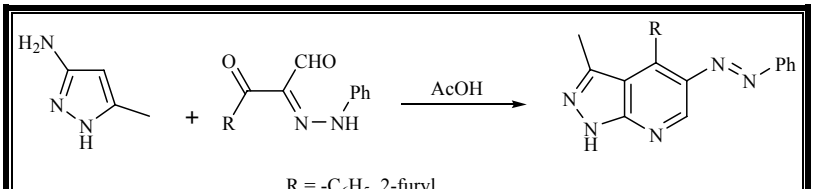


R = **a-f** -C_6H_5, **g-j** 4-$NO_2C_6H_4$, **k-o** Me,
R_1 = **a** -H, **b** 5-Me, **c** 5-F, **d** 5-Br, **e** 5-Cl, **f** 5-NO_2, **g** 5-Me, **h** 5-F, **i** 5-Br, **j** 5-Cl, **k** 5-Me, **l** 5-F, **m** 5-Br, **n** 5-Cl, **o** 5-NO_2

Scheme 2.2.14

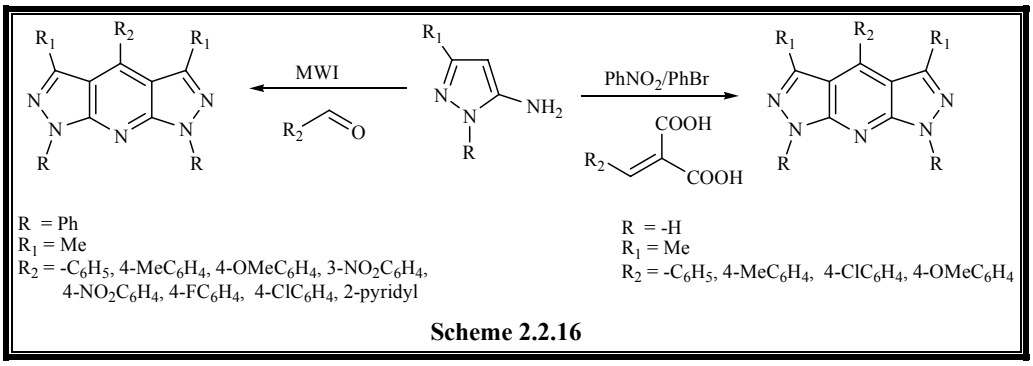
2.2.2.1.1.6 Miscellaneous reactions with 3(5)-aminopyrazoles

Abdel-Khalik et al. have reported reaction of 2-Arylhydrazonopropanals with 5-methyl-1H-pyrazol-3-amine yielding novel 3-substituted pyrazolo[3,4-*b*] pyridines [155] (Scheme 2.2.15).



Scheme 2.2.15

While microwave-assisted reaction of 5-amino-3-methyl-1-phenylpyrazole with aldehydes under solvent-free conditions furnished bispyrazolo[3,4-b:4',3'-e]pyridines [156]. Bispyrazolo[3,4-b:4',3'-e]pyridines were also obtained by refluxing 3-methyl-5-aminopyrazole with arylidenemalonic acid derivatives in nitrobenzene or bromobenzene [157] (Scheme 2.2.16).



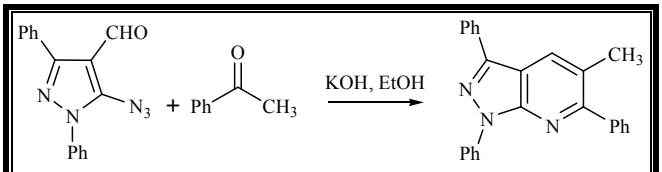
Scheme 2.2.16

2.2.2.1.2 Friedlander Reaction

The classical Friedlander quinoline synthesis is an acid or base catalyzed condensation followed by a cyclodehydration between an aromatic 2-aminoaldehyde or ketone with the carbonyl compound containing a reactive α-methylene group [158, 159]. Friedlander reaction of various 5-amino-pyrazole-4-carboxaldehydes have been efficiently employed in literature for the synthesis of pyrazolo[3,4-b]pyridines with versatile substituents. As active methylene compounds with 5-amino-pyrazole-4-carboxaldehydes; ethyl acetoacetate [160], ethyl 3-oxo-3-phenylpropanoate [161],

acetone [161, 167], acetyl acetone [160, 167], acetophenones [161, 167], diethyl malonate [161], aroylacetonitriles [161-163], aryl/heteroaryl acetonitrile [161, 162], malononitrile [160, 164] have been employed. Abd-El-Latif et al. have reported an interesting condensation between 3-phenyl-5-chloro-1-phenylpyrazole-4-carbaldehyde and cyanoacetamide/cyano-thioacetamide furnishing Friedlander type product 1,3-diphenylpyrazolo[3,4-*b*]pyridine derivatives [165]. Reactions are usually effected in presence of base catalysts like pyridine [160], piperidine [161, 162] or potassium hydroxide [161, 167].

The starting 5-amino-pyrazole-4-carboxaldehydes are generally synthesized by Vilsmeier-Haack formylation of 5-aminopyrazoles [161] or by reduction of 5-azidopyrazole-4-carboxaldehyde with hydrogen sulphide [160, 166] or sodium dithionite [166]. However, Zheng et al.have reported a facile one-pot conversion of 5-azidopyrazole-4-carboxaldehyde to pyrazolo[3,4-*b*]pyridines in presence of alcoholic potassium hydroxide [167] (Scheme 2.2.17).

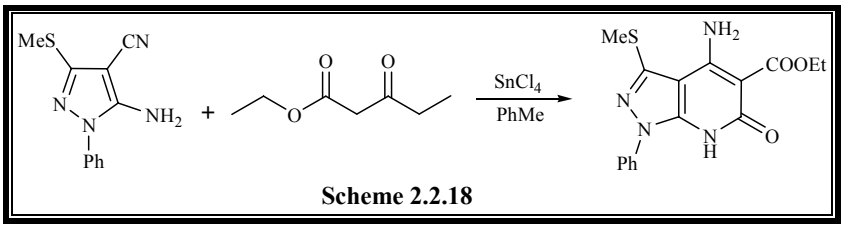


Scheme 2.2.17

2.2.2.1.3 From 4-cyano-5-amino-pyrazoles

Reactions of different substituted 4-cyano-5-amino-pyrazoles with variety of active methylenes or C-H acids are reported as valuable tool for obtaining highly functionalized pyrazolo[3,4-*b*]pyridines. Reactions of 4-cyano-5-amino-pyrazoles with ethyl acetoacetate [168, 169], acetyl acetone [168], diethyl malonate [168, 169], cyanoacetone [170], malononitrile [169] have been reported. The cyclocondensation is generally achieved in basic media like sodium ethoxide [170] or in presence of base like triethyl amine [169].

One interesting report using $SnCl_4$ as a catalyst in toluene for cyclocondensation is published by Zhao et al. [168] (Scheme 2.2.18).



Scheme 2.2.18

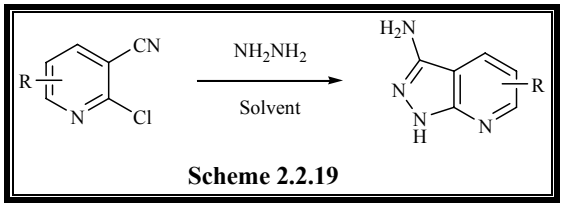
2.2.2. 2. ANNELATION OF PYRAZOLE RING ONTO PYRIDINE

Annelation of pyridine onto pyrazole ring (Route A) is the most commonly employed protocol and offers a degree of flexibility in terms of substitution about the pyridine ring. While Annelation of pyrazole onto pyridine ring (Route B) has remained relatively unexplored and has been confined to pyrazoles containing a methyl-, aryl-, hydroxy- or an amino- group at the 3-position [171]. Route B involves the formation of pyrazole ring from a 3-acetyl-, 3-carboxy- or 3-cyanopyridines bearing a leaving group in the 2-position.

2.2.2. 2.1 From 3-Cyanopyridines

2.2.2. 2.1.1 From 2-halo-3-cyanopyridines

Reaction of 2-chloro-3-cyanopyridines with hydrazine hydrate or its derivatives is widely employed strategy in literature furnishing 3-amino-pyrazolo[3,4-*b*]pyridines (for Route B) [171, 173-178]. Reaction is generally carried out by refluxing 2-chloro-3-cyanopyridines and hydrazine hydrate in suitable solvents viz. EtOH, BuOH, DMF etc (Scheme 2.2.19). However, base catalyst CS_2CO_3 in DMF has been used for cyclization by Lavecchia et al. [173].

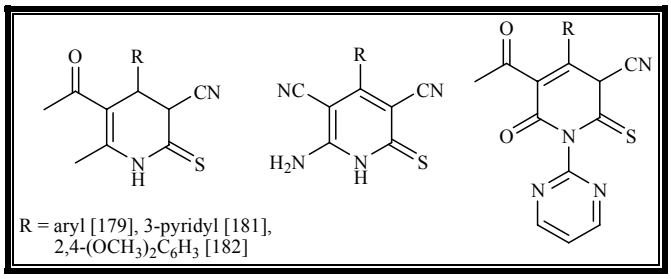


Scheme 2.2.19

2.2.2. 2.1.2 From 2-thioxo-3-cyanopyridines

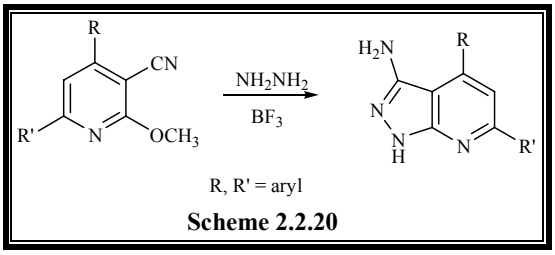
Literature survey also revealed several reports involving reaction of 2-thioxo-3-cyanopyridines [179-182] or its S-methyl derivatives [181, 183, 184] with hydrazine

hydrate for the synthesis of 3-amino-pyrazolo[3,4-*b*]pyridines. Variety of 2-thioxo-3-cyanopyridines have been used by different researchers as shown below.



2.2.2.2.1.3 From 2-alkoxy-3-cyanopyridines

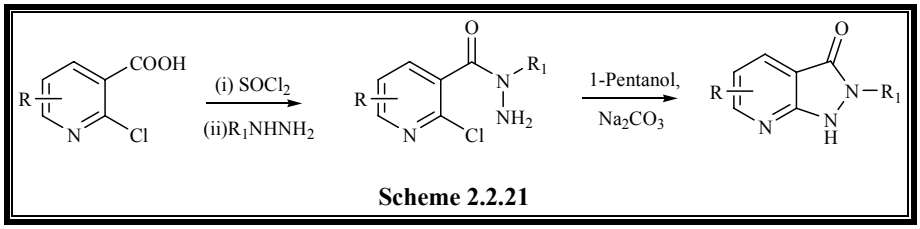
3-amino-pyrazolo[3,4-*b*]pyridines can also be synthesized by the reaction of 2-alkoxy-3-cyanopyridines with hydrazine hydrate or its derivatives [99, 185]. Goda et al. have synthesized 3-amino-pyrazolo[3,4-b]pyridines from 2-alkoxy-3-cyanopyridine using BF_3 as a catalyst [185] (Scheme 2.2.20).



Scheme 2.2.20

2.2.2.2.2. From 3-Carboxylpyridines

Recent literature survey revealed only one report involving reaction starting from 2-chloro-nicotinic acid derivatives yielding 3-oxo-pyrazolo[3,4-*b*]pyridines in three steps [186] (Scheme 2.2.21).

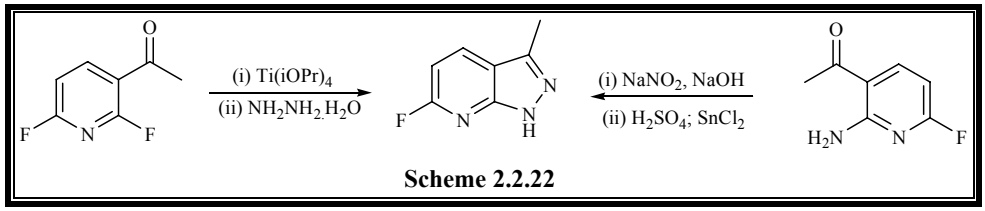


Scheme 2.2.21

2.2.2.2.3 From 3-Acetylpyridines (or 3-Benzoylpyridines)

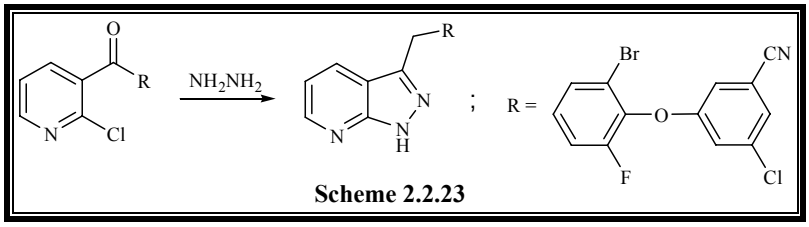
Tucker et al. have reported synthesis of 3-methyl-pyrazolo[3,4-b]pyridines from 1-(2,6-Difluoropyridin-3-yl)ethanone in two steps [98] (Scheme 2.2.22).

Similar reaction have been reported by Zhong et al.via double SNAr reaction of and its derivatives with hydrazine hydrate in one pot using n-BuLi as a base [187] The compound and derivatives have also been synthesized from 1-(2-amino-6-fluoropyridin-3-yl)ethanone [188] (Scheme 2.2.22).



Scheme 2.2.22

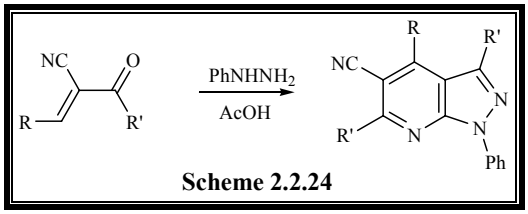
Recently, number of synthetic approaches starting from 3-benzoylpyridines have also been published [189, 190]. For instance, Sweeny et al. have reported synthesis of 3-(substitutedbenzyl)-1H-pyrazolo[3,4-*b*]pyridine from reaction of 1-(2-chloropyridin-3-yl)-2-(aryl)ethanone with hydrazine hydrate [190] (Scheme 2.2.23).



Scheme 2.2.23

2.2.2.3 MISCELLANEOUS SYNTHESIS

Kolosov et al. have reported synthesis of pyrazolo[3,4-*b*]pyridines by reaction of α-cyanochalcones with phenylhydrazine [191] (Scheme 2.2.24).



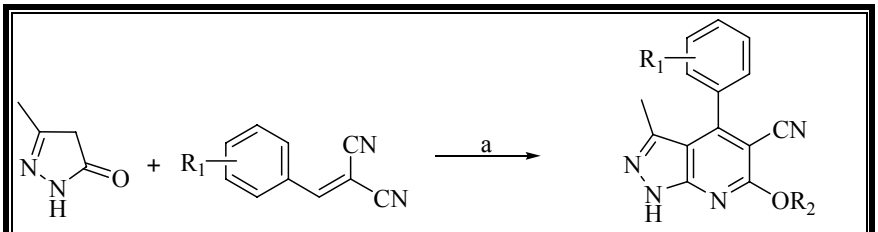
Scheme 2.2.24

2.2.3 Current work

Pyrazolopyridines occupy a special position among fused pyridines due to their diverse biological activities. Pyrazolo[3,4-*b*]pyridine ring system is of special biological interest as isosters of bioactive indoles or indazoles. It has numerous pharmacological and medicinal applications *viz.* antiviral, antidiabetic, antiarrythmic, antitumour, anti-inflammatory etc.

Keeping in mind various biomedical applications and with a view to further assess the pharmacological profile of this class of compounds, two novel series of pyrazolo[3,4-*b*]pyridine-5-carbonitriles (**DDK-A-21 to DDK-A-40**) have been synthesized. The synthesis of pyrazolo[3,4-*b*]pyridine-5-carbonitriles (**DDK-A-21 to DDK-A-30**) and (**DDK-A-31 to DDK-A-40**) was achieved by the reaction of arylidene malononitrile and 3-methyl-1H-pyrazol-5*H*-one in sodium methoxide/methanol and sodium ethoxide/ethanol systems resepectively. Arylidene malononitriles were prepared via the Knoevenagel condensation reaction between different aromatic aldehydes and malononitrile [192]. The products were characterized by FT-IR, mass spectra, ^{1}H NMR and elemental analysis. The newly synthesized compounds were subjected to antimicrobial activity.

2.2.4 Reaction scheme



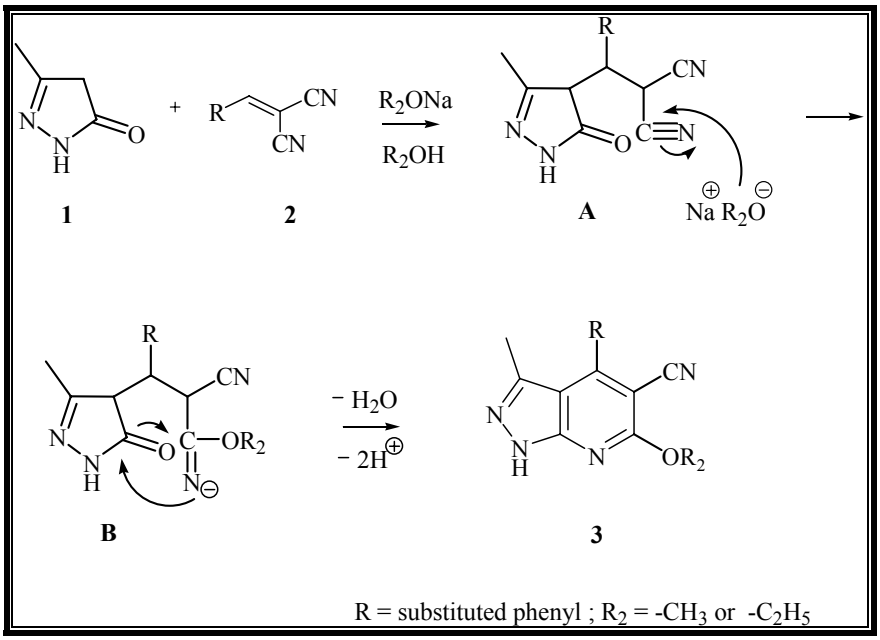
DDK-A-21 to DDK-A-40

Reagents and conditions: (a) R_2ONa, R_2OH, reflux, 10-12 h.

Code	R_1	R_2	M.F.	M.W.	M.P. ºC	Yield %	R_{f1}	R_{f2}
DDK-A-21	H	CH_3	$C_{13}H_{11}N_3OS$	264	222-224	68	0.49	0.66
DDK-A-22	2-Cl	CH_3	$C_{15}H_{11}ClN_4O$	298	218-220	61	0.47	0.73
DDK-A-23	3-Cl	CH_3	$C_{15}H_{11}ClN_4O$	298	237-239	65	0.49	0.71
DDK-A-24	4-Cl	CH_3	$C_{15}H_{11}ClN_4O$	298	193-195	60	0.52	0.74
DDK-A-25	3- NO_2	CH_3	$C_{15}H_{11}N_5O_3$	309	234-236	60	0.50	0.67
DDK-A-26	4- NO_2	CH_3	$C_{15}H_{11}N_5O_3$	309	228-230	65	0.54	0.68
DDK-A-27	4-OCH_3	CH_3	$C_{16}H_{14}N_4O_4$	294	181-183	65	0.53	0.74
DDK-A-28	4- CH_3	CH_3	$C_{16}H_{14}N_4O$	278	261-263	69	0.48	0.65
DDK-A-29	4-F	CH_3	$C_{15}H_{11}FN_4O$	282	236-238	65	0.55	0.66
DDK-A-30	4-OH	CH_3	$C_{15}H_{12}N_4O_2$	280	237-239	60	0.49	0.70
DDK-A-31	H	C_2H_5	$C_{16}H_{14}N_4O$	278	218-220	66	0.53	0.61
DDK-A-32	2-Cl	C_2H_5	$C_{16}H_{13}ClN_4O$	312	229-231	60	0.57	0.65
DDK-A-33	3-Cl	C_2H_5	$C_{16}H_{13}ClN_4O$	312	244-246	64	0.49	0.58
DDK-A-34	4-Cl	C_2H_5	$C_{16}H_{13}ClN_4O$	312	208-210	63	0.52	0.60
DDK-A-35	3- NO_2	C_2H_5	$C_{16}H_{15}N_3O_2S$	323	248-250	61	0.59	0.70
DDK-A-36	4- NO_2	C_2H_5	$C_{15}H_{11}N_5O_3$	323	236-238	65	0.50	0.58
DDK-A-37	4-OCH_3	C_2H_5	$C_{17}H_{16}N_4O_2$	308	196-198	69	0.54	0.61
DDK-A-38	4- CH_3	C_2H_5	$C_{16}H_{14}N_4O$	292	213-215	65	0.55	0.63
DDK-A-39	4-F	C_2H_5	$C_{16}H_{13}FN_4O$	296	229-231	61	0.48	0.57
DDK-A-40	4-OH	C_2H_5	$C_{16}H_{14}N_4O_2$	294	193-195	62	0.49	0.62

TLC Solvent system R_{f1}: Hexane: Ethyl acetate – 5:5,
TLC Solvent system R_{f2}: Chloroform: Methanol – 9:1.

2.2.5 Mechanism



The proposed mechanism involves the Michael addition between **1** and **2** to generate intermediate A, followed by alkoxide nucleophilic attack at one of the nitrile groups of **A** with dehydration and subsequent dehydrogenation to give the pyrazolo[3,4-*b*] pyridine **3** [193].

2.2.6 Experimental

2.2.6.1 Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a Bruker Avance II 400 MHz (for compounds DDK-A-23, DDK-A-29, DDK-A-34, DDK-A-39) and Bruker DRX 300 MHz (for compounds DDK-A-24, DDK-A-37) spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

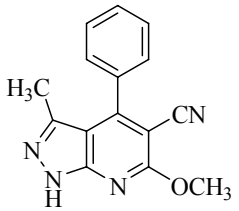
2.2.6.2 Synthesis of 2-(arylidene)malononitriles

Synthesis of 2-(arylidene)malononitriles was achieved using previously published method [192].

2.2.6.3 General procedure for the synthesis of 6-methoxy-3-methyl-4-(aryl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitriles (DDK-A-21 to DDK-A-30)

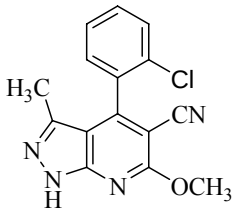
2-(arylidene)malononitrile (0.01 mol) was added to a freshly prepared sodium methoxide solution (0.015 mol of sodium in 15 mL of methanol). 3-methyl-1*H*-pyrazol-5*H*-one (0.01 mol) was then added to this solution. The resulting mixture was heated under reflux for 10-12 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the separated solid was collected by filtration, washed with cold methanol and crystallized from ethanol.

2.2.6.3.1 6-methoxy-3-methyl-4-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-21)



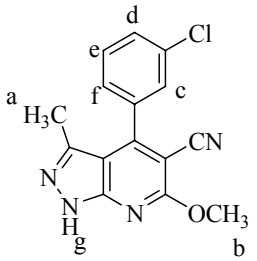
Yield: 68%; m.p. 222-224 ºC; MS: *m/z* 264; Anal. Calcd. for $C_{15}H_{12}N_4O$: C, 68.17; H, 4.58; N, 21.20. Found: C, 68.08; 4.14; N, 21.13%.

2.2.6.3.2 4-(2-chlorophenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-22)



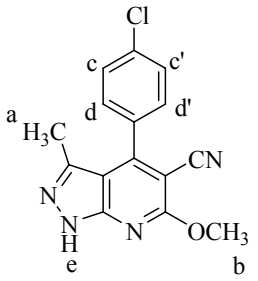
Yield: 61%; m.p. 218-220 ºC; MS: *m/z* 298; Anal. Calcd. for $C_{15}H_{11}ClN_4O$: C, 60.31; H, 3.71; N, 18.76. Found: C, 60.24; H, 3.63; N, 18.70%.

2.2.6.3.3 4-(3-chlorophenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-23)



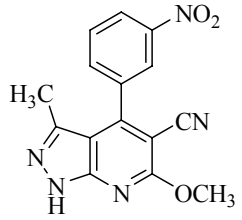
Yield: 65%; m.p. 237-239 ºC; IR (cm^{-1}): 3286 (N-H stretching of secondary amine), 3041 (C-H stretching of aromatic ring), 2947 (C-H asymmetrical stretching of CH_3 group), 2852 (C-H symmetrical stretching of CH_3 group), 2218 (C≡N stretching of nitrile group), 1637 (C=N stretching of pyridine ring), 1599, 1546 and 1510 (C=C stretching of aromatic ring), 1452 (C-H asymmetrical deformation of CH_3 group), 1377 (C-H symmetrical deformation of CH_3 group), 1301 (C-N stretching of secondary amine), 1232 (C-O-C asymmetrical stretching of OCH_3 group), 1095 (C-O-C symmetrical stretching OCH_3 group), 995 (C-H in plane bending for aromatic ring), 794 (C-Cl stretching), 727 (C-H out of plane bending for 1,3-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.53 (s, 3H, H_a), 3.98 (s, 3H, H_b), 7.4 (s, 1H, H_c), 7.50-7.53 (m, 2H, $H_{d, f}$), 7.39-7.42 (m, 1H, H_e), 8.00 (s, 1H, H_g); MS: *m/z* 298; Anal. Calcd. for $C_{15}H_{11}ClN_4O$: C, 60.31; H, 3.71; N, 18.76. Found: C, 60.231; H, 3.64; N, 18.69%.

2.2.6.3.4 4-(4-chlorophenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-24)



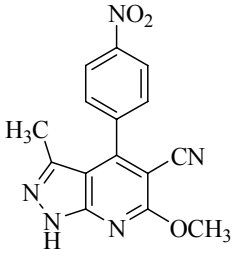
Yield: 60%; m.p. 193-195 ºC; IR (cm^{-1}): 3240 (N-H stretching of secondary amine), 3039 (C-H stretching of aromatic ring), 2947 (C-H asymmetrical stretching of CH_3 group), 2884 (C-H symmetrical stretching of CH_3 group), 2218 (C≡N stretching of nitrile group), 1645 (C=N stretching of pyridine ring), 1597, 1548 and 1512 (C=C stretching of aromatic ring), 1454 (C-H asymmetrical deformation of CH_3 group), 1379 (C-H symmetrical deformation of CH_3 group), 1305 (C-N stretching), 1230 (C-O-C asymmetrical stretching of OCH_3 group), 1091 (C-O-C symmetrical stretching OCH_3 group), 727 (C-Cl stretching), 840 (C-H out of plane bending for para-substituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.50 (s, 3H, H_a), 3.85 (s, 3H, H_b), 7.33-7.36 (d, 2H, $H_{c, c'}$, J = 8.4 Hz), 7.50-7.53 (d, 2H, $H_{d, d'}$, J = 8.4 Hz), 7.90 (s, 1H, H_e); MS: *m/z* 298; Anal. Calcd. for $C_{15}H_{11}ClN_4O$: C, 60.31; H, 3.71; N, 18.76. Found: C, 60.24; H, 3.64; N, 18.68%.

2.2.6.3.5 6-methoxy-3-methyl-4-(3-nitrophenyl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-25)



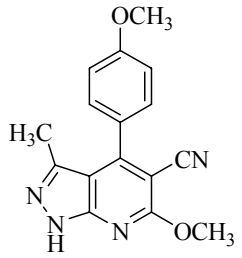
Yield: 60%; m.p. 234-236 ºC; MS: *m/z* 309; Anal. Calcd. for $C_{15}H_{11}N_5O_3$: C, 58.25; H, 3.58; N, 22.64. Found: C, 58.18; H, 3.52; N, 22.57%.

2.2.6.3.6 6-methoxy-3-methyl-4-(4-nitrophenyl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-26)



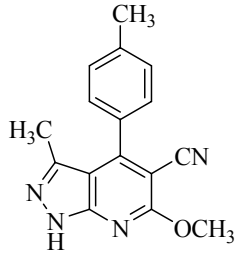
Yield: 65%; m.p. 228-230 ºC; MS: *m/z* 309; Anal. Calcd. for $C_{15}H_{11}N_5O_3$: C, 58.25; H, 3.58; N, 22.64. Found: C, 58.17; H, 3.50; N, 22.56%.

2.2.6.3.7 6-methoxy-4-(4-methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-27)



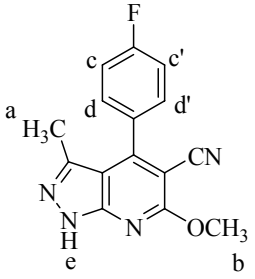
Yield: 65%; m.p. 181-183 ºC; MS: *m/z* 294; Anal. Calcd. for $C_{16}H_{14}N_4O_4$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.23; H, 4.73; N, 18.96%.

2.2.6.3.8 6-methoxy-3-methyl-4-p-tolyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-28)



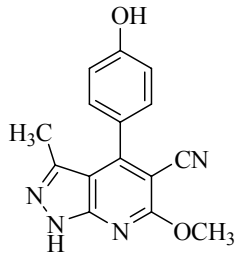
Yield: 69%; m.p. 261-263 ºC; MS: *m/z* 278; Anal. Calcd. for $C_{16}H_{14}N_4O$: C, 69.05; H, 5.07; N, 20.13. Found: C, 68.96; H, 4.99; N, 20.06%.

2.2.6.3.9 4-(4-fluorophenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-29)



Yield: 65%; m.p. 236-238 ºC; IR (cm^{-1}): 3271 (N-H stretching of secondary amine), 3032 (C-H stretching of aromatic ring), 2918 (C-H asymmetrical stretching of CH_3 group), 2858 (C-H symmetrical stretching of CH_3 group), 2218 (C≡N stretching of nitrile group), 1599, 1545 and 1516 (C=C stretching of aromatic ring), 1437 (C-H asymmetrical deformation of CH_3 group), 1301 (C-H symmetrical deformation of CH_3 group), 1232 (C-O-C asymmetrical stretching of OCH_3 group), 1090 (C-O-C symmetrical stretching OCH_3 group), 1020 (C-F stretching), 839 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.53 (s, 3H, H_a), 3.91 (s, 3H, H_b), 7.32-7.36 (t, 2H, $H_{c,c'}$), 7.48-7.52 (m, 2H, $H_{d,d'}$), 7.93 (s, 1H, H_e); MS: *m/z* 282; Anal. Calcd. for $C_{15}H_{11}FN_4O$: C, 63.83; H, 3.93; N, 19.85. Found: C, 63.77; H, 3.87; N, 19.77%.

2.2.6.3.10 4-(4-hydroxyphenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-30)

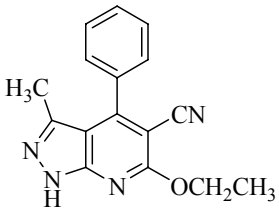


Yield: 60%; m.p. 237-239 ºC; MS: *m/z* 280; Anal. Calcd. for $C_{15}H_{12}N_4O_2$: C, 64.28; H, 4.32; N, 19.99. Found: C, 64.19; H, 4.26; N, 19.91%.

2.2.6.4 General procedure for the synthesis of 6-ethoxy-3-methyl-4-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitriles (DDK-A-31 to DDK-A-40)

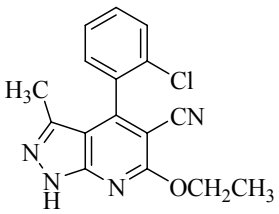
2-(arylidene)malononitrile (0.01 mol) was added to a freshly prepared sodium methoxide solution (0.015 mol of sodium in 15 mL of ethanol). 3-methyl-1H-pyrazol-5*H*-one (0.01 mol) was then added to this solution. The resulting mixture was heated under reflux for 10-12 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the separated solid was collected by filtration, washed with cold methanol and crystallized from ethanol.

2.2.6.4.1 6-ethoxy-3-methyl-4-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-31)



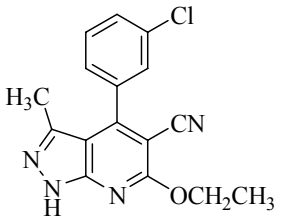
Yield: 66%; m.p. 218-220 ºC; MS: *m/z* 278; Anal. Calcd. for $C_{16}H_{14}N_4O$: C, 69.05; H, 5.07; N, 20.13. Found: C, 68.97; H, 4.99; N, 20.07%.

2.2.6.4.2 4-(2-chlorophenyl)-6-ethoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-32)



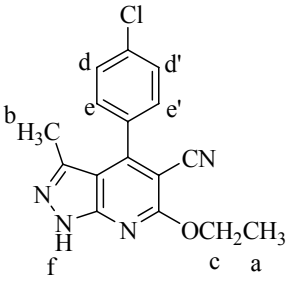
Yield: 60%; m.p. 229-231 ºC; MS: *m/z* 312; Anal. Calcd. for $C_{16}H_{13}ClN_4O$: C, 61.44; H, 4.19; N, 17.91. Found: C, 61.38; H, 4.14; N, 17.84%.

2.2.6.4.3 4-(3-chlorophenyl)-6-ethoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-33)



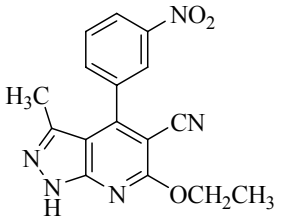
Yield: 64%; m.p. 244-246 ºC; MS: *m/z* 312; Anal. Calcd. for $C_{16}H_{13}ClN_4O$: C, 61.44; H, 4.19; N, 17.91. Found: C, 61.37; H, 4.12; N, 17.85%.

2.2.6.4.4 4-(4-chlorophenyl)-6-ethoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-34)



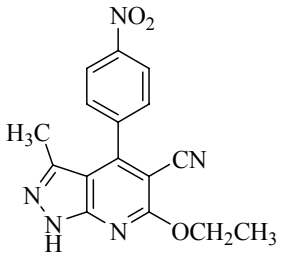
Yield: 63%; m.p. 208-210 ºC; IR (cm^{-1}): 3236 (N-H stretching of secondary amine), 3043 (C-H stretching of aromatic ring), 2985 (C-H asymmetrical stretching of CH_3 and CH_2 group), 2860 (C-H symmetrical stretching of CH_3 and CH_2 group), 2218 (C≡N stretching of nitrile group), 1635 (C=N stretching of pyridine ring), 1539 and 1494 (C=C stretching of aromatic ring), 1440 (C-H asymmetrical deformation of CH_3 group), 1330 (C-H symmetrical deformation of CH_3 group), 1301 (C-N stretching), 1247 (C-O-C asymmetrical stretching of OCH_3 group), 1091 (C-O-C symmetrical stretching OCH_3 group), 719 (C-Cl stretching), 833 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 1.53-1.56 (t, 1H, H_a), 2.53 (s, 3H, H_b), 4.51-4.53 (q, 2H, H_c), 7.33-7.35 (d, 2H, $H_{d, d'}$, J = 8.32 Hz), 7.56-7.58 (d, 2H, $H_{e, e'}$, J = 8.52 Hz), 8.00 (s, 1H, H_f); MS: *m/z* 312; Anal. Calcd. for $C_{16}H_{13}ClN_4O$: C, 61.44; H, 4.19; N, 17.91. Found: C, 61.38; H, 4.12; N, 17.82%.

2.2.6.4.5 6-ethoxy-3-methyl-4-(3-nitrophenyl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-35)



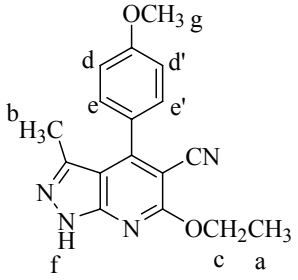
Yield: 61%; m.p. 248-250 ºC; MS: *m/z* 323; Anal. Calcd. for $C_{16}H_{13}N_5O_3$: C, 59.44; H, 4.05; N, 21.66;. Found: C, 59.39; H, 3.98; N, 21.59;%.

2.2.6.4.6 6-ethoxy-3-methyl-4-(4-nitrophenyl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-36)



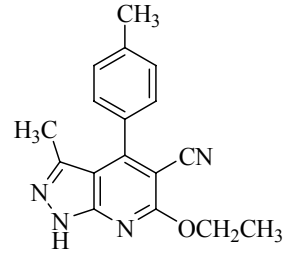
Yield: 65%; m.p. 236-238 ºC; MS: *m/z* 323; Anal. Calcd. for $C_{15}H_{11}N_5O_3$: C, 58.25; H, 3.58; N, 22.64. Found: C, 58.17; H, 3.50; N, 22.56%.

2.2.6.4.7 6-ethoxy-4-(4-methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-37)



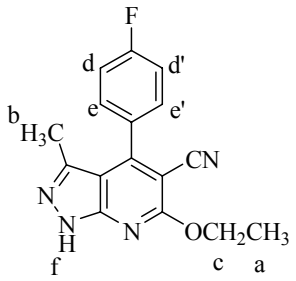
Yield: 69%; m.p. 196-198 ºC; IR (cm^{-1}): 3285 (N-H stretching of secondary amine), 3010 (C-H stretching of aromatic ring), 2972 (C-H asymmetrical stretching of CH_3 group), 2933 (C-H asymmetrical stretching of CH_2 group), 2843 (C-H symmetrical stretching of CH_3 and CH_2 group), 2218 (C≡N stretching of nitrile group), 1597 & 1541 (C=C stretching of aromatic ring), 1452 (C-H asymmetrical deformation of CH_3 group), 1311 (C-H symmetrical deformation of CH_3 group), 1301 (C-N stretching), 1251 (C-O-C asymmetrical stretching of OCH_3 group), 1026 (C-O-C symmetrical stretching OCH_3 group), 977 (C-H in plane bending for aromatic ring), 837 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 1.31-1.36 (t, 1H, H_a), 2.49 (s, 3H, H_b), 3.83 (s, 1H, H_g), 4.39-4.45 (q, 2H, H_c), 7.09-7.11 (d, 2H, $H_{d,d'}$, J = 8.4 Hz), 7.46-7.49 (d, 2H, $H_{e,e'}$, J = 8.1 Hz), 7.90 (s, 1H, H_f); MS: *m/z* 308; Anal. Calcd. for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.17. Found: C, 66.16; H, 5.18; N, 18.09%.

2.2.6.4.8 6-ethoxy-3-methyl-4-p-tolyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-38)



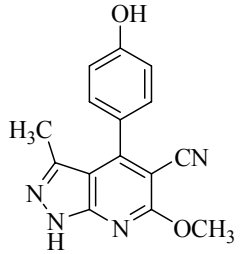
Yield: 65%; m.p. 213-215 ºC; MS: *m/z* 292; Anal. Calcd. for $C_{16}H_{14}N_4O$: C, 69.85; H, 5.52; N, 19.17. Found: C, 69.79; H, 5.44; N, 19.09%.

2.2.6.4.9 4-(4-fluorophenyl)-6-ethoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-39)



Yield: 61%; m.p. 229-231 ºC; IR (cm^{-1}): 3246 (N-H stretching of secondary amine), 3032 (C-H stretching of aromatic ring), 2989 (C-H asymmetrical stretching of CH_3 and CH_2 group), 2868 (C-H symmetrical stretching of CH_3 and CH_2 group), 2218 (C≡N stretching of nitrile group), 1637 (C=N stretching of pyridine ring), 1599, 1546 and 1510 (C=C stretching of aromatic ring), 1452 (C-H asymmetrical deformation of CH_3 group), 1377 (C-H symmetrical deformation of CH_3 group), 1301 (C-N stretching), 1232 (C-O-C asymmetrical stretching of OCH_3 group), 1095 (C-O-C symmetrical stretching OCH_3 group), 1027 (C-F stretching), 995 (C-H in plane bending for aromatic ring), 840 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 1.41-1.44 (t, 1H, H_a), 2.58 (s, 3H, H_b), 4.45-4.51 (q, 2H, H_c), 7.21-7.25 (t, 2H, $H_{d, d'}$), 7.51-7.54 (m, 2H, $H_{e, e'}$), 8.00 (s, 1H, H_f); MS: *m/z* 296; Anal. Calcd. for $C_{16}H_{13}FN_4O$: C, 64.86; H, 4.42; N, 18.91. Found: C, 64.78; H, 4.35; N, 18.85%.

2.2.6.4.10 4-(4-hydroxyphenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-40)



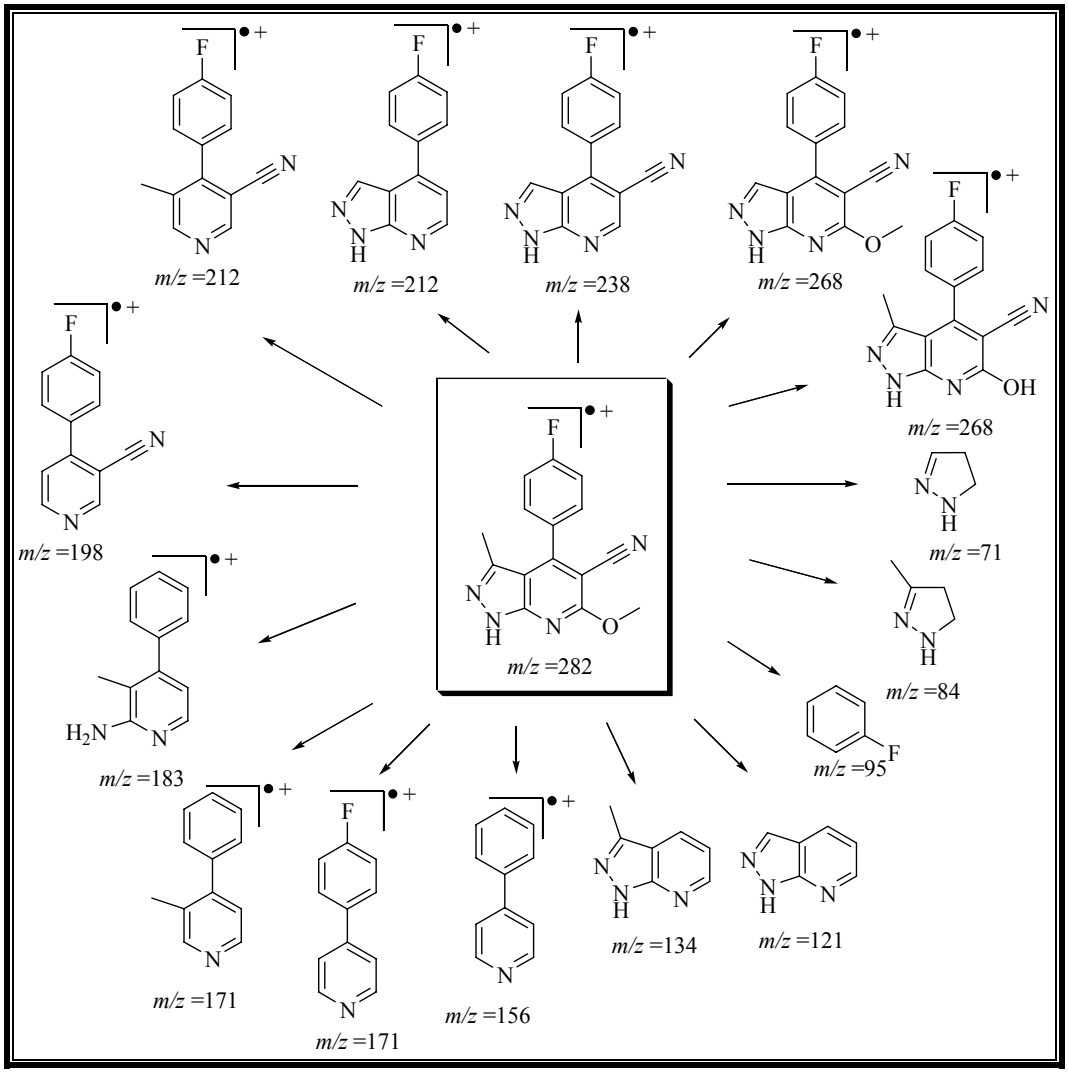
Yield: 62%; m.p. 193-195 ºC; MS: *m/z* 294; Anal. Calcd. for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.23; H, 4.72; N, 18.97%.

2.2.7 Spectral discussion

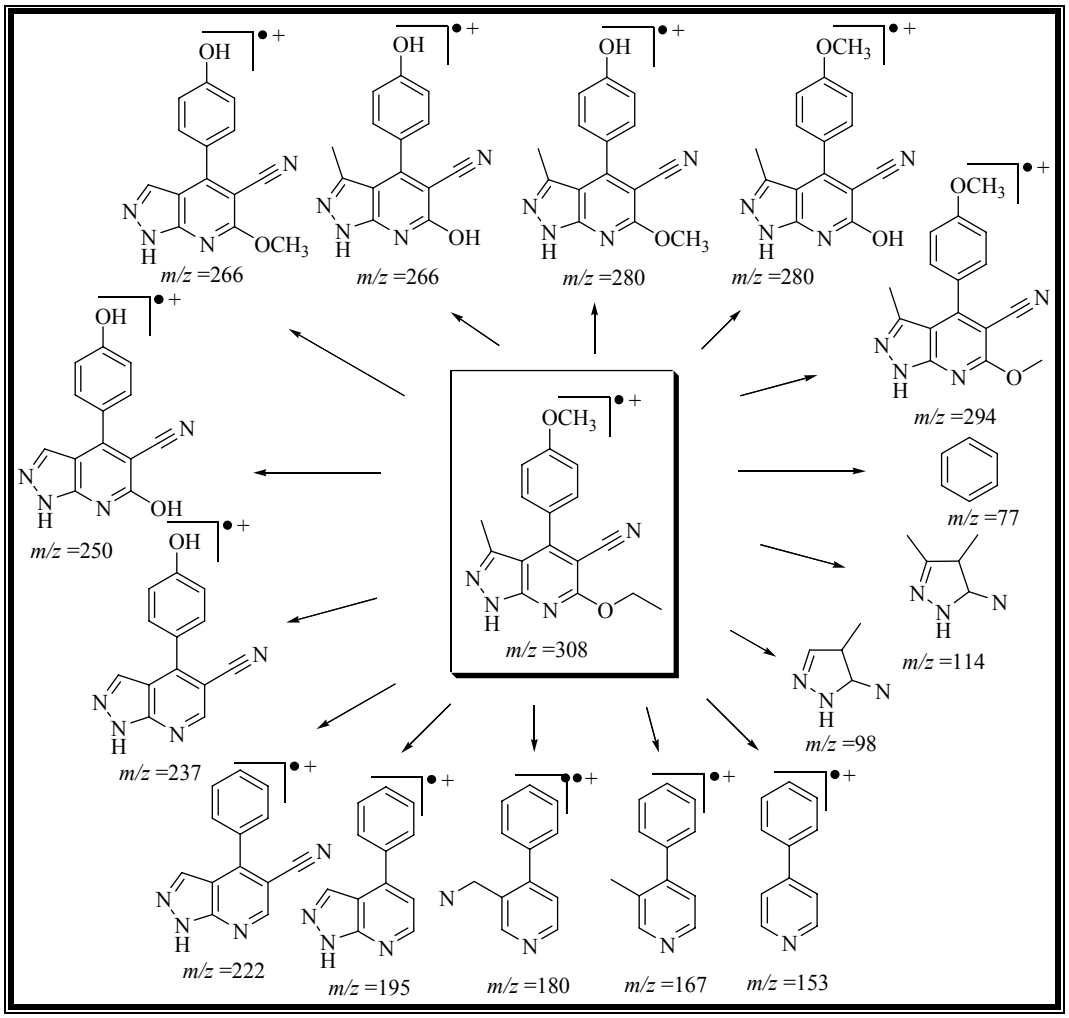
2.2.7.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of respective compound. Mass fragmentation pattern for a representative compound of each series is depicted below.

2.2.7.1.1 Mass fragmentation pattern for DDK-A-29



2.2.7.1.2 Mass fragmentation pattern for DDK-A-37



2.2.7.2. IR spectral study

IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For pyrazolo[3,4-b]pyridine-5-carbonitriles (**DDK-A-21 to DDK-A-40**), a characteristic band of nitrile group was observed in the range of 2191-2218 cm^{-1}. Confirmatory bands of C-O-C asymmetrical stretching at 1232-1247 cm^{-1} and C-O-C symmetrical stretching at 1076-1095 cm^{-1} were observed for methoxy and ethoxy groups. Also, N-H stretching band of secondary amine was observed at 3246-3285 cm^{-1} suggesting formation of desired products (**DDK-A-21 to DDK-A-40**).

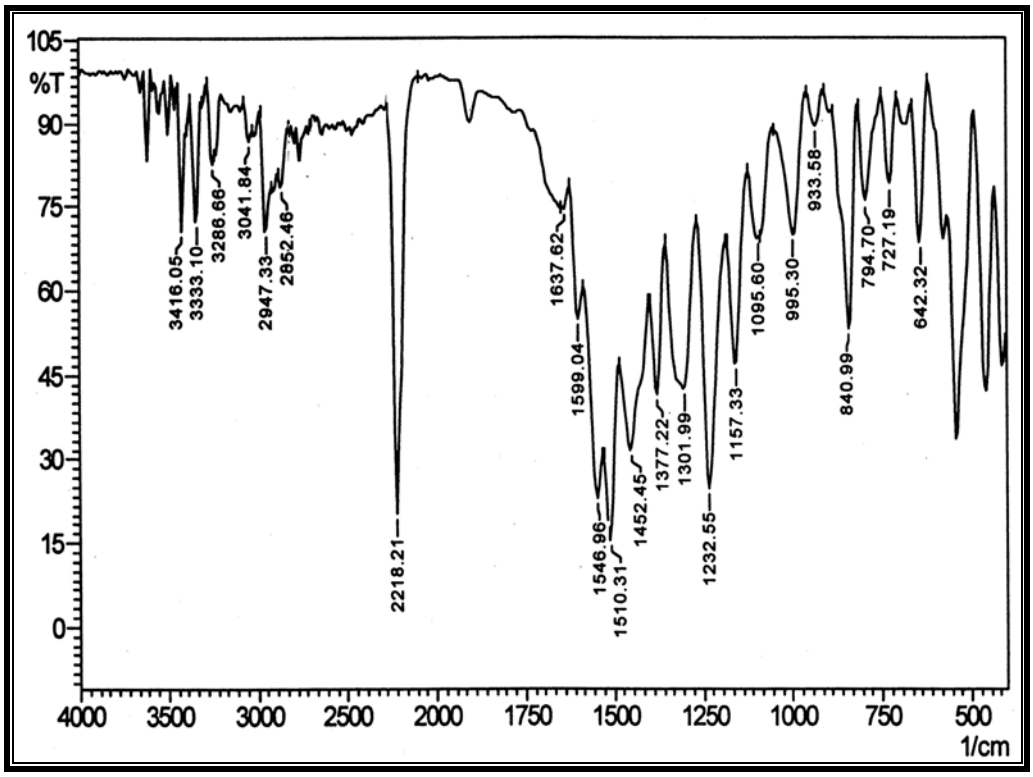
2.2.7.3 ^{1}H NMR spectral study

^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

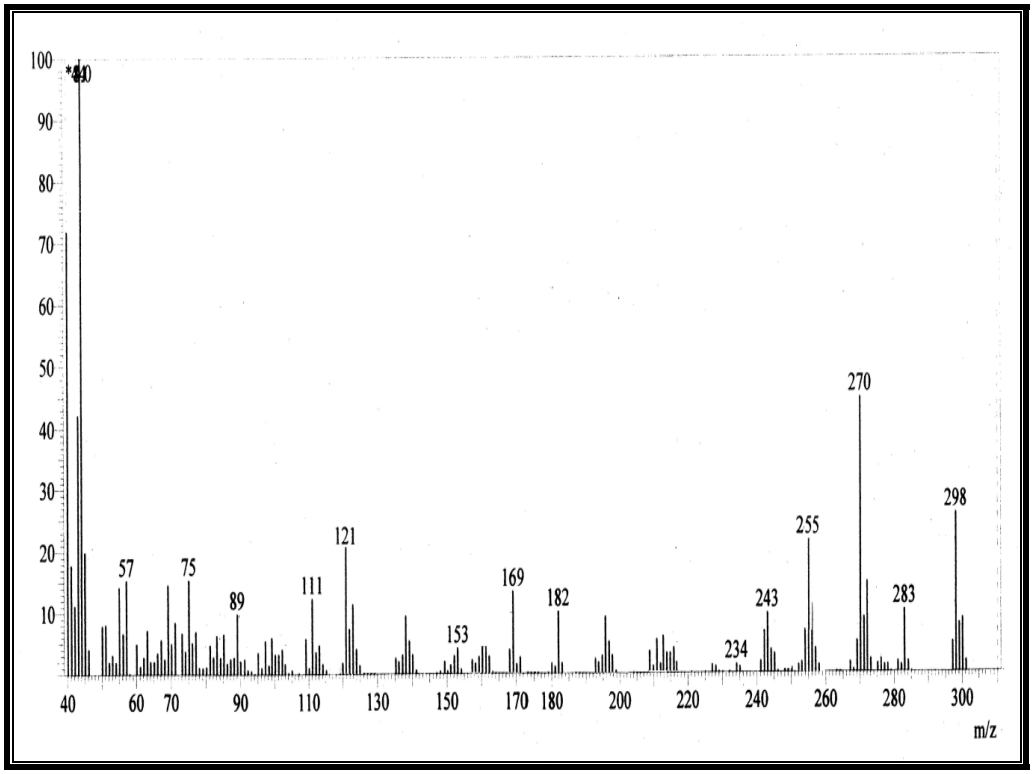
For pyrazolo[3,4-b]pyridine-5-carbonitriles (**DDK-A-21 to DDK-A-30**), characteristic singlet was observed for methoxy group at 3.85-3.98 δ ppm confirming the formation of 2-methoxy-pyrazolo[3,4-*b*]pyridine-5-carbonitriles.. The aromatic ring protons were observed at 7.32-7.53 δ ppm and *J* value were found to be in accordance with substitution pattern on phenyl ring. The singlet for secondary amine (-NH) proton was observed at 7.90-8.00 δ ppm.

While, for pyrazolo[3,4-*b*]pyridine-5-carbonitriles (**DDK-A-31 to DDK-A-40**), characteristic triplet-quartet pattern was observed for ethoxy group. A signal as quartet at 1.31-1.56 δ ppm corresponding to three methyl (O-CH_2-C$\underline{H}_3$) protons and a triplet at 4.39-4.53 δ ppm corresponding to two methylene (O-C$\underline{H}_2$-CH_3) protons was observed confirming the formation of 2-ethoxy-pyrazolo[3,4-*b*]pyridine-5-carbonitriles. The aromatic ring protons were observed at 7.09-7.58 δ ppm and *J* value were found to be in accordance with substitution pattern on phenyl ring. The singlet for secondary amine (-NH) proton was observed at 7.90-8.00 δ ppm.

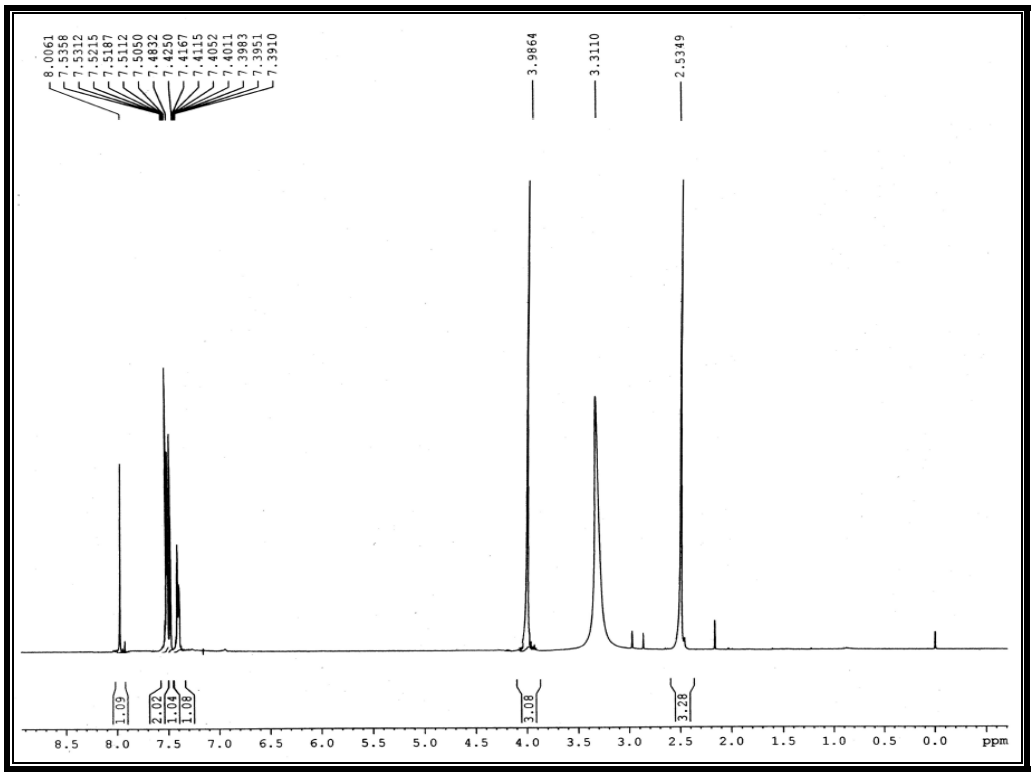
IR spectrum of DDK-A-23



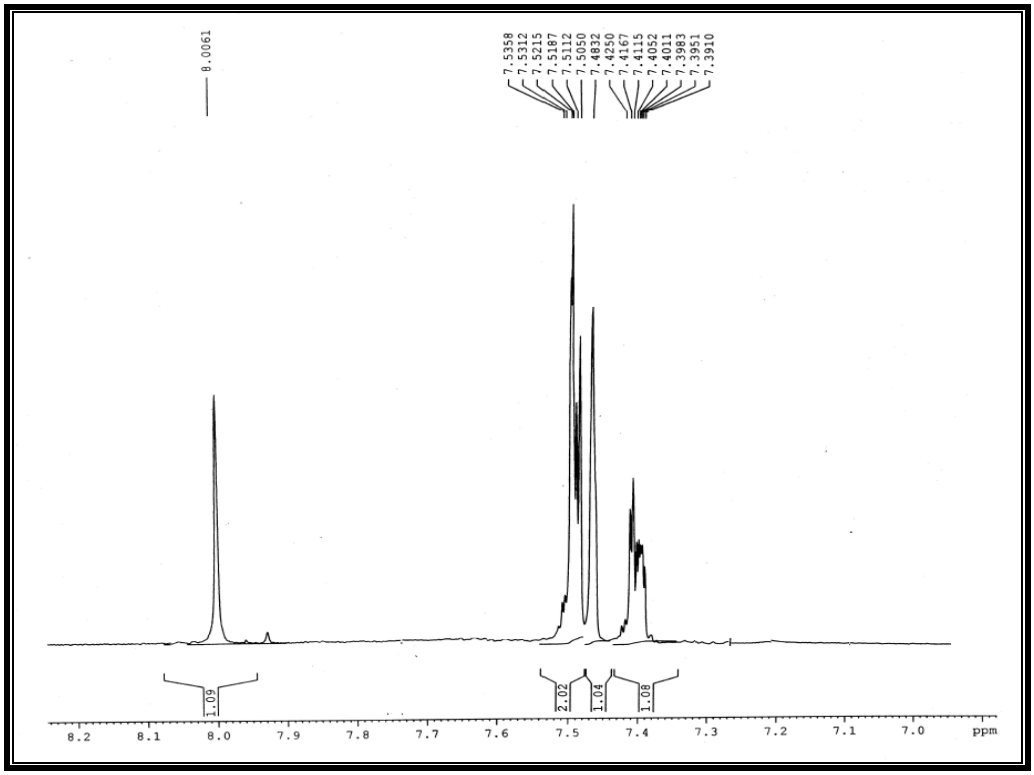
Mass spectrum of DDK-A-23



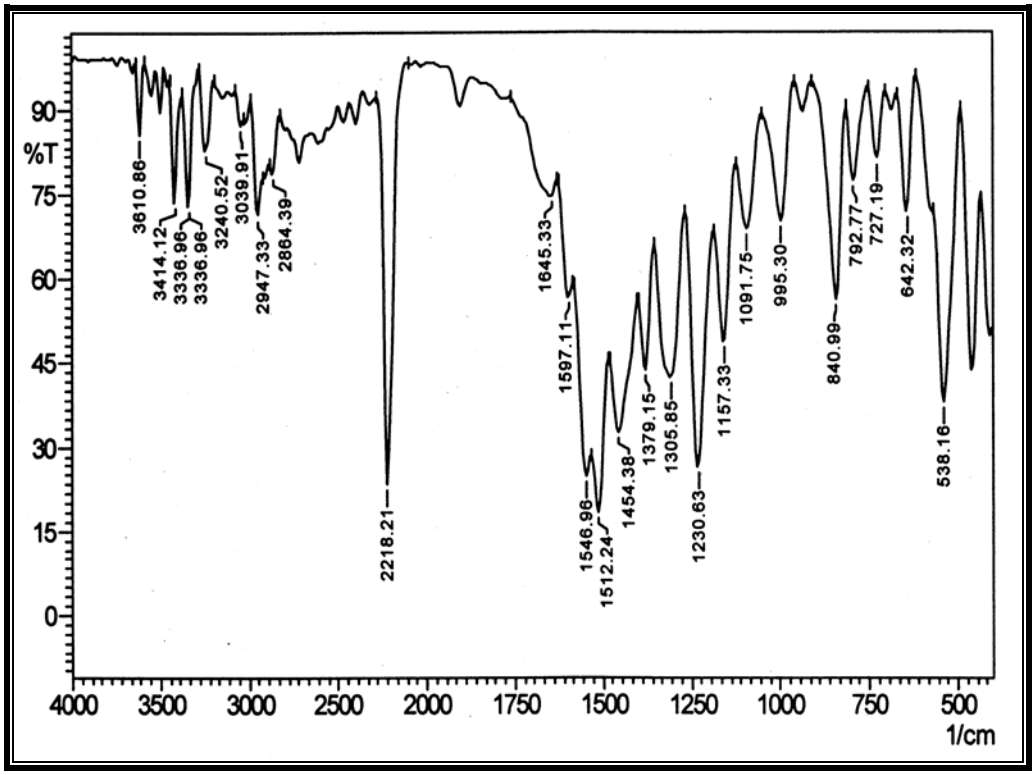
^{1}H NMR spectrum of DDK-A-23



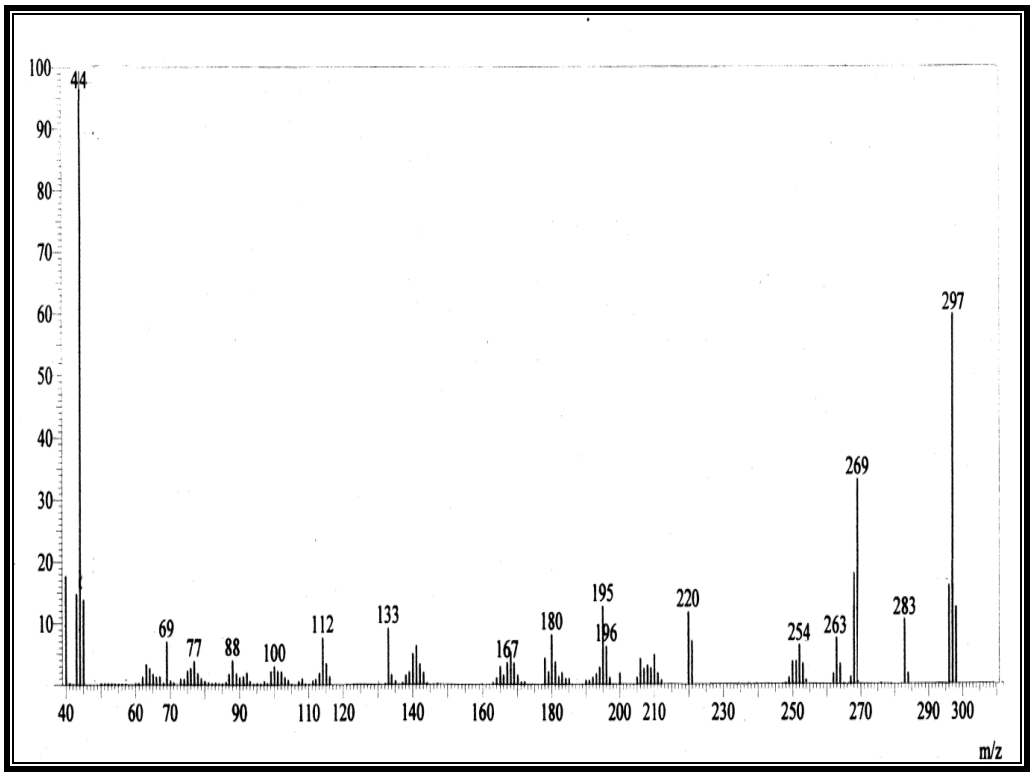
Expanded ^{1}H NMR spectrum of DDK-A-23



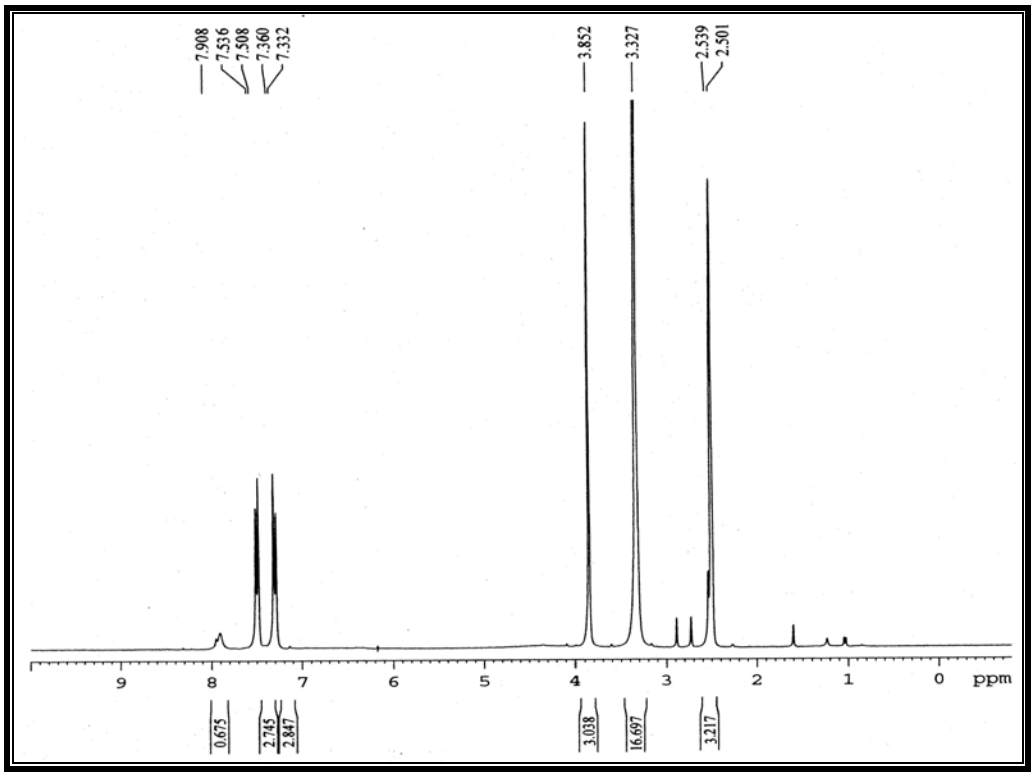
IR spectrum of DDK-A-24



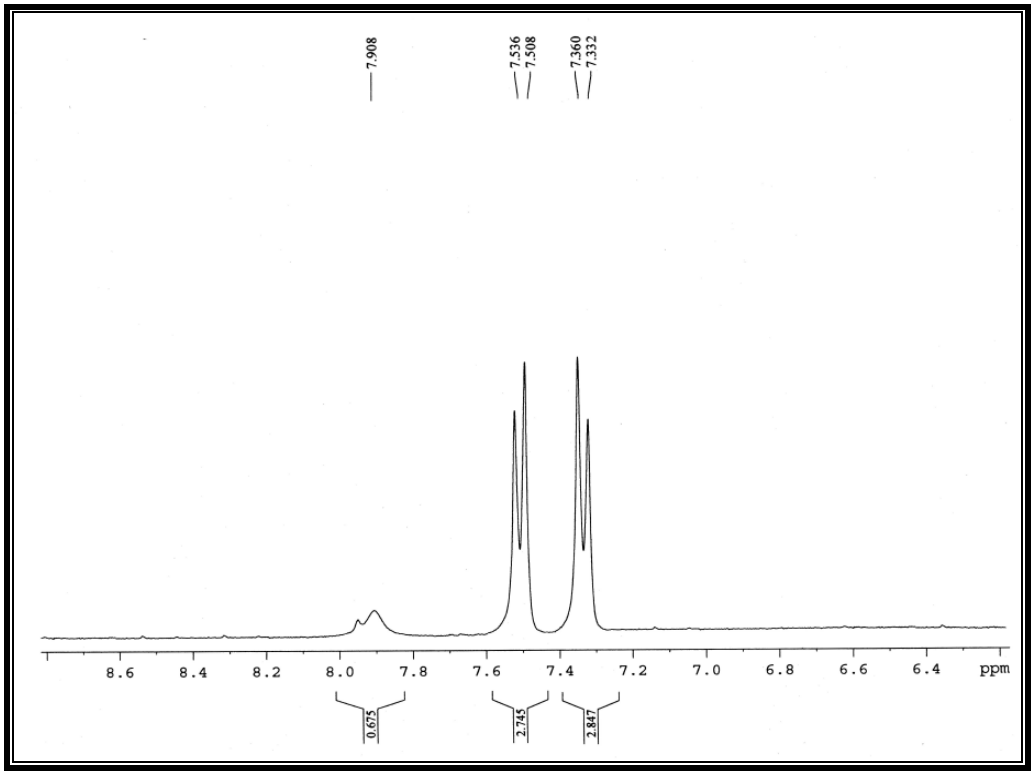
Mass spectrum of DDK-A-24



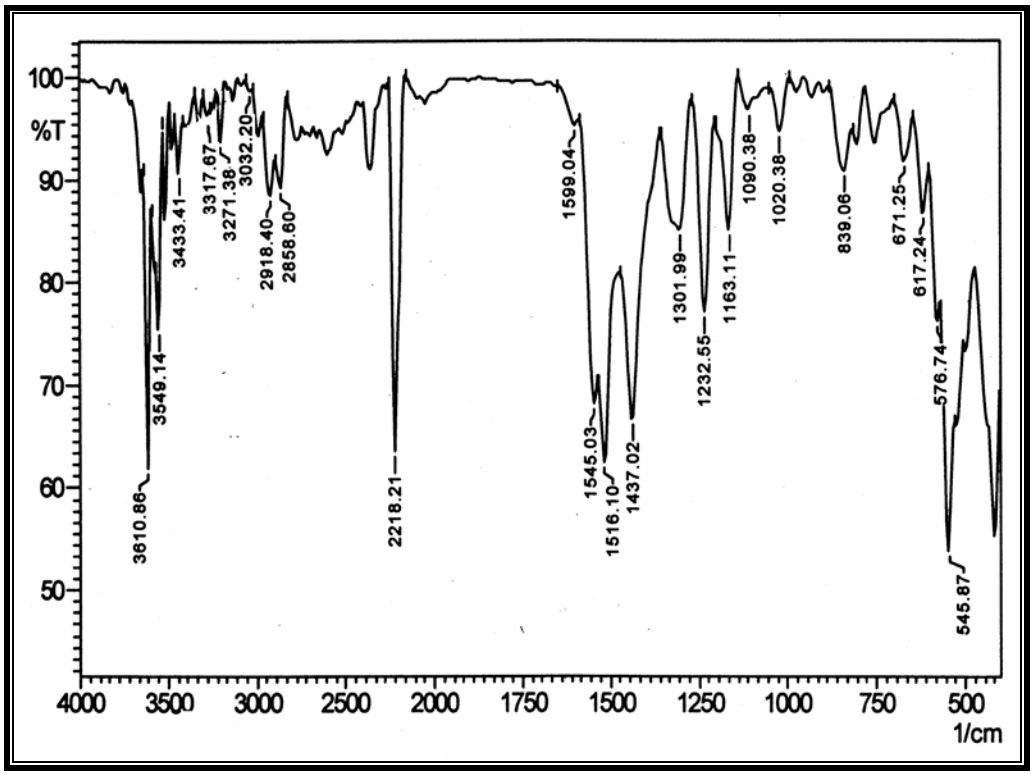
^{1}H NMR spectrum of DDK-A-24



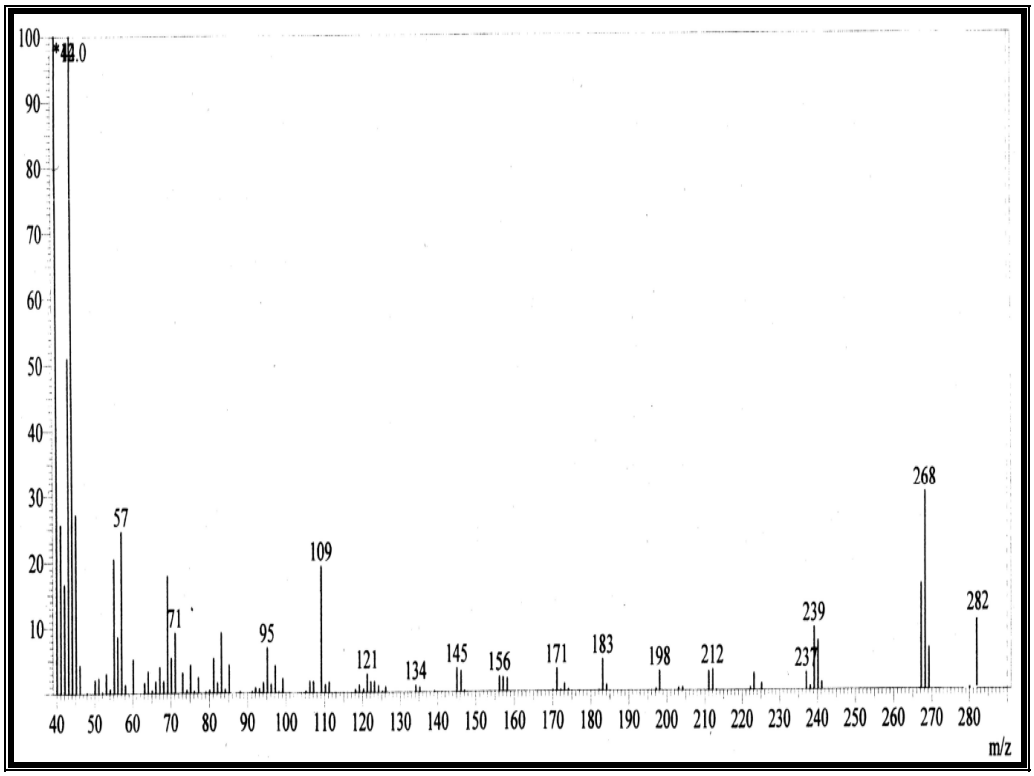
Expanded ^{1}H NMR spectrum of DDK-A-24



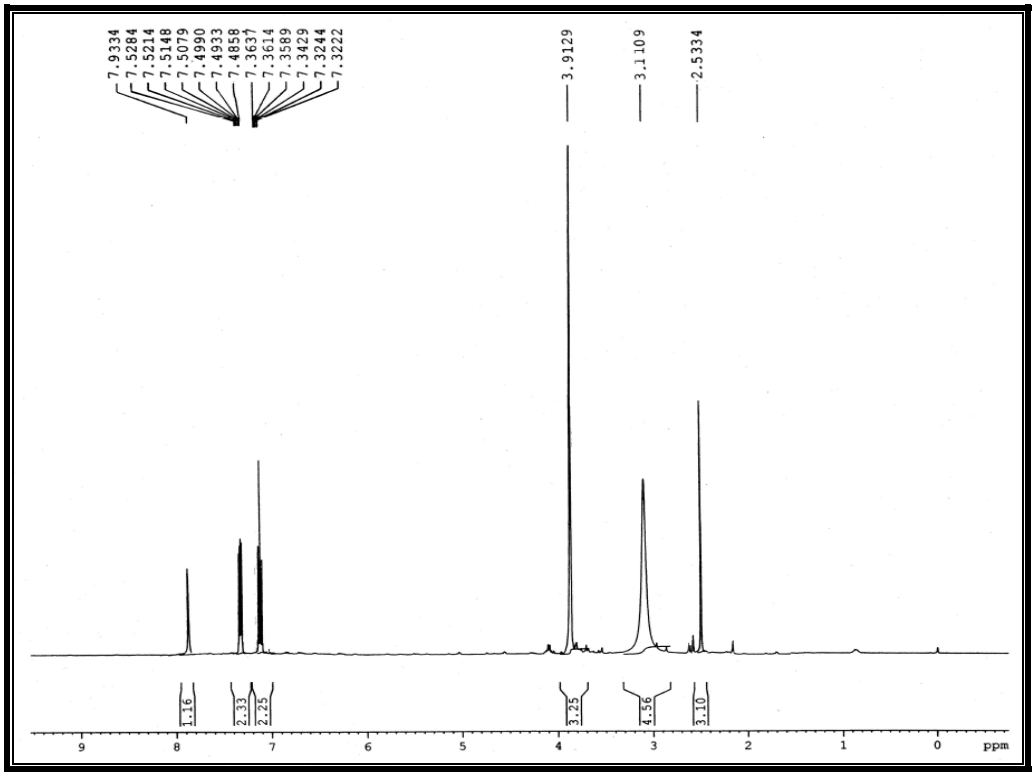
IR spectrum of DDK-A-29



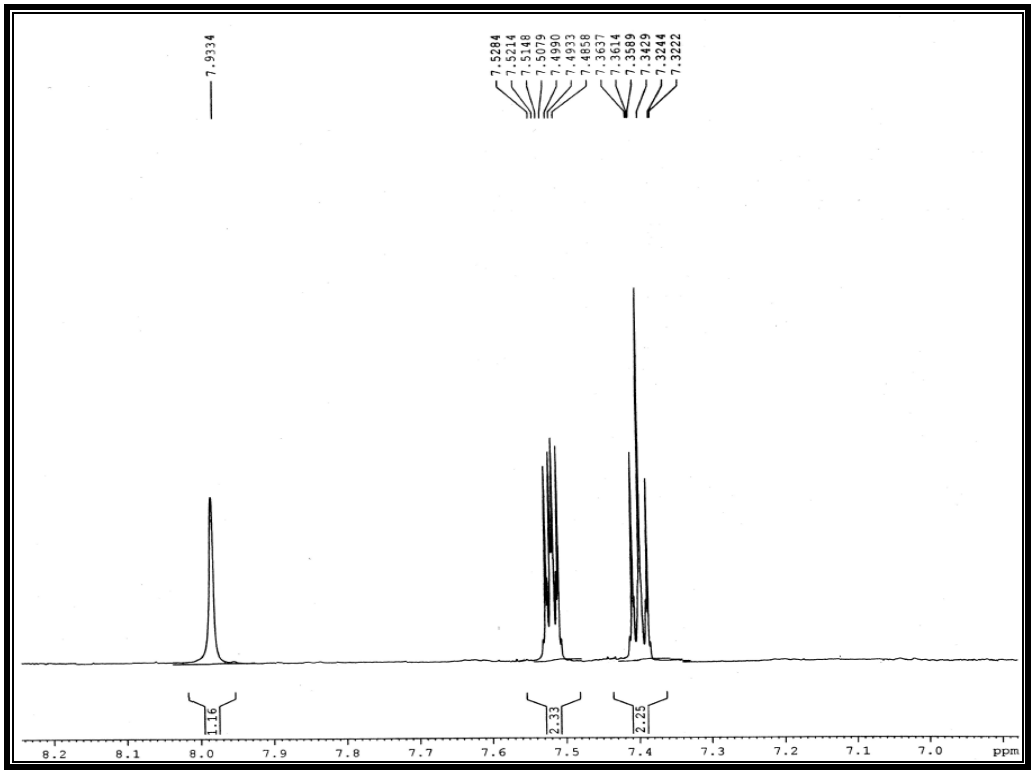
Mass spectrum of DDK-A-29



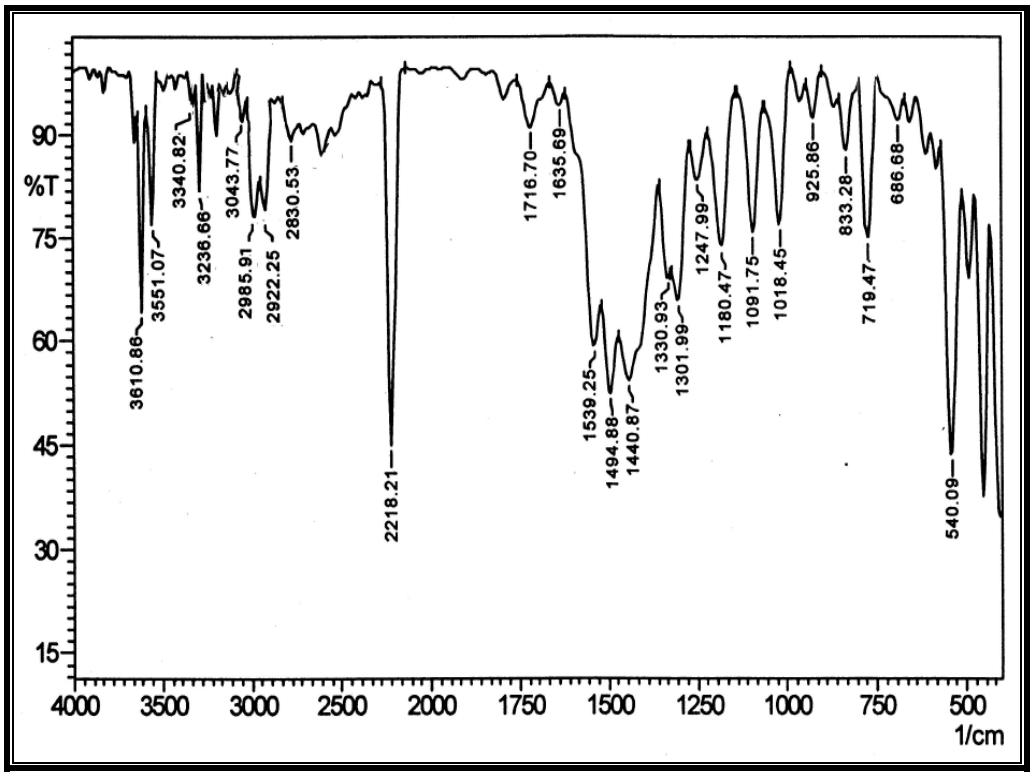
^{1}H NMR spectrum of DDK-A-29



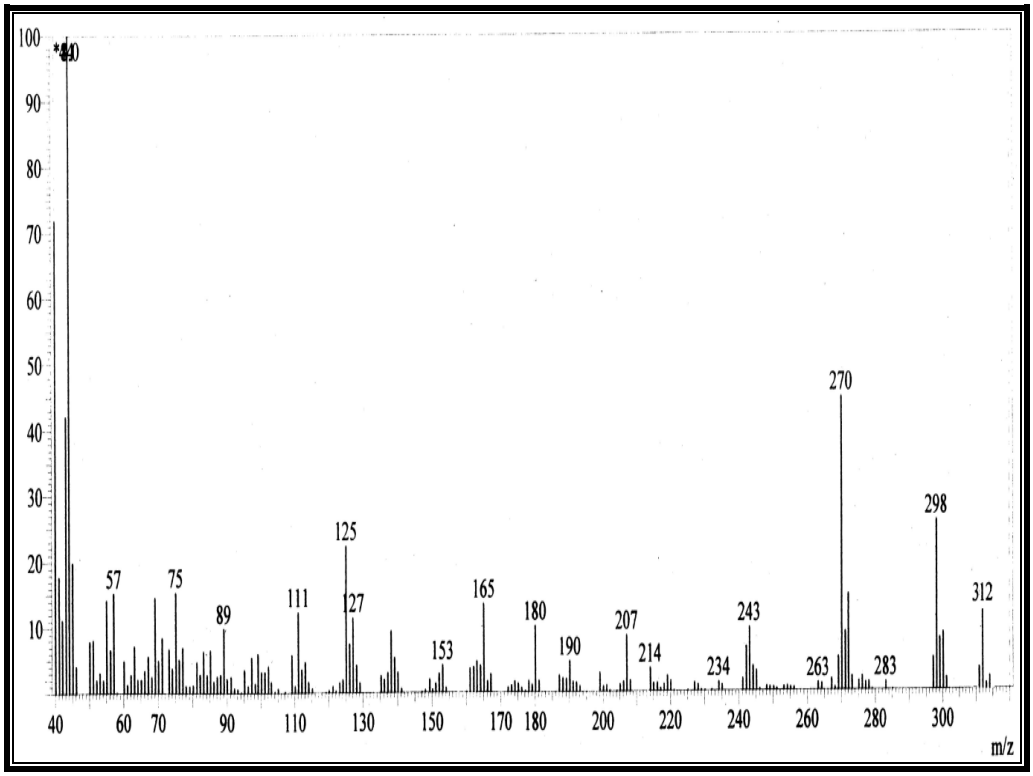
Expanded ^{1}H NMR spectrum of DDK-A-29



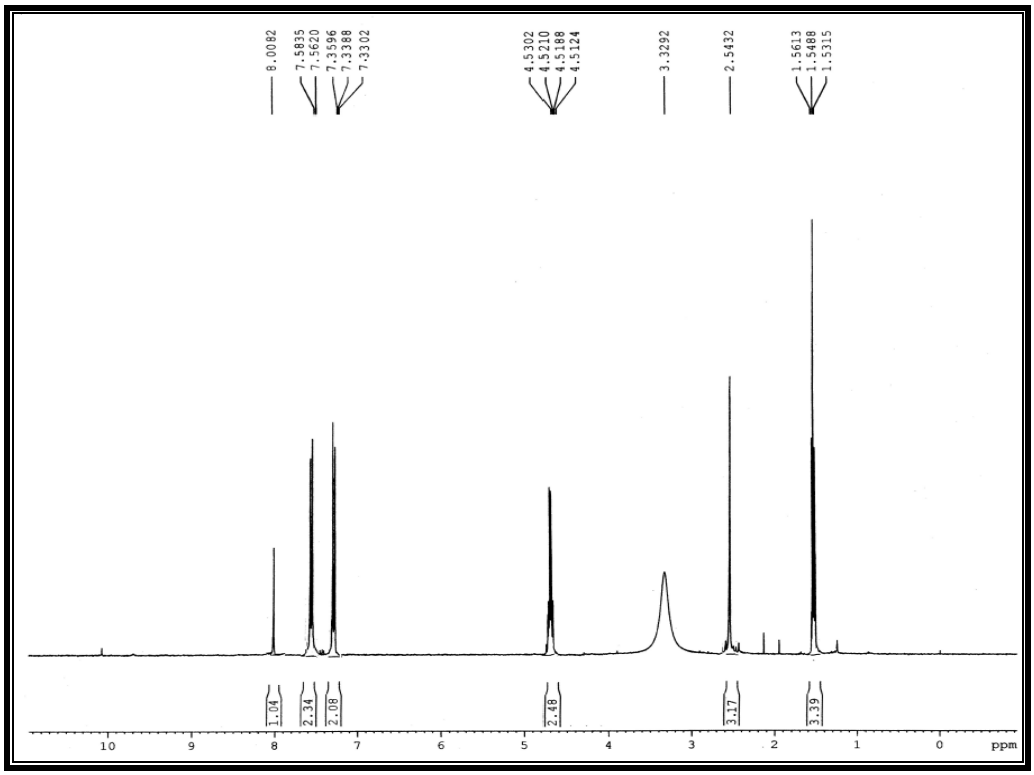
IR spectrum of DDK-A-34



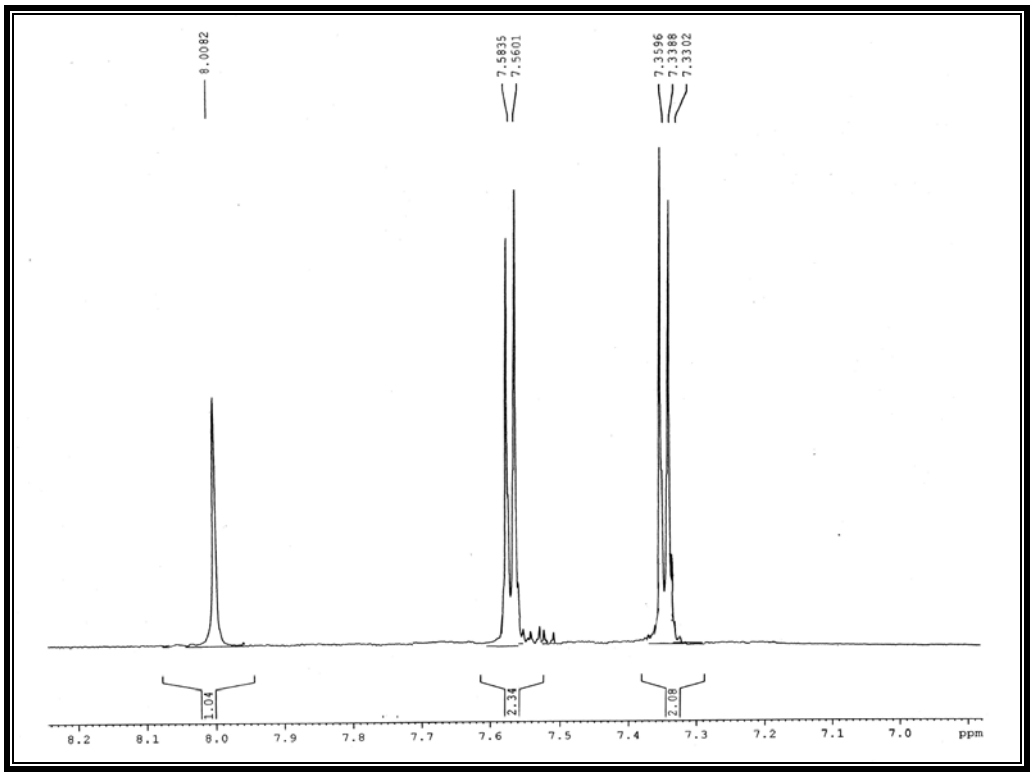
Mass spectrum of DDK-A-34



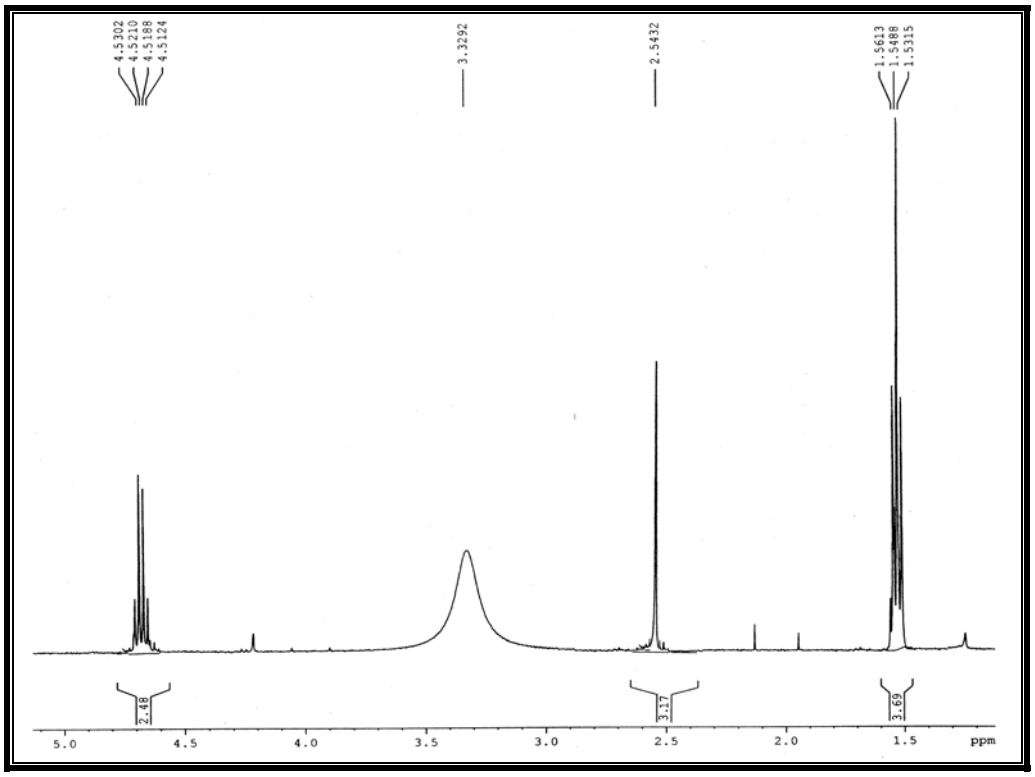
^{1}H NMR spectrum of DDK-A-34



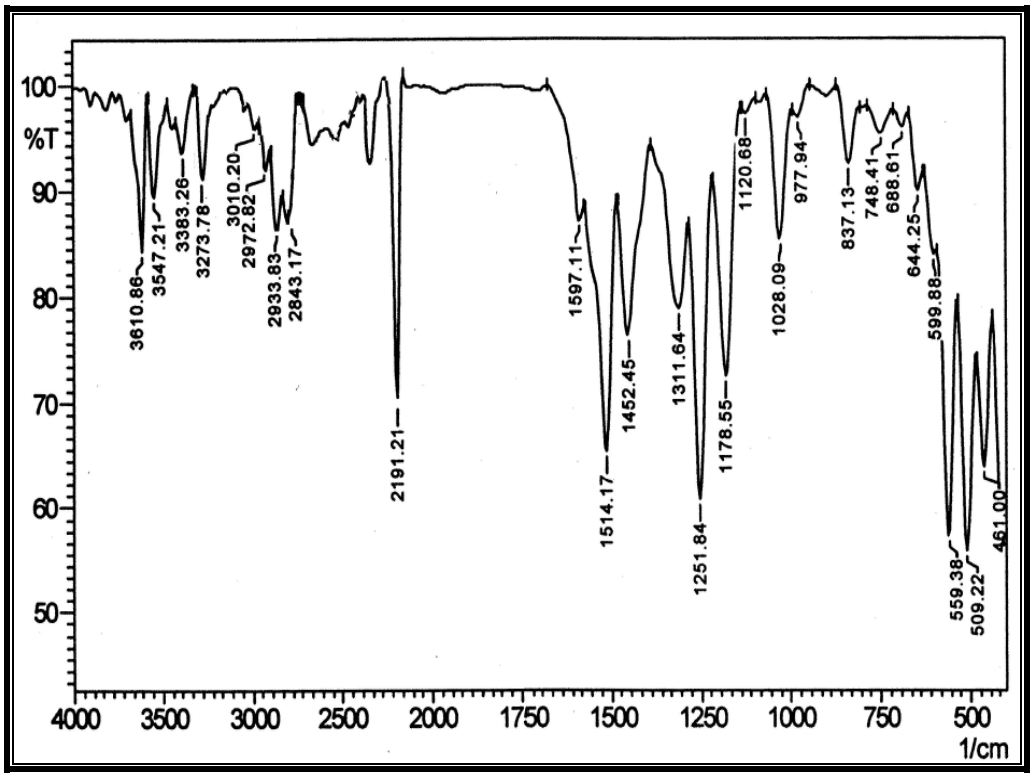
Expanded ^{1}H NMR spectrum of DDK-A-34



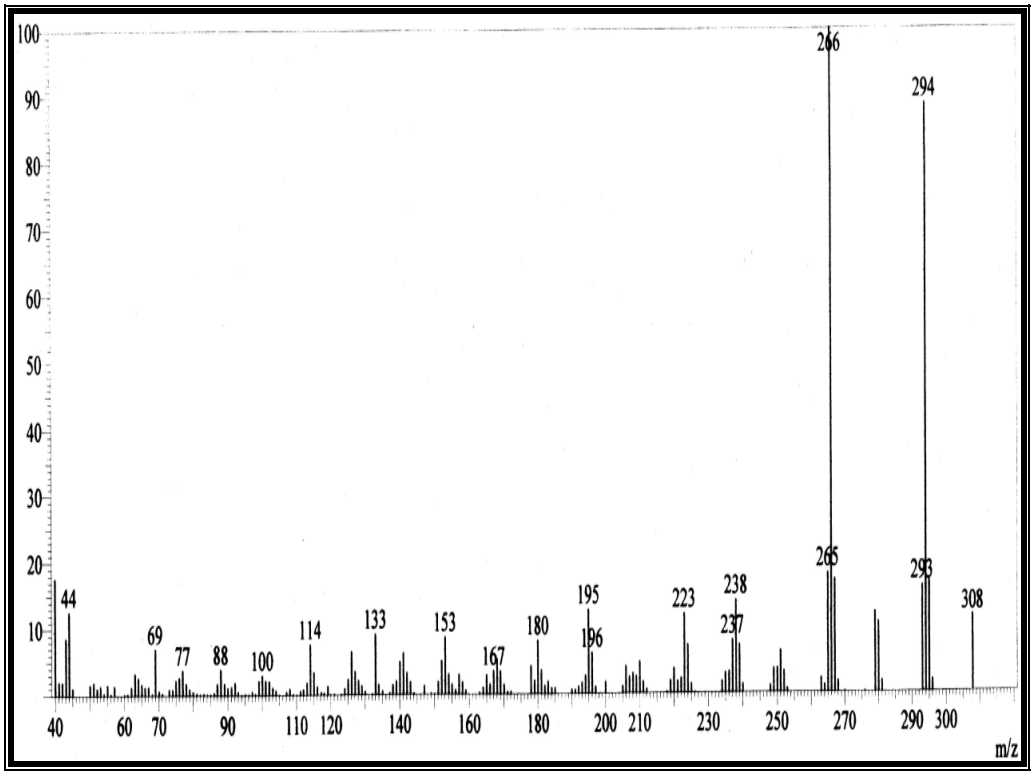
Expanded ^{1}H NMR spectrum of DDK-A-34



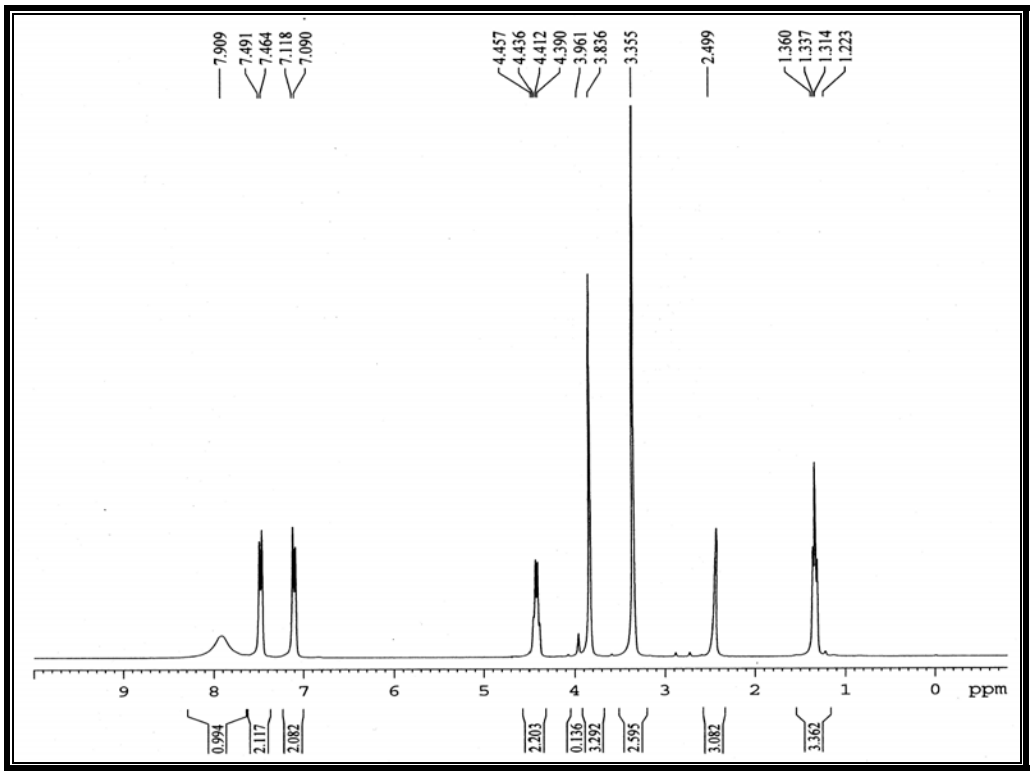
IR spectrum of DDK-A-37



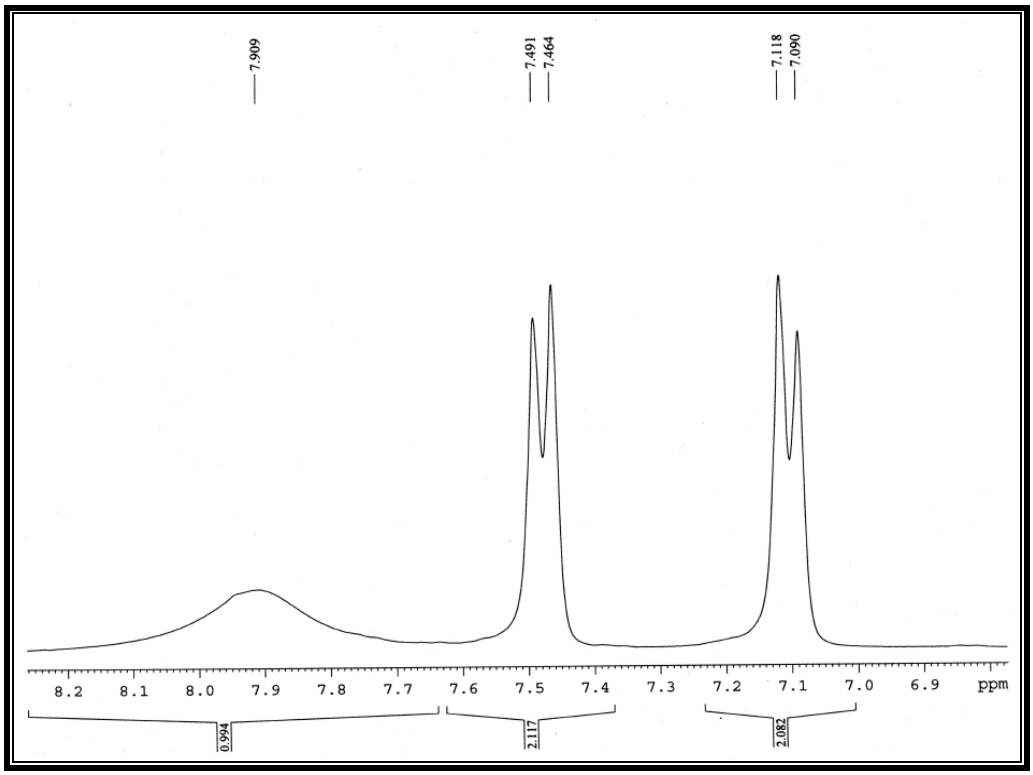
Mass spectrum of DDK-A-37



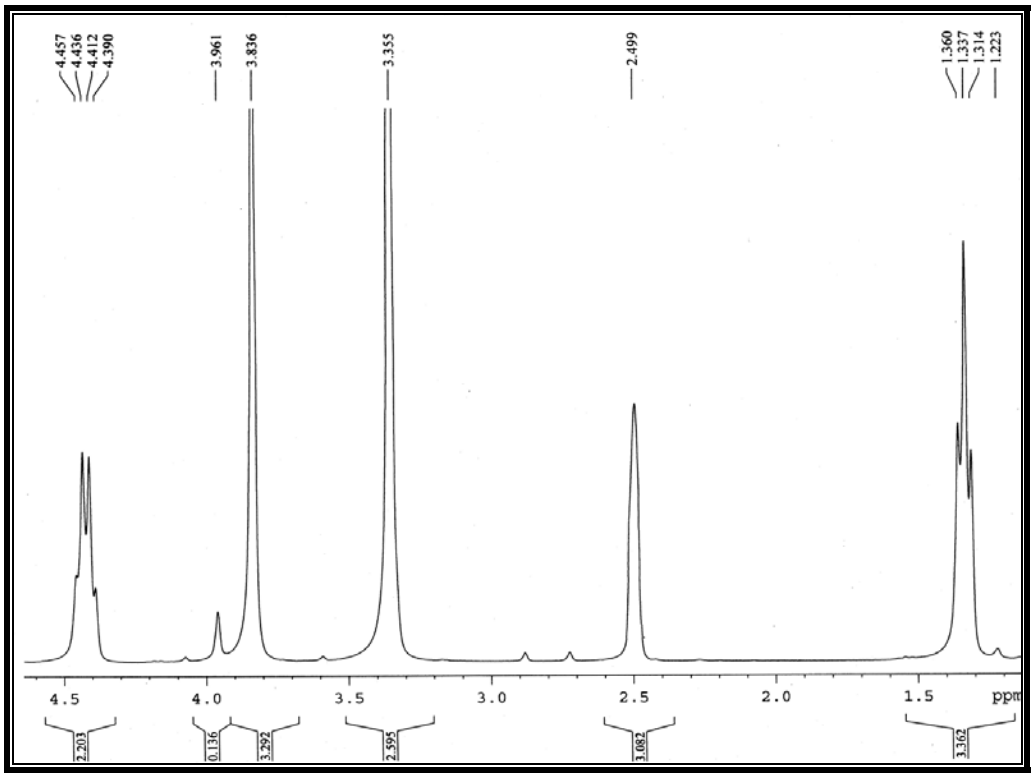
^{1}H NMR spectrum of DDK-A-37



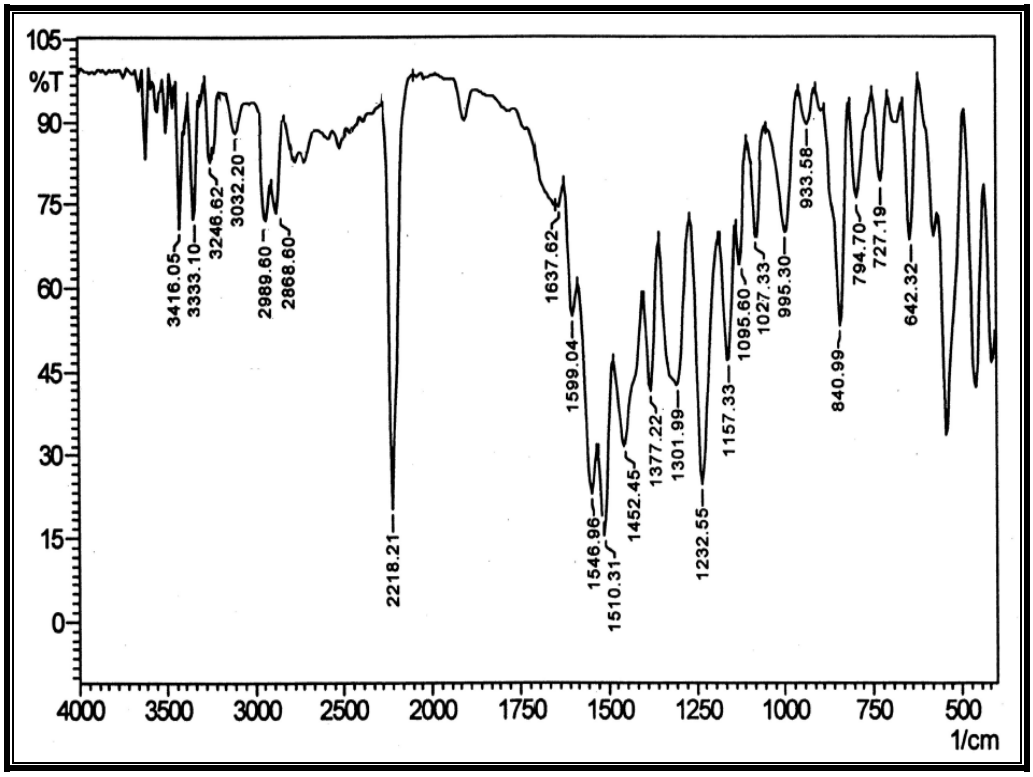
Expanded ^{1}H NMR spectrum of DDK-A-37



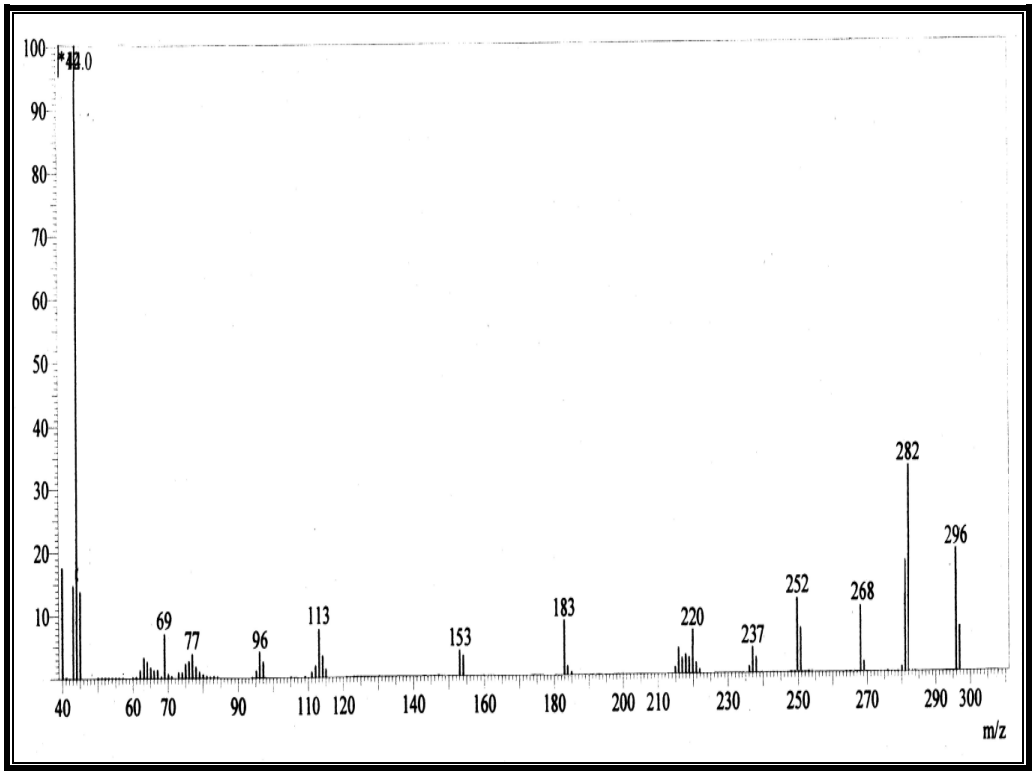
Expanded ^{1}H NMR spectrum of DDK-A-37



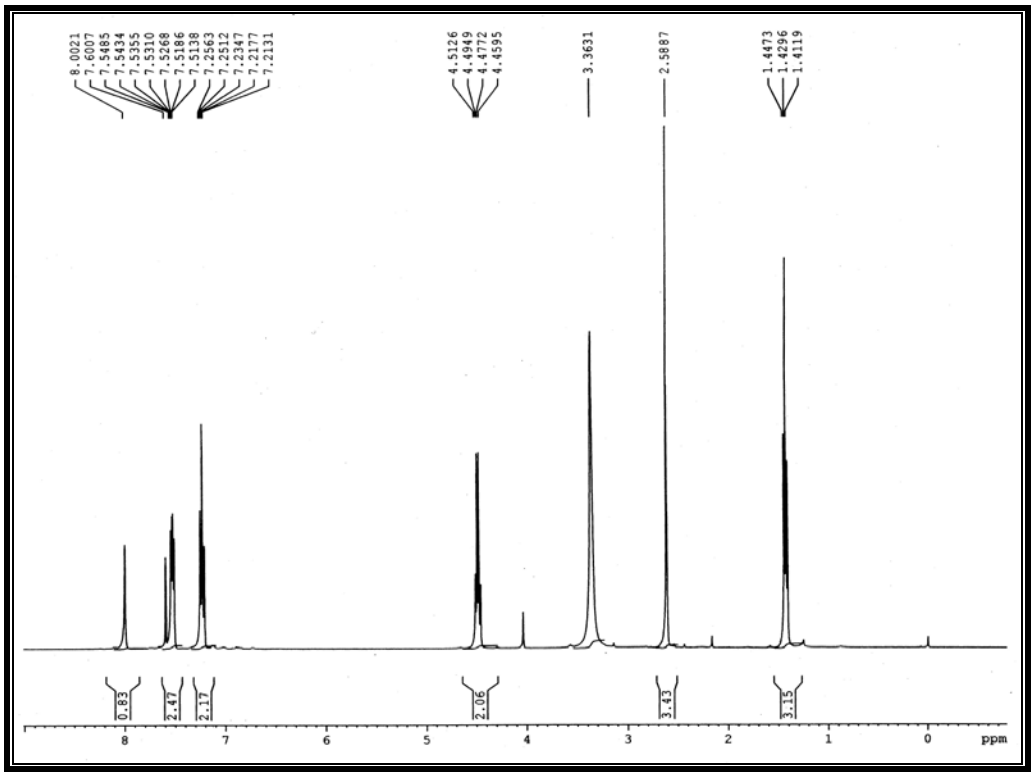
IR spectrum of DDK-A-39



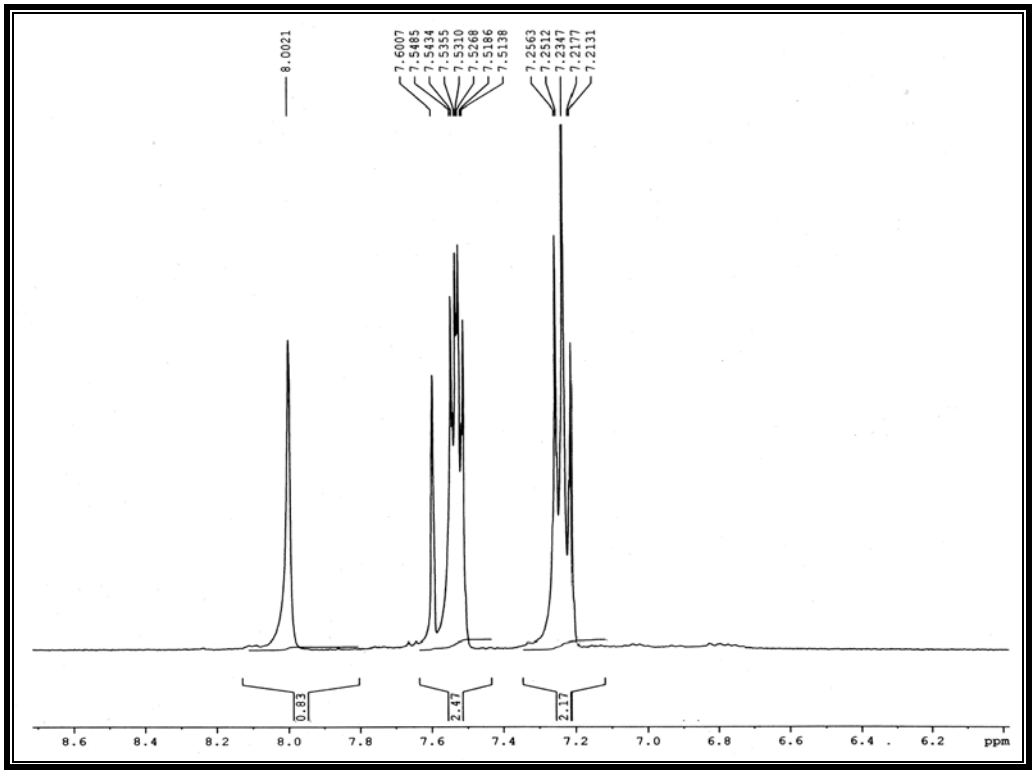
Mass spectrum of DDK-A-39



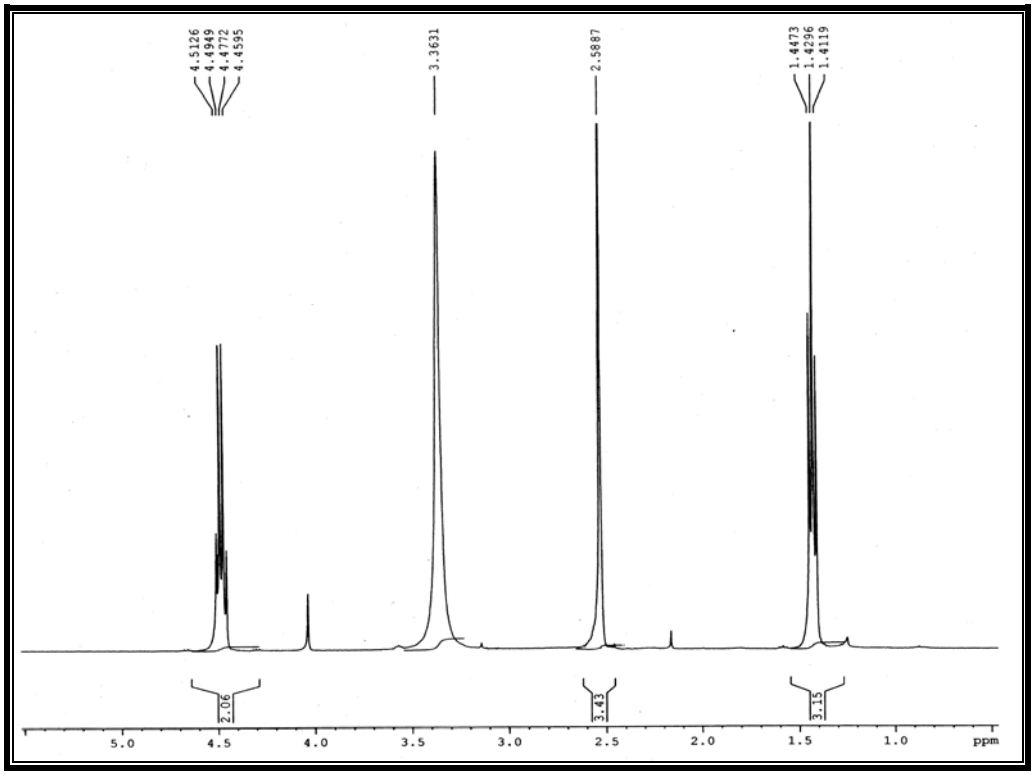
^{1}H NMR spectrum of DDK-A-39



Expanded ^{1}H NMR spectrum of DDK-A-39



Expanded ^{1}H NMR spectrum of DDK-A-39



2.2.8 Biological evaluation

2.2.8.1 Antimicrobial evaluation

All of the synthesized compounds (**DDK-A-21 to DDK-A-40**) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [88] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using microdilution broth method according to NCCLS standards [88(a)]. Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations. In primary screening 1000 μg mL^{-1}, 500 μg mL^{-1} and 250 μg mL^{-1} concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution at 125 μg mL^{-1}, 62.5 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1}, and 6.250 μg mL^{-1} concentration against all microorganisms. The tubes were inoculated with 10^8 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table 1.

Table 1. Antibacterial and antifungal activity of synthesized compounds (DDK-A-21 to DDK-A-40)

Code	Minimum inhibition concentration (μg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	***S. p.***	***E.c.***	***P.a.***	***C. a.***	***A. n.***	***A.c.***
DDK-A-21	50	25	125	500	1000	1000	500
DDK-A-22	250	250	100	500	100	500	500
DDK-A-23	100	500	125	1000	500	1000	1000
DDK-A-24	250	1000	250	500	500	250	250
DDK-A-25	50	125	250	62.5	1000	1000	500
DDK-A-26	500	500	500	>1000	100	500	250
DDK-A-27	1000	500	1000	1000	500	500	100
DDK-A-28	500	1000	1000	500	500	100	500
DDK-A-29	62.5	100	250	125	1000	>1000	1000
DDK-A-30	1000	500	500	1000	500	100	500
DDK-A-31	500	1000	250	1000	500	500	>1000
DDK-A-32	1000	250	500	500	500	100	250
DDK-A-33	100	125	100	62.5	>1000	1000	1000
DDK-A-34	100	>1000	500	1000	>1000	500	1000
DDK-A-35	25	500	250	100	500	1000	>1000
DDK-A-36	1000	100	100	500	250	100	250
DDK-A-37	125	100	100	500	500	250	1000
DDK-A-38	500	500	1000	>1000	1000	500	125
DDK-A-39	125	100	50	250	500	1000	500
DDK-A-40	100	1000	250	1000	1000	500	1000
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

2.2.8.2 Antimycobacterial, anticancer and antiviral evaluation

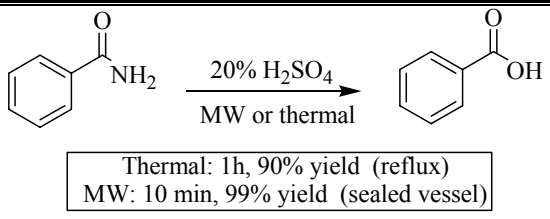
Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds (**DDK-A-21 to DDK-A-40**) is currently under investigation and results are awaited.

Chapter 3
Microwave assisted synthesis of heterocycles-an overview

3.1 Microwave-Assisted Organic Synthesis (MAOS) – A Brief History

While fire is now rarely used in synthetic chemistry, it was not until Robert Bunsen invented the burner in 1855 that the energy from this heat source could be applied to a reaction vessel in a focused manner. The Bunsen burner was later superseded by the isomantle, the oil bath or the hot plate as a means of applying heat to a chemical reaction. In the past few years, heating and driving chemical reactions by microwave energy has been an increasingly popular theme in the scientific community [1, 2].

Microwave energy, originally applied for heating foodstuffs by Percy Spencer in the 1940s, has found a variety of technical applications in the chemical and related industries since the 1950s, in particular in the food-processing, drying and polymer industries. Other applications range from analytical chemistry (microwave digestion, ashing and extraction) [3] to biochemistry (protein hydrolysis, sterilization) [3], pathology (histoprocessing, tissue fixation) [4] and medical treatments (diathermy) [5]. Somewhat surprisingly, microwave heating has only been implemented in organic synthesis since the mid-1980s. The first reports on the use of microwave heating to accelerate organic chemical transformations (MAOS) were published by the groups of Richard Gedye (Scheme 3.1) [6] and Raymond J. Giguere/George Majetich [7] in 1986.



Scheme 3.1 Hydrolysis of benzamide: The first published example (1986) of microwave-assisted organic synthesis.

In those early days, experiments were typically carried out in sealed teflon or glass vessels in a domestic household microwave oven without any temperature or pressure measurements. The results were often violent explosions due to the rapid uncontrolled heating of organic solvents under closed-vessel conditions. In the 1990s, several groups started to experiment with solvent-free microwave chemistry (so-called dry-media reactions), which eliminated the danger of explosions [8]. Here, the reagents were pre-adsorbed onto either an essentially microwave-transparent (i.e., silica, alumina or clay) or strongly absorbing (i.e., graphite) inorganic support, that additionally may have been doped with a catalyst or reagent. Particularly in the early days of MAOS, the solvent-free approach was very popular since it allowed the safe use of domestic microwave ovens and standard open-vessel technology. While a large number of interesting transformations using "dry-media" reactions have been published in the literature [8], technical difficulties relating to non-uniform heating, mixing and the precise determination of the reaction temperature remained unresolved, in particular when scale-up issues needed to be addressed.

Alternatively, microwave-assisted synthesis has been carried out using standard organic solvents under open-vessel conditions. If solvents are heated by microwave irradiation at atmospheric pressure in an open vessel, the boiling point of the solvent typically limits the reaction temperature that can be achieved. In order to nonetheless achieve high reaction rates, high-boiling microwave-absorbing solvents have been frequently used in open-vessel microwave synthesis [9]. However, the use of these solvents presented serious challenges in relation to product isolation and recycling of the solvent. Because of the recent availability of modern microwave reactors with on-line monitoring of both temperature and pressure, MAOS in dedicated sealed vessels using standard solvents-a technique pioneered by Christopher R. Strauss in the mid-1990s [10]-has been celebrating a comeback in recent years. This is clearly evident surveying the recently published (since 2001) literature in the area of controlled microwave-assisted organic synthesis (MAOS). It appears that the combination of rapid heating by microwaves with sealed-vessel (autoclave) technology will most likely be the method of choice for performing MAOS on a laboratory scale in the future. Importantly, recent innovations in microwave reactor technology now allow controlled parallel and automated sequential

processing under sealed-vessel conditions, and the use of continuous- or stop-flow reactors for scale-up purposes.

Since the early days of microwave synthesis, the observed rate accelerations and sometimes altered product distributions compared to oil-bath experiments have led to speculation on the existence of so-called "specific" or "non-thermal" microwave effects [11]. Historically, such effects were claimed when the outcome of a synthesis performed under microwave conditions was different from that of the conventionally heated counterpart at the same apparent temperature. Reviewing the present literature [12], it appears that today most scientists agree that in the majority of cases the reason for the observed rate enhancements is a purely thermal/kinetic effect, i.e., a consequence of the high reaction temperatures that can rapidly be attained when irradiating polar materials in a microwave field, although effects that are caused by the unique nature of the microwave dielectric heating mechanism ("specific microwave effects") clearly also need to be considered. While for the medicinal chemist in industry this discussion may seem largely irrelevant, the debate on "microwave effects" is undoubtedly going to continue for many years in the academic world. Regardless of the nature of the observed rate enhancements, microwave synthesis has now truly matured and has moved from a laboratory curiosity in the late 1980s to an established technique in organic synthesis, heavily used in both academia and industry.

The initially slow uptake of the technology in the late 1980s and 1990s has been attributed to its lack of controllability and reproducibility, coupled with a general lack of understanding of the basics of microwave dielectric heating. The risks associated with the flammability of organic solvents in a microwave field and the lack of available dedicated microwave reactors allowing for adequate temperature and pressure control were major concerns. Important instrument innovations now allow for careful control of time, temperature and pressure profiles, paving the way for reproducible protocol development, scale-up and transfer from laboratory to laboratory and from scientist to scientist. Today, microwave chemistry is as reliable as the vast arsenal of synthetic methods that preceded it. Since 2001, therefore, the number of publications related to MAOS has increased dramatically, to such a level that it might be assumed that, in a few years, most chemists will probably use microwave energy to heat chemical reactions on a laboratory scale [1, 2]. Not only is

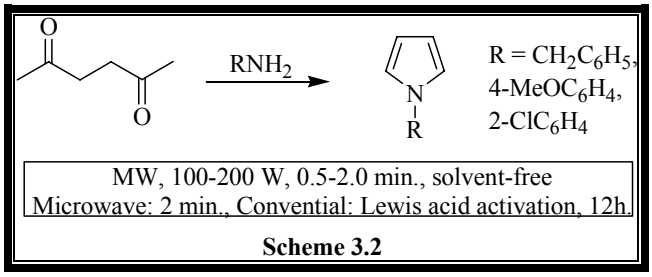
direct microwave heating able to reduce chemical reaction times significantly, but it is also known to reduce side reactions, increase yields and improve reproducibility. Therefore, many academic and industrial research groups are already using MAOS as a technology for rapid reaction optimization, for the efficient synthesis of new chemical entities or for discovering and probing new chemical reactivity.

3.2 Applications of microwaves in heterocyclic ring formation

3.2.1 Five-membered heterocyclic rings

3.2.1.1 Pyrroles

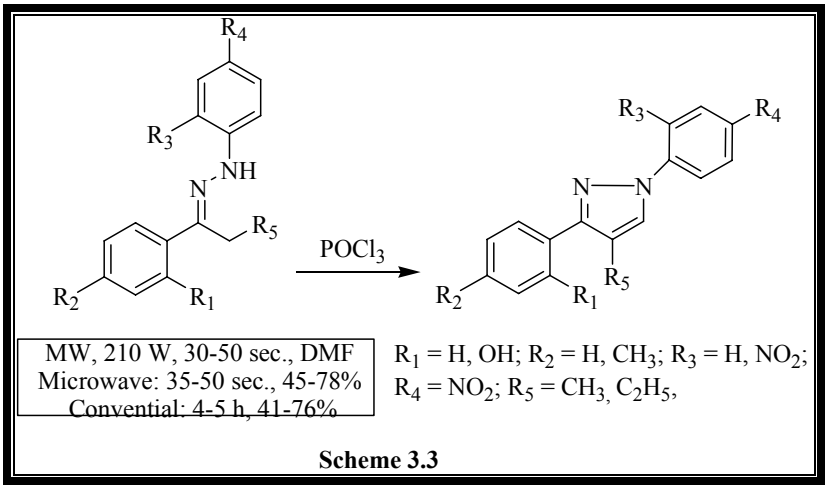
The classical Paal-Knorr cyclization of 1,4-diketones to give pyrroles is dramatically speeded- up under microwave irradiation and high yields are obtained as shown in Scheme 3.2 [13].



Scheme 3.2

3.2.1.2 Pyrazoles

Another recent application of microwaves in cyclization is the preparation of pyrazoles from hydrazones using the Vilsmeier cyclization method by treatment with

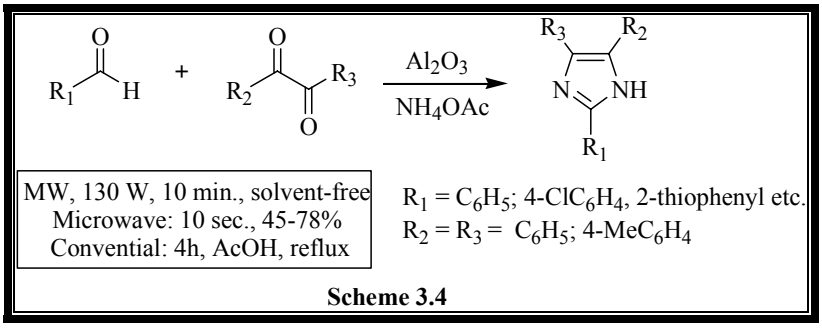


Scheme 3.3

$POCl_3$ and DMF [14]. As shown in Scheme 3.2, once again the reaction is speeded-up by factors of several 100-fold.

3.2.1.3 Imidazoles

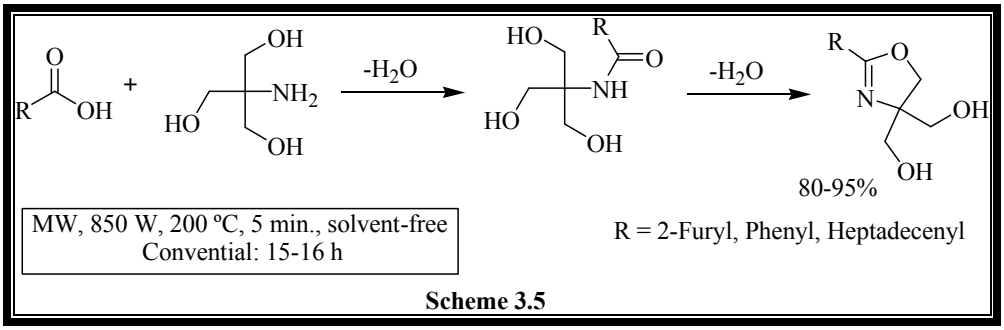
An important classical preparation of imidazoles is from an α-diketone, an aldehyde and ammonia. Here again, excellent yields can be obtained in reaction times of a few minutes as shown in Scheme 3.4 [15].



Scheme 3.4

3.2.1.4 Oxazolines

The example of Scheme 3.5, the preparation of oxazolines shows that partially saturated five-membered rings can also be prepared advantageously using microwaves [16].

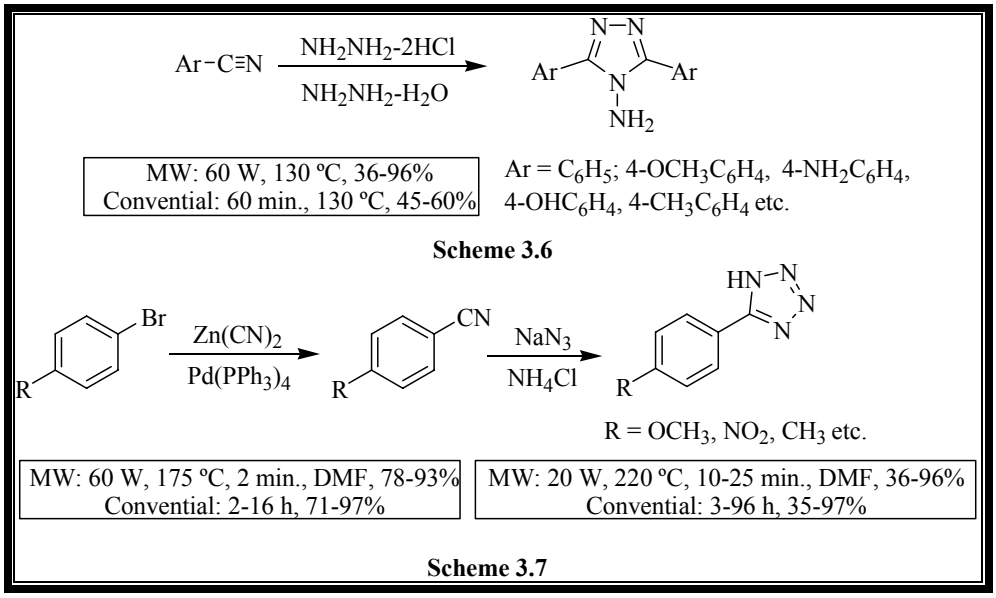


Scheme 3.5

3.2.1.5 Triazoles and Tetrazoles

Schemes 3.6 and 3.7 show the overview of five-membered rings with illustrations of the advantageous preparation of 1,2,4–triazoles [17] and tetrazoles [18] respectively using microwaves. Notice that in Scheme 3.6 the starting aryl cyanides are also made

by a Pd-catalyzed but microwave-enhanced replacement of aryl bromides using zinc cyanide.

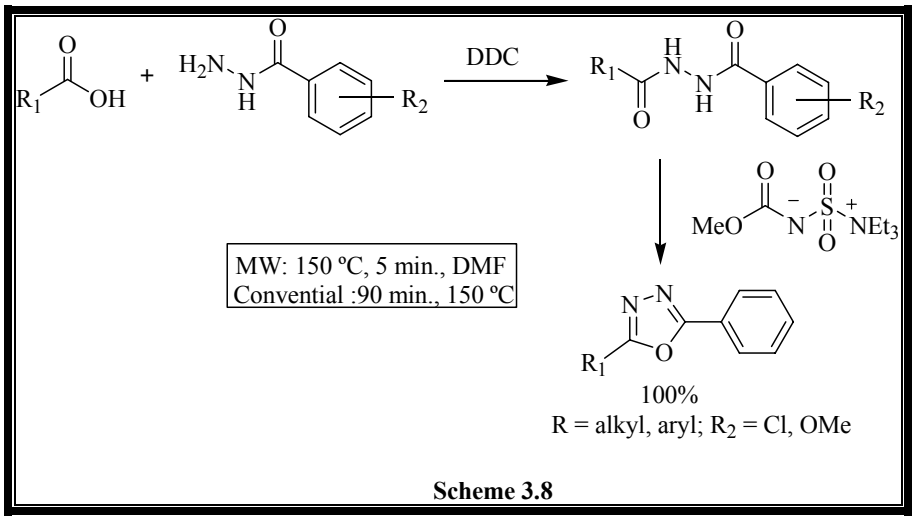


Scheme 3.6

Scheme 3.7

3.2.1.6 Oxadiazoles

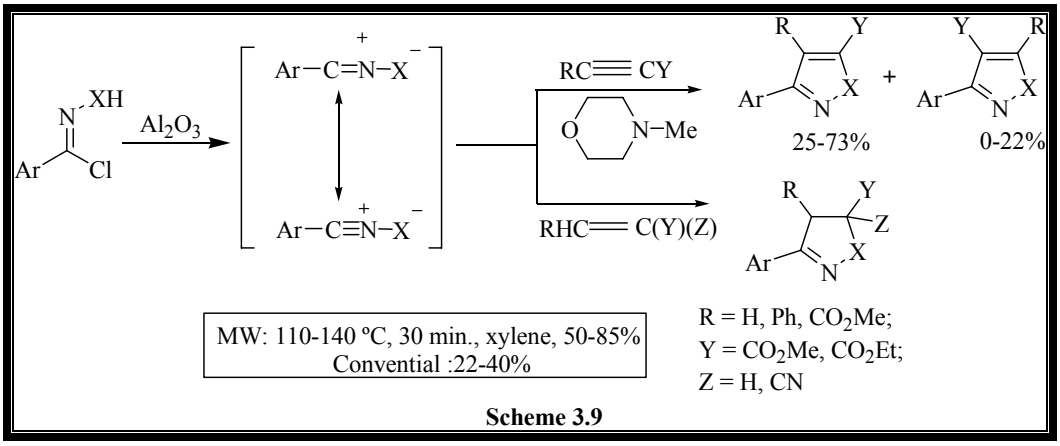
The dehydration of unsymmetrical diacylhydrazines (themselves prepared by a conventional Mitsunobu reaction) using Burgess's reagent is shown in Scheme 3.8 to give 1,3,4-oxadiazoles rapidly under microwave irradiation [19].



Scheme 3.8

3.2.1.7 Isoxazolines and pyrazolines

The acceleration of 1,3-dipolar cycloaddition reactions to give isoxazolines and pyrazolines by the addition of activated olefins to nitrile oxides or nitrile imides, respectively, is illustrated in Scheme 3.9; the resulting compounds are obtained in far high yield than under conventional conditions [20].

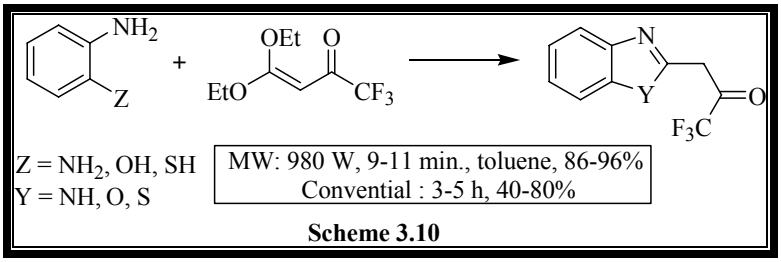


Scheme 3.9

3.2.2 Benzo-derivatives of five-membered rings

3.2.2.1 Benz- imidazoles, -oxazoles, and –thiazoles

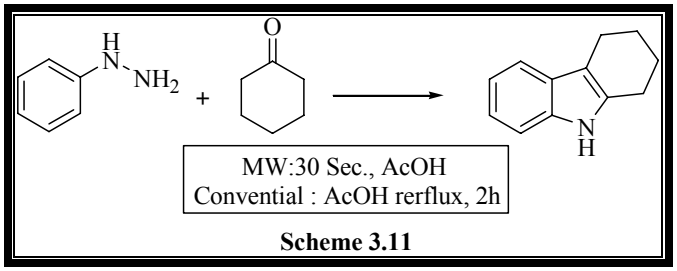
Ring closure reactions of appropriate *o*-substituted anilines to give benzimidazoles, benzoxazoles, and benzthiazoles takes place much faster and in significantly high yield under microwave conditions than conventionally [21] as shown in Scheme 3.10.



Scheme 3.10

3.2.2.2 Indoles

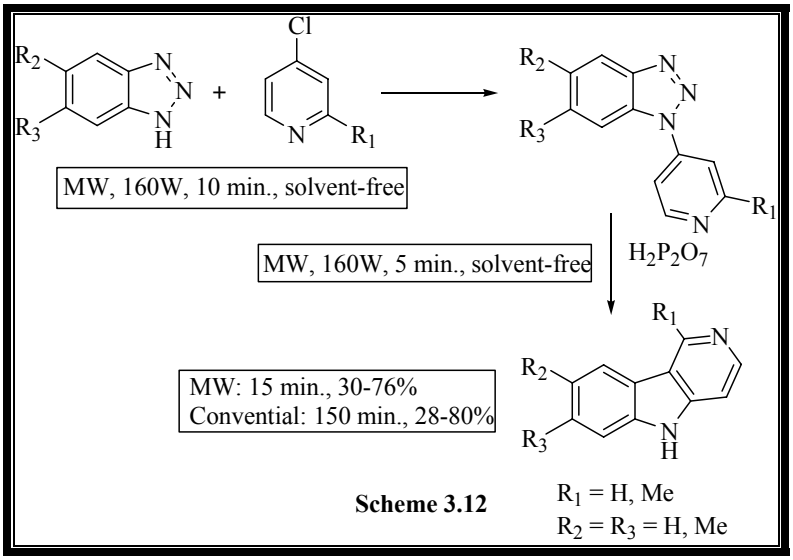
The classical Fischer-indole synthesis from an aryl hydrazine and a ketone is speeded-up by several 100-fold as documented in Scheme 3.11 [22].



Scheme 3.11

3.2.2.3 γ-Carbolines

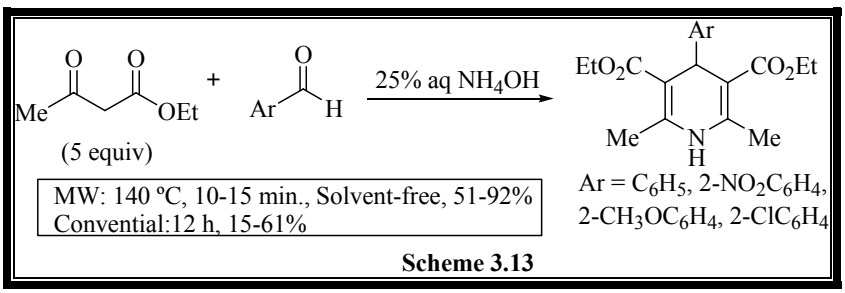
The Graebe-Ullmann synthesis which converts 1-arylbenzotriazoles into carbazoles or their heterocyclic analogs is also accelerated under microwave conditions as shown in Scheme 3.12 where the 1-(4-pyridyl)benzotriazole is converted into a γ-carboline [23].



Scheme 3.12

3.2.3 Six-membered rings

3.2.3.1 Dihydropyridines

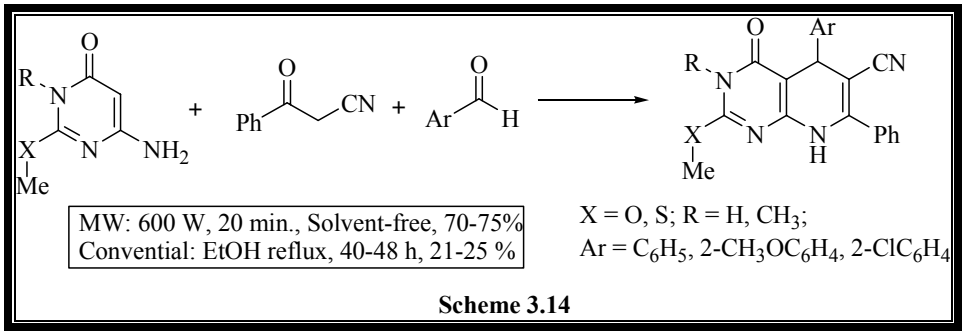


Scheme 3.13

The Hantzsch dihydropyridine synthesis remains one of the most important routes to pyridine ring systems. Under conventional conditions long periods of heating are required and yields are poor to moderate. Microwaves dramatically reduce the heating times and also significantly increase the yields as shown in Scheme 3.13 [24].

3.2.3.2 Dihydropyridopyrimidinones

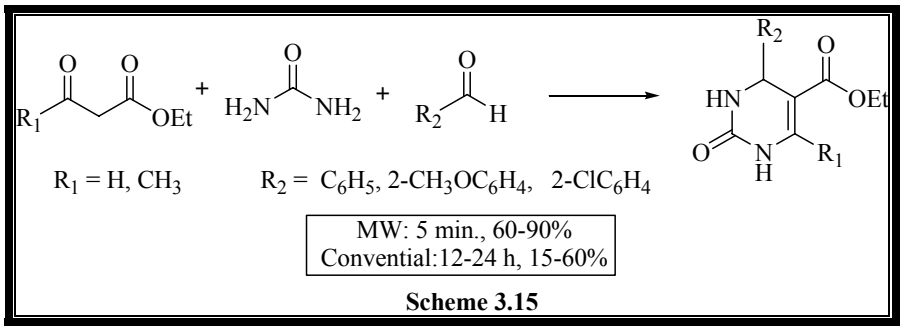
Dihydropyridopyrimidinones have been produced by ring annulations of aminopyrimidinones. Once again the reaction time is dramatically reduced and yields are much better with the solvent-free microwave conditions (Scheme 3.14) [25].



Scheme 3.14

3.2.3.3 Dihydropyrimidines

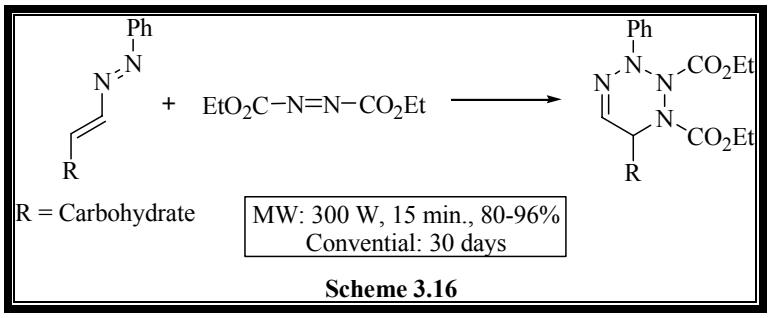
The Biginelli reaction is important for the preparation of dihydropyrimidine derivatives and excellent results are found for reactions carried out with microwave enhancement (Scheme 3.15) [19].



Scheme 3.15

3.2.3.4 Tetrazines

The Diels-Alder reaction between aza-olefins and aza-dicarboxylic ester to give tetrazines is speeded-up by a factor of 1000 by microwave enhancement as shown in Scheme 3.16 [26].

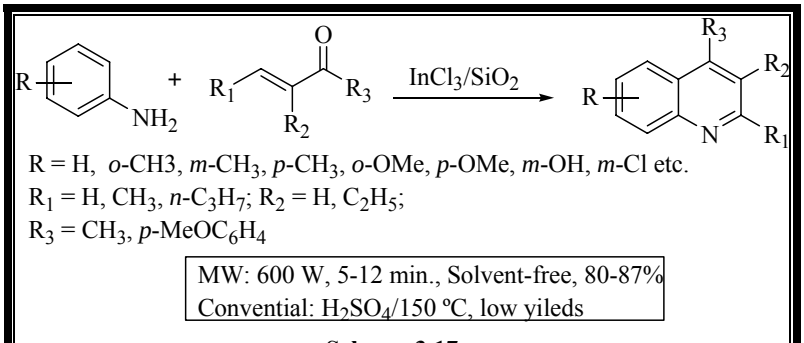


Scheme 3.16

3.2.4 Polycyclic six-membered rings

3.2.4.1 Quinolines

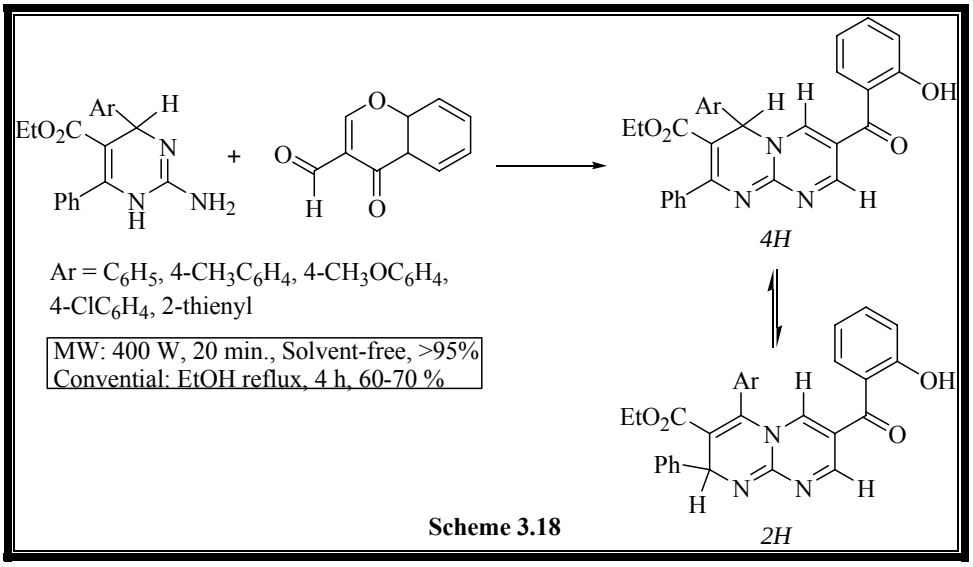
The Skraup synthesis has a bad reputation as it involves very messy conditions and gives only low yields of quinolines when carried out conventionally. Recently, it has been reported that microwave enhancement reduces the reaction time to a few minutes and allows high yields to be isolated (Scheme 3.17) [27].



Scheme 3.17

3.2.4.2 Pyrimido[1,2-*a*]pyrimidines

Pyrimido[1,2-*a*]pyrimidines are prepared from dihydroaminopyrimidines and chromone-3-aldehydes as is shown in Scheme 3.18 [28]. Although the conventional reaction must proceed in refluxing ethanol, reactions are much faster and better yields have been obtained with microwaves.

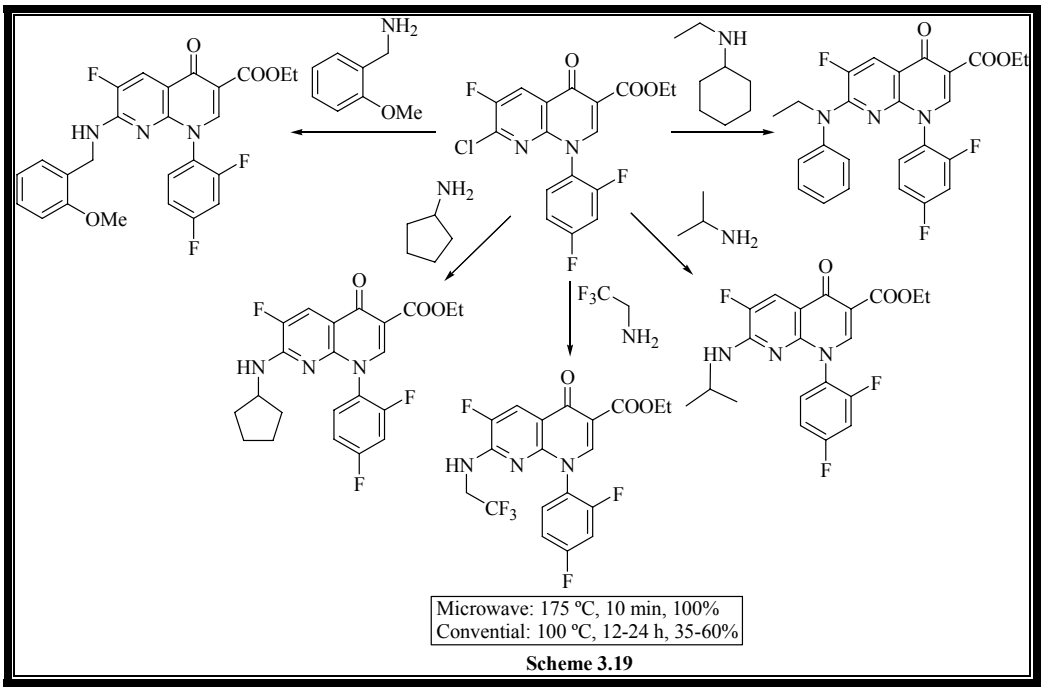


Scheme 3.18

3.2.5 Nucleophilic Substitutions

3.2.5.1 Heterocyclic *C*-alkylations

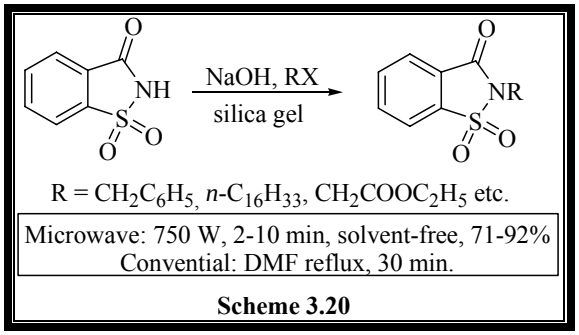
Nucleophilic substitution reactions can be speeded-up very considerably as is illustrated in Scheme 3.19 for a chloro-naphthyridine derivative [29].



Scheme 3.19

3.2.5.2 Heterocyclic *N*-alkylations

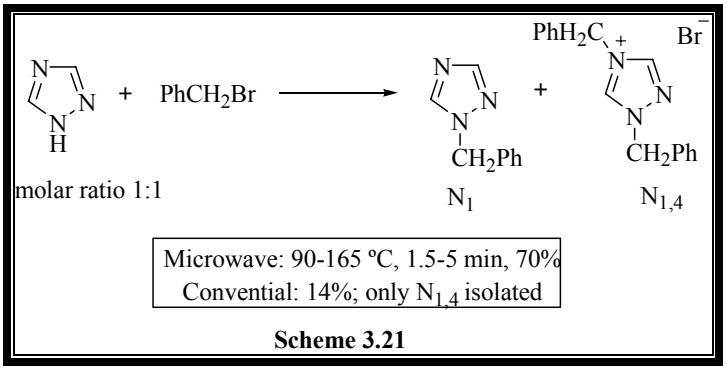
Another class of nucleophilic substitution is involved in heterocyclic *N*-alkylation which we have illustrated in Scheme 3.20. This shows that nucleophilic substitution on the nitrogen atom of saccharin is significantly speeded-up by microwave irradiation [19].



Scheme 3.20

3.2.5.3 Selective-alkylation

In Scheme 3.21, the results presented indicate that selectivity is achieved in the *N* alkylation of 1,2,4-triazole under microwave conditions where only the N_1-alkyl derivative was formed in contradistinction to the conventional conditions which give a considerable amount of the di-1,4-benzylated compound [30].

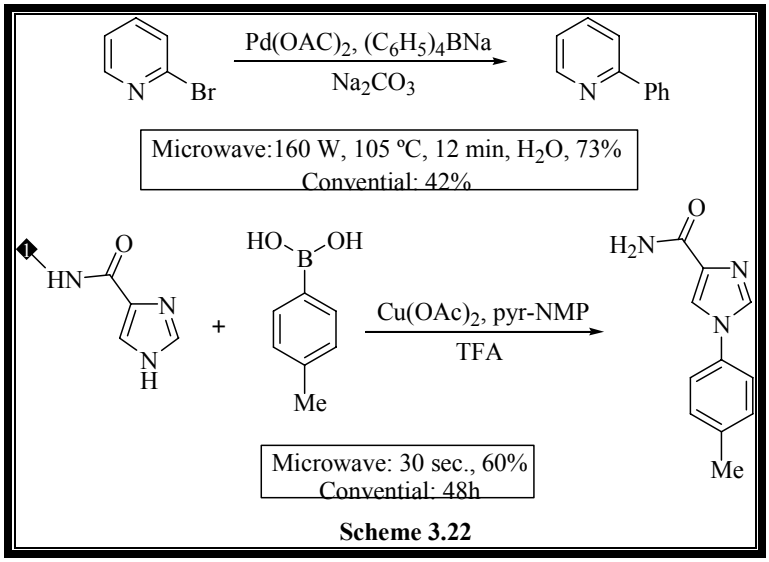


Scheme 3.21

3.2.5.4 Transition metal cross-coupling

An important type of nucleophilic substitution reactions which are recently much exploited are comprised of transition metal cross-coupling. A Suzuki coupling is shown at the top of Scheme 3.22 to give significantly better yield in the presence of

microwave irradiation [31]. At the bottom of Scheme 3.22 another Suzuki coupling is shown, which was speeded-up by a factor of 100 [32].

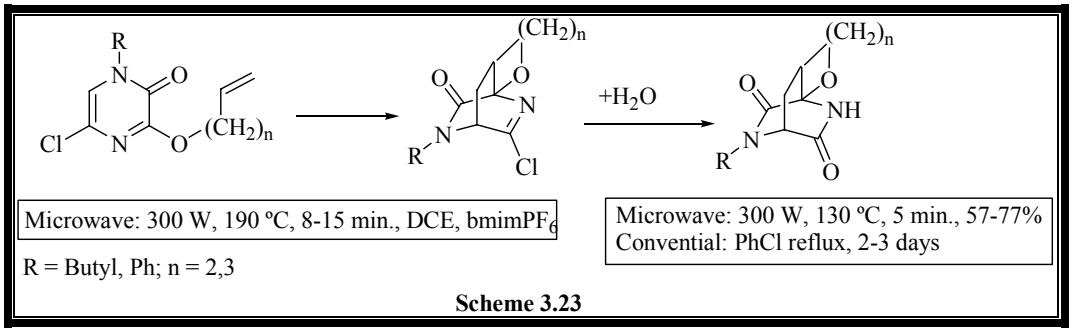


Scheme 3.22

3.2.6 Hetero-Diels−Alder reactions

3.2.6.1 Intramolecular reactions

We have already seen one example of a hetero-Diels−Alder reaction involving acyclic components. Hetero-Diels−Alder reactions involving cyclic components which lead to polycyclic ring systems are of great importance. An intramolecular example shown in Scheme 3.23 indicates that the reaction was accelerated by a factor of around 1000 by microwave irradiation [33].

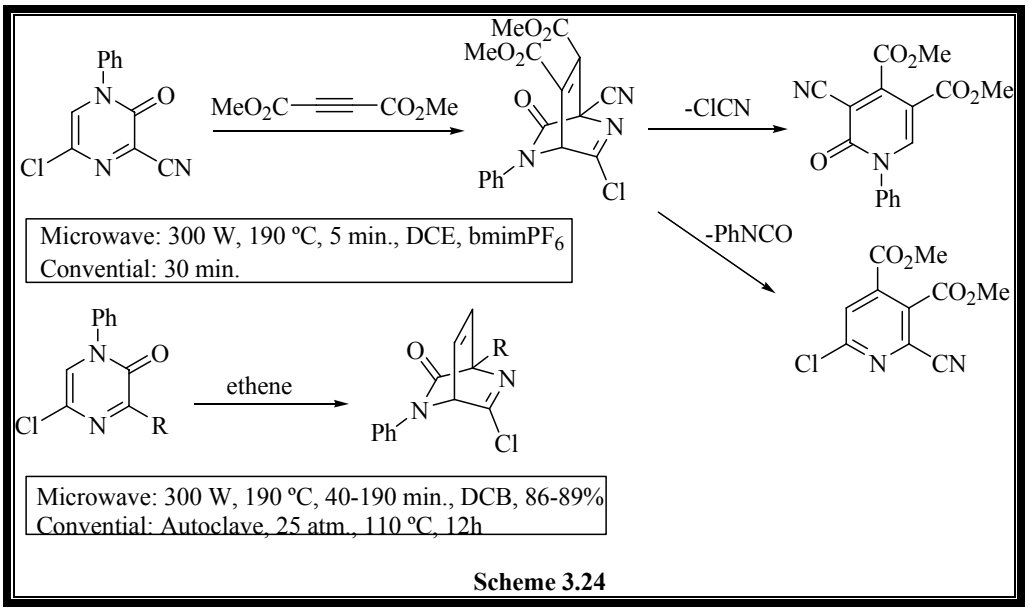


Scheme 3.23

3.2.6.2 Intermolecular reactions

Scheme 3.24 shows two impressive examples of rate enhancement for intermolecular hetero-Diels−Alder reactions [33]. In the first example on the top of Scheme 3.24 the

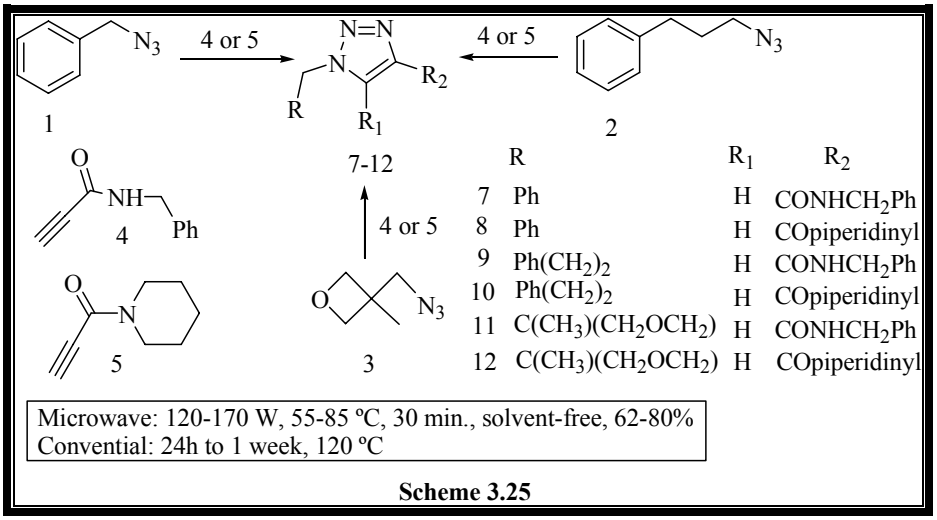
initial reaction is followed by elimination thus involving the conversion of a pyrazine derivative into a pyridine. Perhaps more impressive is the lower example in Scheme 3.24 where an autoclave is required under conventional conditions but which can be dispensed with when microwave acceleration is utilized.



Scheme 3.24

3.2.7 1,3-Dipolar cycloaddition reactions

3.2.7.1 Synthesis of *C*-carbamoyl-1,2,3-triazoles

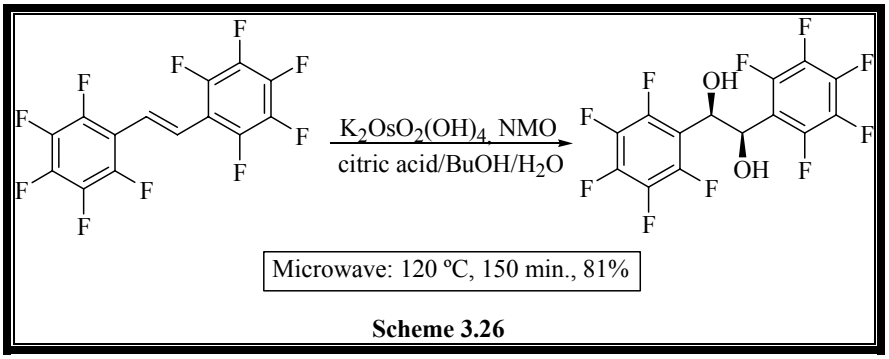


Scheme 3.25

There is one recent report which has involved microwave induced 1,3-dipolar cycloaddition of organic azides to acetylenic amides. As shown in Scheme 3.25, these reactions were achieved under microwave conditions in a reasonable time at temperatures of around 70±15 ºC [34]. Under conventional conditions the times were roughly 100 times as long and the temperature had to be taken up to 120 ºC [35].

3.2.8 Oxidation

The osmium-catalyzed dihydroxylation reaction, the addition of osmium tetroxide to olefins to produce a vicinal diol, is one of the most selective and reliable organic transformations. Recent work by Sharpless, Fokin, and coworkers [36] has uncovered that electron-deficient olefins can be converted into the corresponding diols much more efficiently when the reaction medium is kept acidic (Scheme 3.26).



Scheme 3.26

3.3. Concluding remarks

For more than a century, heterocycles have constituted one of the largest areas of research in organic chemistry. The presence of heterocycles in all kinds of organic compounds of interest in biology, pharmacology, optics, electronics, material sciences, and so on is very well known. Among them, sulfur and nitrogen-containing heterocyclic compounds have maintained the interest of researchers and their unique structures led to several applications in different areas.

The presence of heteroatoms results in significant changes in the cyclic molecular structure, due to the availability of unshared pairs of electrons, and in the reactivity, compared with the parent aromatic hydrocarbons. In contrast to the number and variety of such heterocycles, the number of synthetic methods to afford sulfur and nitrogen-containing molecules is, in practice, restricted to the availability of the

appropriate sulfur or nitrogen reagent. Sometimes the preparation of theses heterocyclic systems by conventional ways is difficult work that implies many synthetic steps and extensive starting material.

For all these reasons, the various possibilities offered by the microwave technology are particularly attractive where fast, high-yielding protocols and the avoidance or facilitation of purification are highly desirable. Despite the area of microwave-assisted chemistry being about 20 years old, the technique has only recently received widespread global acceptance of microwave-assisted synthesis of sulfur and nitrogen-containing heterocycles in the academic and industrial communities [37]. This is a consequence of the recent availability of commercial microwave systems specific for synthesis, which offers improved opportunities for reproducibility, rapid synthesis, rapid reaction optimization and the potential discovery of new chemistries. The beneficial effects of microwave irradiation are finding an increased role in process chemistry, especially in cases when usual methods require forcing conditions or prolonged reaction times. Microwaves have also shown an advantage where processes involve sensitive reagents or when products may decompose under prolonged reaction conditions.

All above observations led us to explore microwave-assisted synthesis of our aimed heterocyclic scaffolds viz. acridine-1,8-diones, 5,6,7,8-tetrahydroquinolines and 7,8-dihydroquinolines. The results are described in the following chapters:

Chapter 4: Studies on Microwave Assisted Synthesis of Acridines

Chapter 5: Studies on Microwave Assisted Synthesis of Polyhydroquinolines

3.4 References and notes

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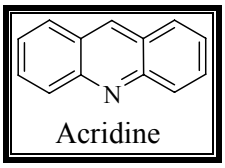
Chapter 4
Studies on microwave assisted synthesis of acridines

4.1 Introduction

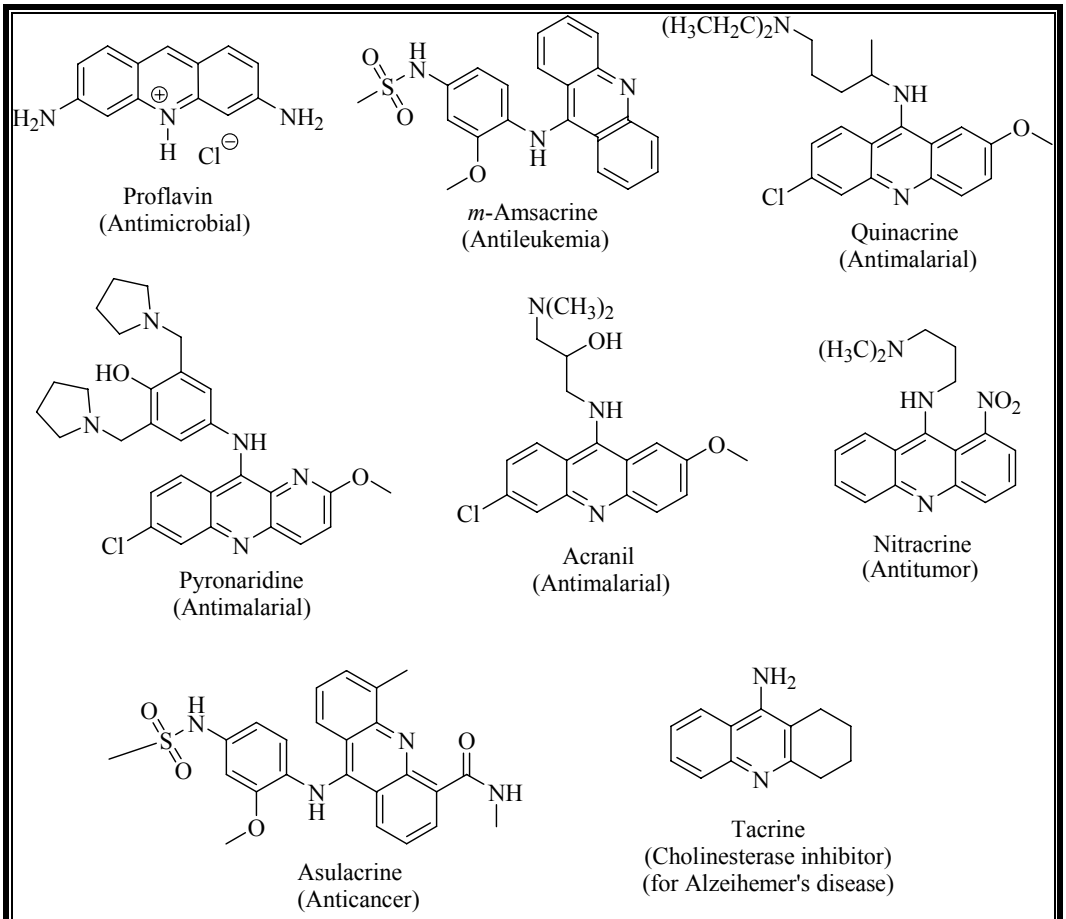
Acridine ($C_{13}H_9N$) is a nitrogen heterocycle, which is structurally related to anthracene with one of the central CH groups replaced by nitrogen.



Acridine

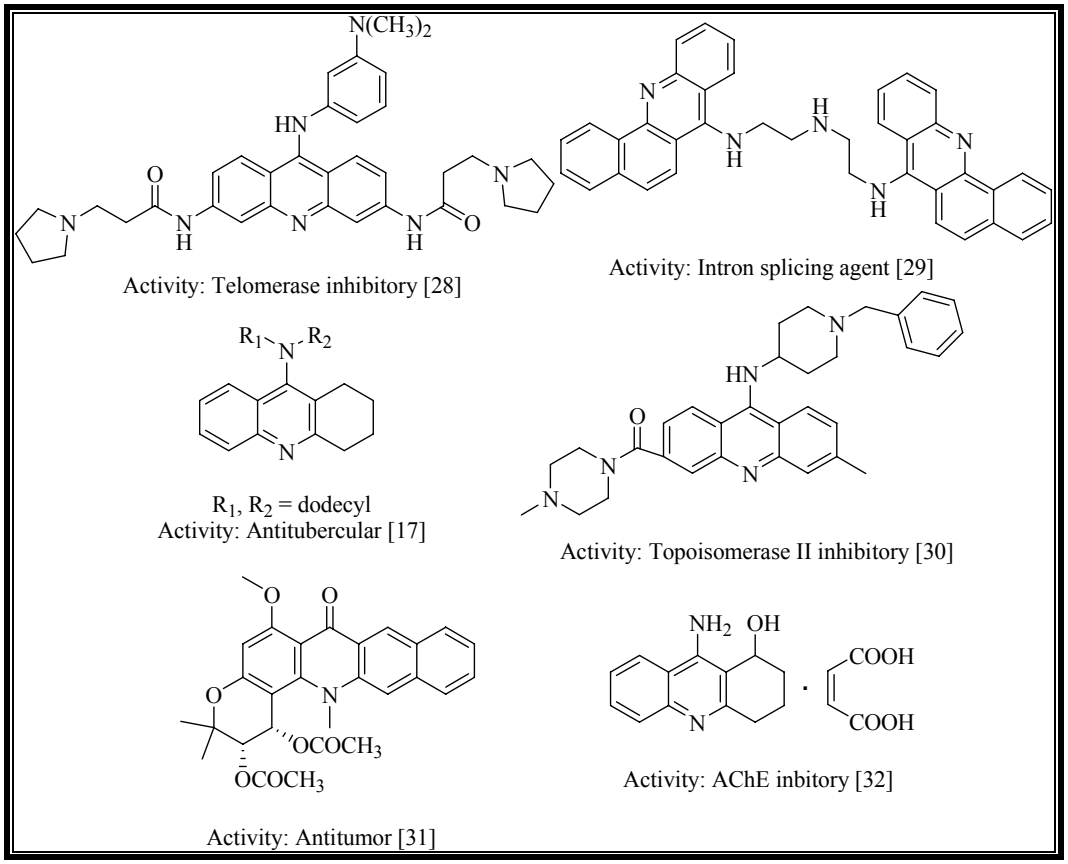
The acridines were first developed as dyes and during the early 20th century their pharmacological properties were evaluated. At this time, proflavin was used as a topical antibacterial and antifungal agent [1]. In the 1940's and to the present day, the acridines (e.g., quinacrine, pyronaridine and acranil) have been used as anti-malarial drugs [2]. The first acridine-based therapeutic agents specifically designed for cancer treatment were developed during the 1970's. These efforts led to the development of *m*-amsacrine, a 9-anilinoacridine introduced into clinical use in 1976 [3]. Accordingly, this acridine has been clinically utilized as a single agent or in combination with other antineoplastic drugs in the treatment of acute nonlymphocytic, lymphocytic [4, 5], and acute myeloid [6, 7] leukemias. However, *m*-amsacrine has not generally been effective in the treatment of solid tumors [8].

Asulacrine is an inhibitor of topoisomerase II, which has shown potential against breast and lung cancer [9, 10]. Nitracrine (Ledakrin) is another acridine antineoplastic agent used in mammary and ovarian tumors. It also inhibits RNA synthesis [11].



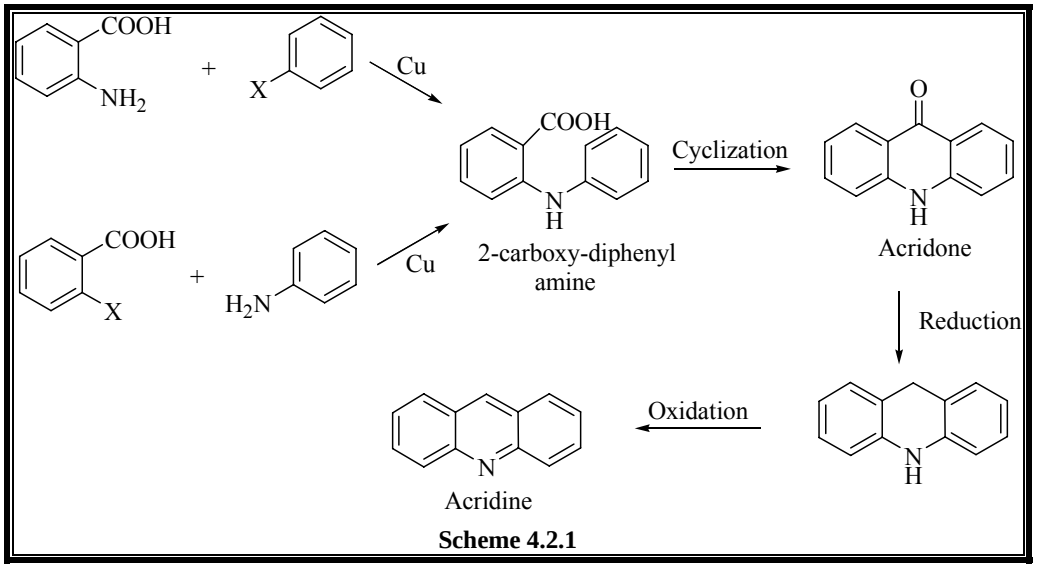
Some Acridine Derivatives in Clinical Use

Acridines and their fused derivatives posses wide spectrum biological activities viz. antibacterial [12], fungicidal [13], antimicrobial [14], anti-tubercular [15-17], antimalarial [18], anti-tumor [19, 20], anti-cancer [21], acetylcholinesterase inhibitory [22, 23], vasorelaxing [24], anti-viral [25]. The antitumor and anti-infectious activities of acridines are mainly related to their capacity to reversibly bind with DNA [26]. Due to their planar polycyclic structure, they have been shown to intercalate between DNA double-strands, to interfere with DNA regulatory enzymes such as topoisomerase I and II and to disrupt DNA functions in cells [27]. Some examples of published derivatives of acridines with their biological activities are shown in following figure.



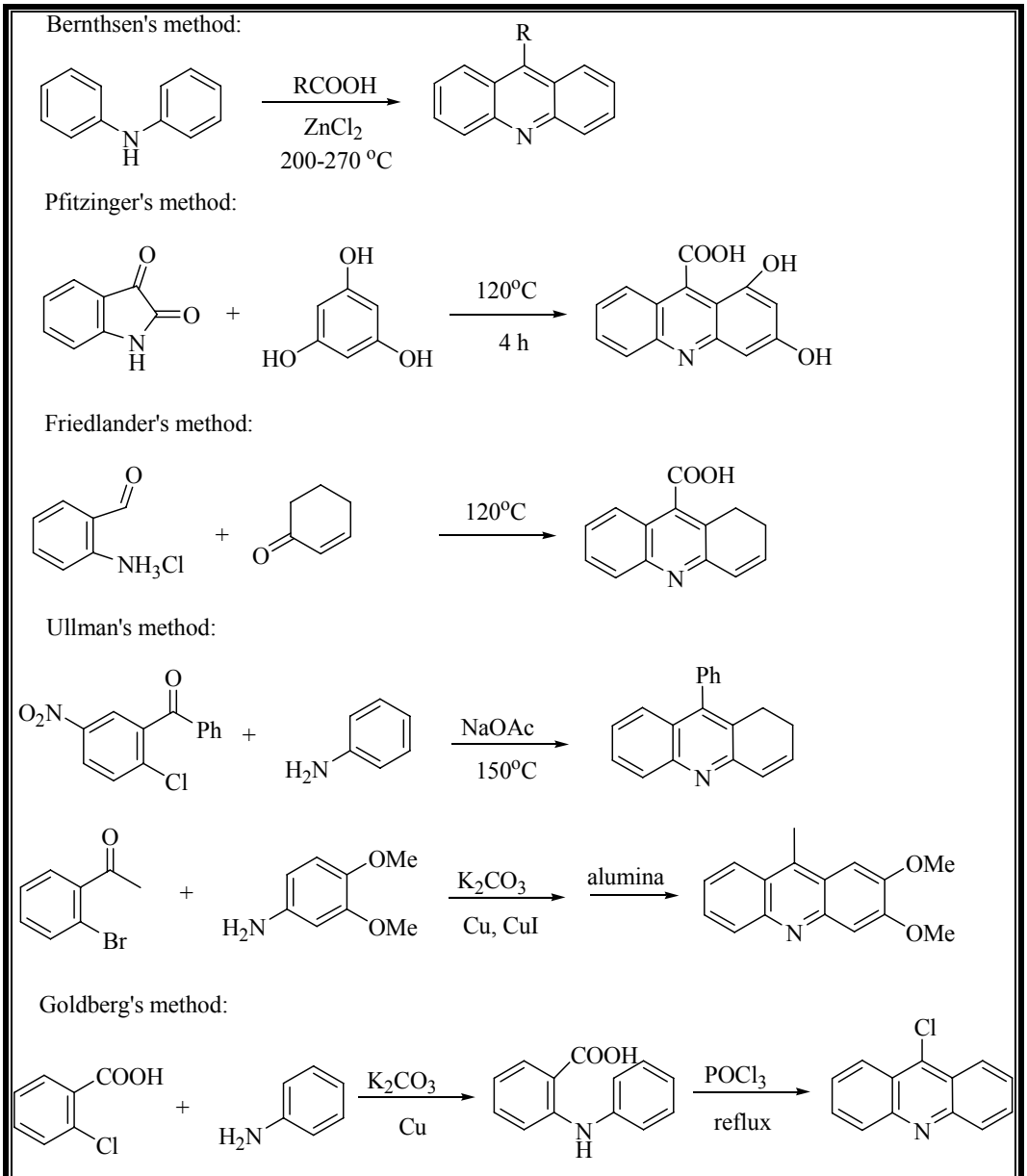
4.2 Reported synthetic strategies

Acridine is prepared from either anthranilic acid derivatives and aryl halides or from 2-chlorobenzoic acid and aryl amines in four steps [1] (Scheme 4.2.1).



Scheme 4.2.1

Other general synthetic strategies include Bernthsen's method, Pfitzinger's method, Friedlander's method, Ullman's method and Goldberg's method [1] (Scheme 4.2.2).

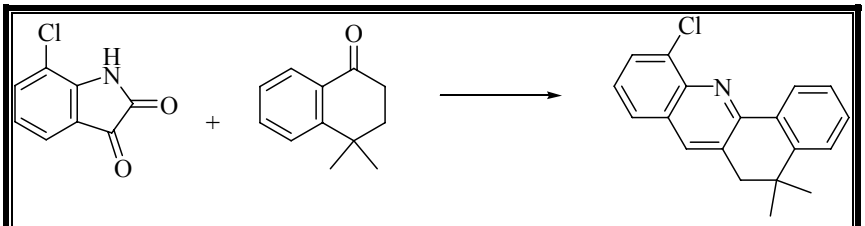


Scheme 4.2.2

The above mentioned strategies have been modified by different researchers to obtain diversely functionalized acridines or to improve the reaction conditions. Bernthsen's method was modified by Seijas et al. using microwave assistance, which

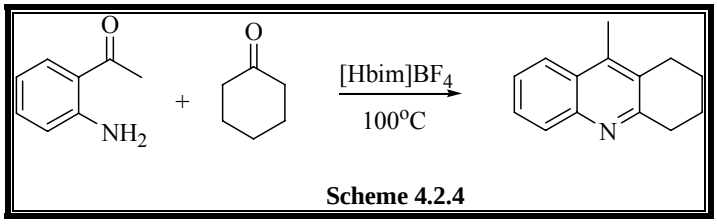
reduced the reaction time drastically from hours to minutes and avoided the use of higher temperatures (200-270 ºC) [33].

Buu-Hoi et al. synthesized 2-chloro-5,6-dihydro-9-methylbenz]acridine-7-carboxylic acid by Pfitzinger's method using 5-methylisatin and 7-Chloro-3,4-dihydro-l(2*H*)-naphthalenone as starting materials [34]. While Cromwell and Bell have reported synthesis of 11-chloro-5,5-dimethyl-5,6-dihydro-benzo[*c*]acridine by Pfitzinger's method by the reaction of 7-chloroisatin with 4,4-dimethyl-3,4-dihydro-2H-naph- thalen-1-one [35] (Scheme 4.2.3). Recent literature survey also revealed few examples of acridine synthesis by Pfitzinger's method [36, 37].



Scheme 4.2.3

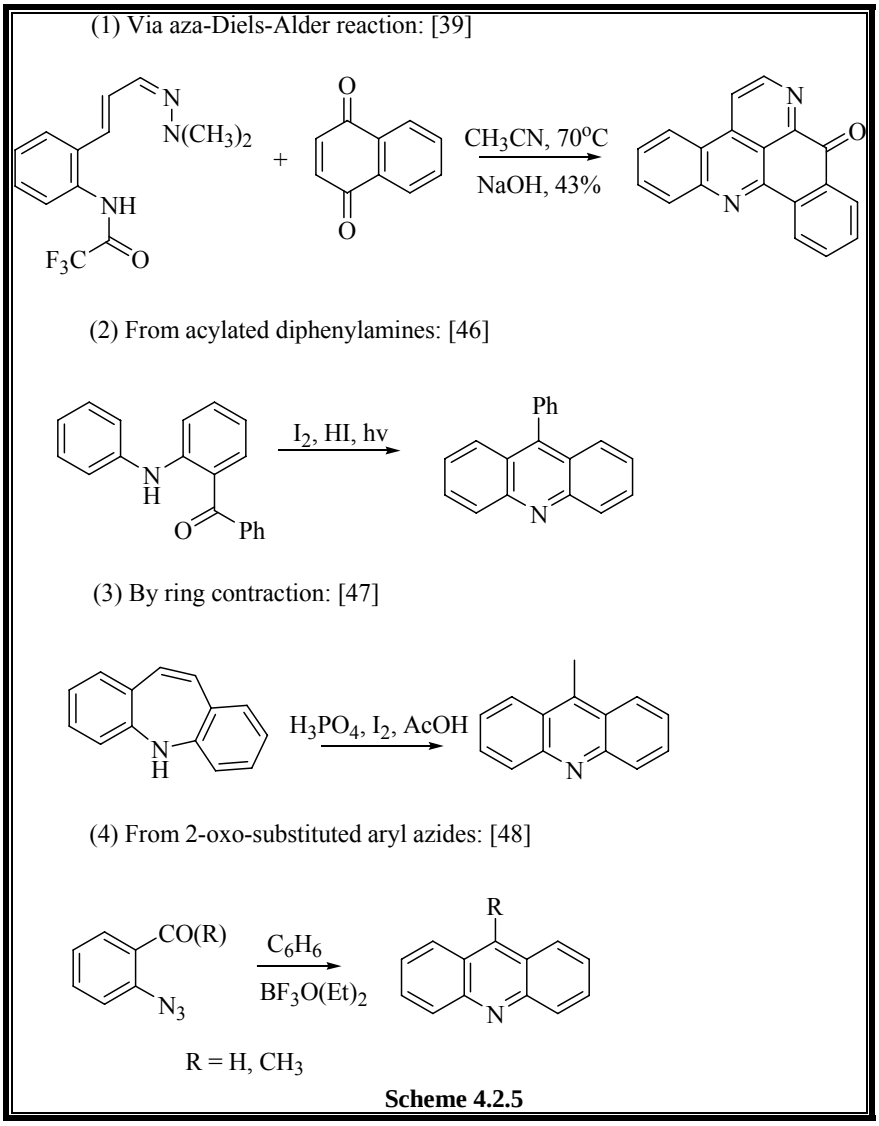
Palimkar et al. have reported ionic liquid 1-n-butylimidazolium tetrafluoroborate ([Hbim]BF_4) promoted Friedlander annulation between 2-aminoacetophenone and cyclic ketones [38] (Scheme 4.2.4). Other researchers have also utilised Friedlander reaction for the synthesis of different fused acridines [39-41].



Scheme 4.2.4

A recent report on synthesis of 2-(2-Arylidenaminothiazol-4-yl)acridines has utilized Goldberg's method sequence [42]. Literature survey also revealed number of examples of acridine synthesis using Ullman reaction at one or other stages of the reaction [43-45].

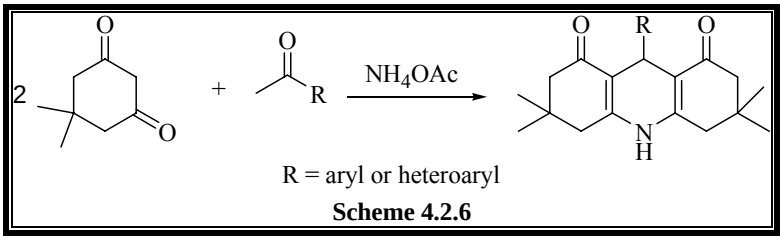
Several other synthetic approaches have been reported by different researchers, few of which [39, 46-48] are summarized in (Scheme 4.2.5).



Scheme 4.2.5

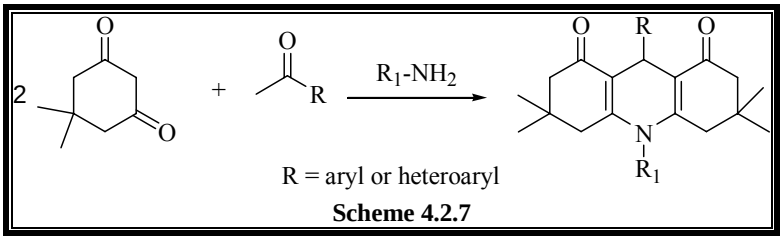
Polyhydroacridines and polyhydroacridine-1,8-diones are polyfunctionalized 1,4-dihydropyridine type derivatives. 1,4-Dihydropyridines (1,4-DHPs) are well-known compounds because of their biological activities [49, 50]. The chemical modifications on the DHP ring, such as different substituents [49] or heteroatoms [51], have allowed the study of the extended structure and activity relationship and also provided some insight into the molecular interactions at the receptor level. In this context, polyhydroacridine-1,8-diones are also increasingly receiving attention due to their likeness in properties with those of 1,4-dihydropyridines. As a consequence, the interest of organic chemists in the synthesis or structure modifications of acridinedione derivatives remains high. Several reports describing the synthesis of

polyhydroacridines have been published recently. The synthesis of polyhydroacridine-1,8-diones by the reaction of 5,5-dimethylcyclohexane-1,3-dione (dimedone) with different aldehydes and ammonium acetate (Scheme 4.2.6) by heating in conventional solvents [52], by solvent-free conventional heating [53], by microwave-assisted synthesis [54], by reactions in aqueous media using ammonium chloride, or $Zn(OAc)_2 \cdot 2H_2O$ or *L*-proline as catalysts [55], and by using ionic liquids [56] have been reported.



Scheme 4.2.6

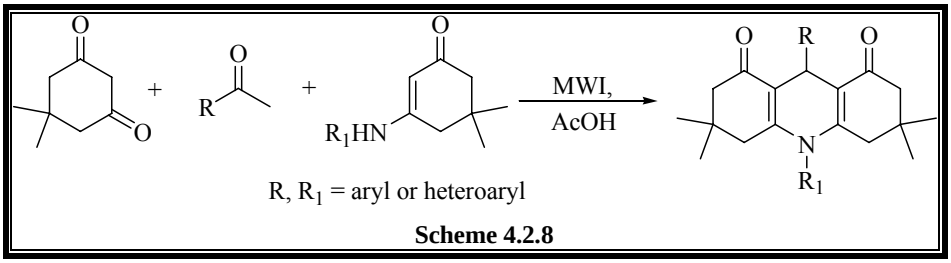
The synthesis of *N*-aryl-polyhydroacridine-1,8-diones by the reaction of 5,5-dimethylcyclohexane-1,3-dione (dimedone) with different aldehydes and amines (Scheme 4.2.7) using microwave-assisted synthesis [57, 58], by reactions in aqueous media using p-dodecylbenezenesulfonic acid [59], sodium dodecyl sulfate (SDS) [60] or proline [61] as catalysts, and by using ionic liquids [62, 63] have been reported.



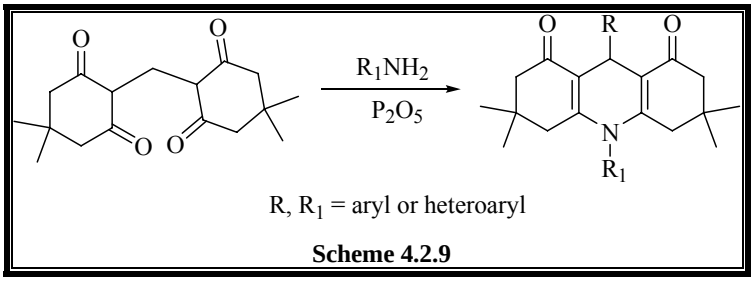
Scheme 4.2.7

Reactions of enaminoketones with aldehydes in 20% P_2O_5 solution in isopropyl alocohol also furnish *N*-aryl-polyhydroacridine-1,8-diones [64, 65]. Reactions of enaminones with aldehydes and dimedone also furnished the same products under microwave irradiation using solvent acetic acid [66] (Scheme 4.2.8). Reaction of methylene-bis(5,5-dimethylcyclohexane-1,3-dione) with amines also affords *N*-substituted-polyhydroacridine-1,8-diones [67, 68] (Scheme 4.2.9).

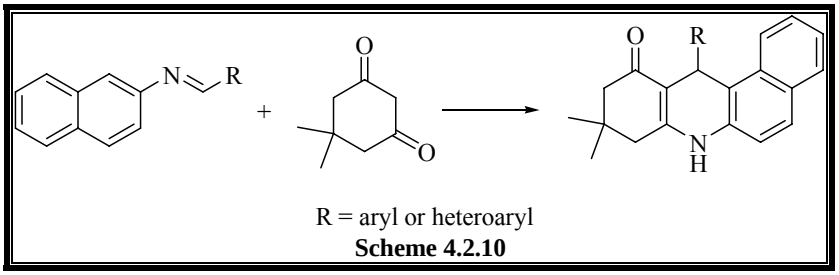
Synthesis of polyhydroacridine-1,8-diones from Schiff base derivatives of amines with dimedone is also well documented [69-71] (Scheme 4.2.10).



Scheme 4.2.8



Scheme 4.2.9



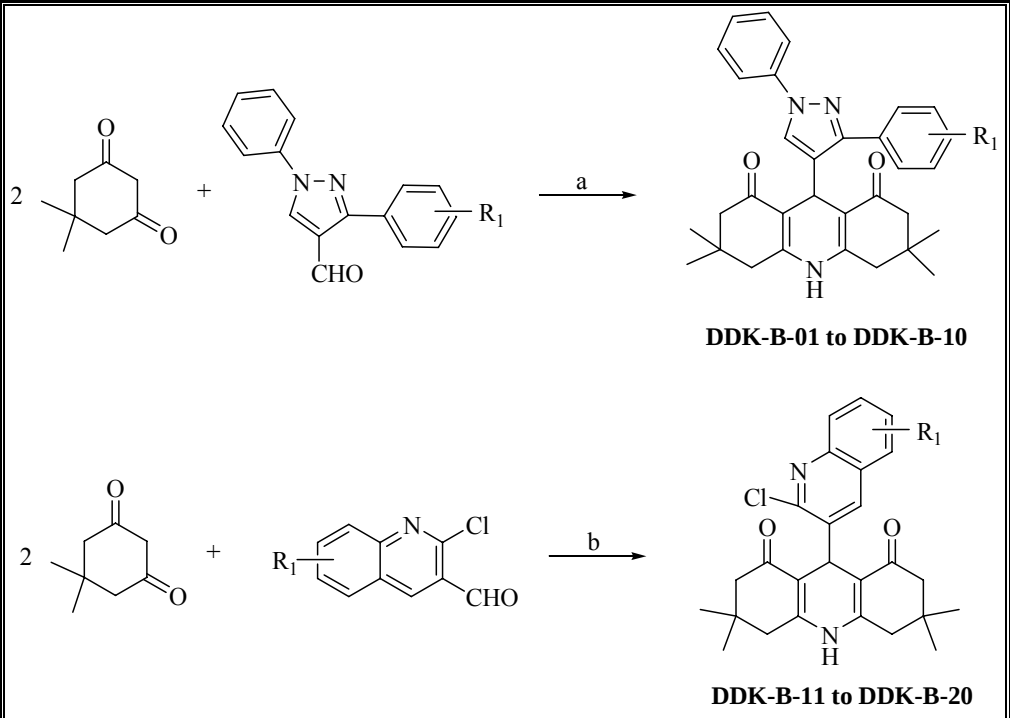
Scheme 4.2.10

3.3 Current Work

The chemistry of acridine and its derivatives has been studied for over a century due to their diverse biological activities *viz*, antitubercular, antimalarial, antitumour, vasorelaxing, antiviral etc. Polyhydroacridine-1,8-diones are polyfunctionalized 1,4-dihydropyridine type derivatives. 1,4-Dihydropyridines (1,4-DHPs) are well-known compounds because of their biological activities [49-51]. The chemical modifications on the DHP ring, such as different substituents [49] or heteroatoms [51], have allowed the study of the extended structure and activity relationship and also provided some insight into the molecular interactions at the receptor level. In this context, polyhydroacridine-1,8-diones are also increasingly receiving attention due to their likeness in properties with those of 1,4-dihydropyridines.

In view of these observations and with a view to further assess the pharmacological profile of this class of compounds; two novel series of acridines (**DDK-B-01 to DDK-B-20**) are synthesized. The synthesis of pyridine-3-carbonitriles (**DDK-B-01 to DDK-B-10**) and (**DDK-B-11 to DDK-B-20**) was achieved by microwave-assisted one pot reaction of two moles of 5,5-dimethylcyclohexane-1,3-dione (dimedone) with one mole of substituted 2-chloro-quinoline-3-carbaldehyde and 3-(aryl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde respectively in presence of excess of ammonium acetate. The products were characterized by FT-IR, mass, ^{1}H NMR spectroscopy and elemental analyses. The newly synthesized compounds were subjected to antimicrobial activity.

4.4 Reaction scheme

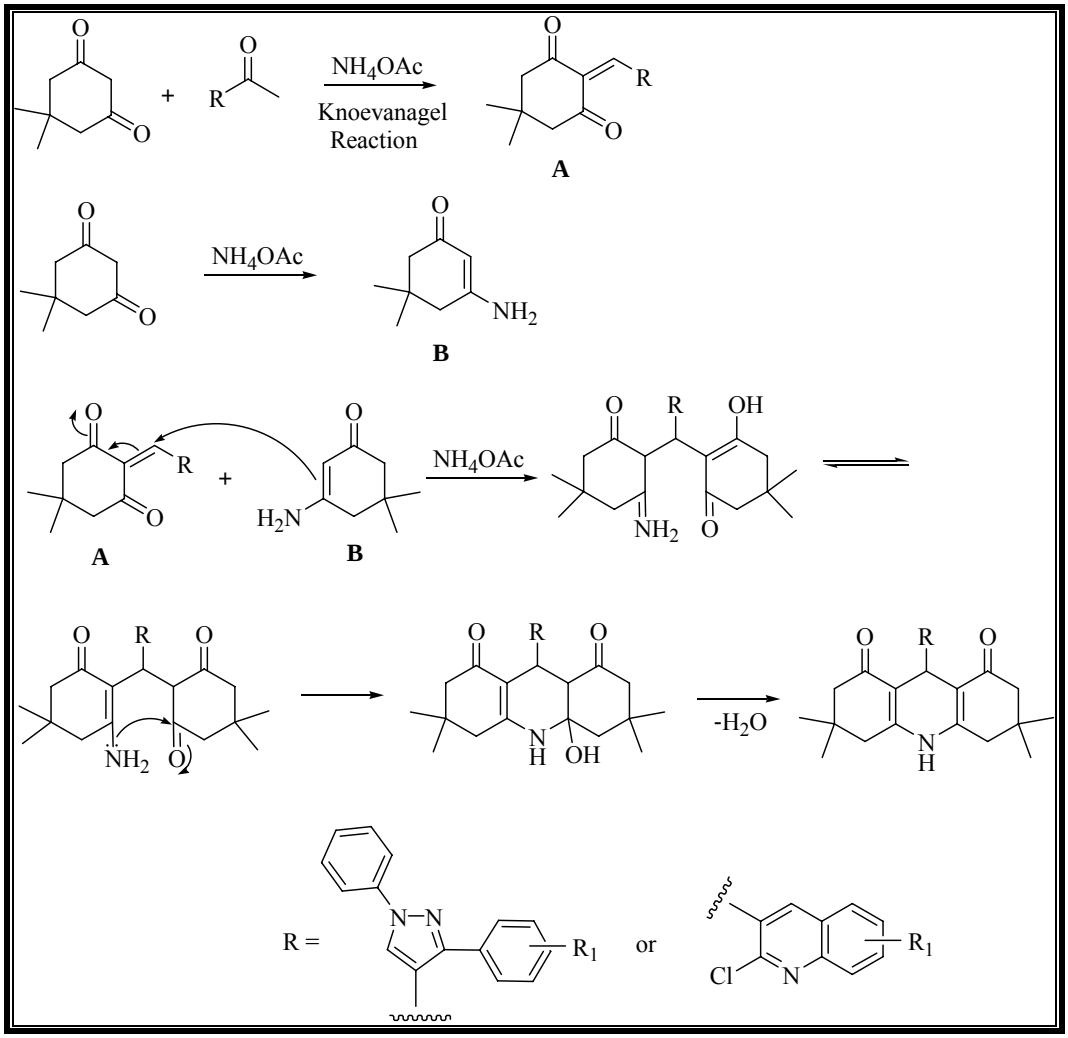


Reagents and conditions: (a) NH_4OAc, MWI, 4-5 mins. ; (b) NH_4OAc, MWI, 2-3 mins.

Code	R_1	M.F.	M.W.	M.P. ºC	Yield %	R_{f1}	R_{f2}
DDK-B-01	H	$C_{32}H_{33}N_3O_2$	491	203-205	75	0.45	0.64
DDK-B-02	4-Cl	$C_{32}H_{32}ClN_3O_2$	526	196-198	81	0.48	0.68
DDK-B-03	3-NO_2	$C_{32}H_{32}ClN_4O_4$	536	216-218	78	0.51	0.69
DDK-B-04	4-NO_2	$C_{32}H_{32}ClN_4O_4$	536	233-235	77	0.50	0.65
DDK-B-05	2-OCH_3	$C_{33}H_{35}N_3O_3$	521	191-193	70	0.47	0.67
DDK-B-06	4-OCH_3	$C_{33}H_{35}N_3O_3$	521	218-220	83	0.54	0.71
DDK-B-07	2-OH	$C_{32}H_{33}N_3O_3$	507	179-181	68	0.53	0.72
DDK-B-08	4-OH	$C_{32}H_{33}N_3O_3$	507	193-195	69	0.51	0.67
DDK-B-09	4-F	$C_{32}H_{32}FN_3O_2$	509	254-256	82	0.54	0.71
DDK-B-10	4-Br	$C_{32}H_{32}BrN_3O_2$	570	244-246	79	0.52	0.69
DDK-B-11	4-F	$C_{26}H_{26}ClFN_2O_2$	452	209-211	75	0.51	0.63
DDK-B-12	3-Cl	$C_{26}H_{26}Cl_2N_2O_2$	469	237-239	72	0.54	0.67
DDK-B-13	4-Cl	$C_{26}H_{26}Cl_2N_2O_2$	469	243-245	79	0.51	0.56
DDK-B-14	2-OCH_3	$C_{27}H_{29}ClN_2O_3$	464	228-230	69	0.53	0.60
DDK-B-15	3-OCH_3	$C_{27}H_{29}ClN_2O_3$	464	249-251	71	0.59	0.70
DDK-B-16	4-OCH_3	$C_{27}H_{29}ClN_2O_3$	464	238-240	78	0.53	0.59
DDK-B-17	3-CH_3	$C_{27}H_{29}ClN_2O_2$	448	182-184	70	0.51	0.62
DDK-B-18	4-CH_3	$C_{27}H_{29}ClN_2O_2$	448	217-219	76	0.53	0.65
DDK-B-19	4-NO_2	$C_{26}H_{26}ClN_3O_4$	479	226-228	72	0.52	0.60
DDK-B-20	4-Br	$C_{26}H_{26}BrClN_2O_2$	513	247-249	74	0.57	0.67

TLC Solvent system R_{f1}: Hexane: Ethyl acetate – 4:6,
TLC Solvent system R_{f2}: Chloroform:Methanol – 9.0:1.0.

4.5 Mechanism



The probable mechanism involves the synthesis of acridines via formation of two intermediates **A** & **B** as suggested by Da-quing et al. [60] and Venkatesan et al. [61]. It is likely that the first step is Knoevenagel reaction between dimedone and aldehyde resulting in the formation of intermediate **A**. While condensation of another dimedone molecule with ammonium ion results in the formation of enamine intermediate **B**. Then, the Michael addition, cyclization, and dehydration between intermediates **A** and **B** take place respectively and give the product acridine.

4.6 Experimental

4.6.1 Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. Microwave assisted reactions were carried out in QPro-M microwave synthesizer. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a Bruker Avance 400 MHz (for compounds DDK-B-02, DDK-B-06, DDK-B-09, DDK-B-11 and DDK-B-18) and Varian 400 MHz (for compound B-13) spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

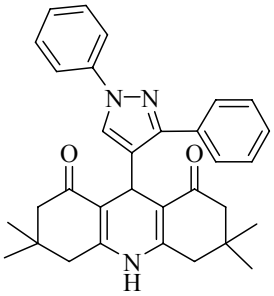
4.6.2 Synthesis of 3-(aryl)-1-phenyl-1H-pyrazole-4-carbaldehydes

Synthesis of substituted 3-(aryl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes was achieved using previously published method [72].

4.6.3 General procedure for the synthesis of 9-(3-(aryl)-1-phenyl-1H-pyrazol-4-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethylacridine-1,8(2H,5H,9H,10H)-diones (DDK-B-01 to DDK-B-10)

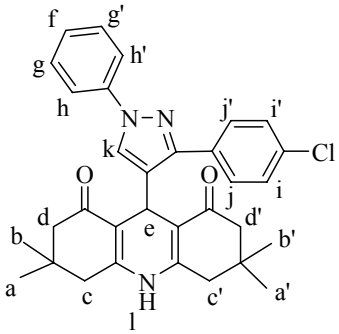
A mixture of the 5,5-dimethylcyclohexane-1,3-dione (dimedone) (0.01 mol), 3-(aryl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (0.005 mol) and ammonium acetate (0.08 mol) was irradiated under microwave irradiation at 120 °C for 4-5 min. The microwave irradiation was operated in 30-second cycles. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the reaction mass was poured into ice-cold water, the product was filtered, washed with water, dried and crystallized from ethanol-DMF (9:1) mixture.

4.6.3.1 9-1,3-diphenyl-1H-pyrazol-4-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl acridine-1,8(2H,5H,9H,10H)-dione (DDK-B-01)



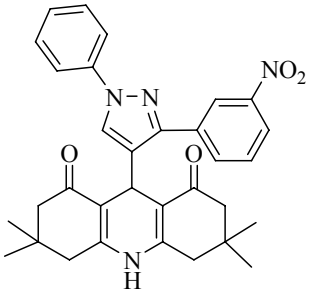
Yield: 73%; m.p. 203-205 ºC; MS: *m/z* 491; Anal. Calcd. for $C_{32}H_{33}N_3O_2$: C, 78.18; H, 6.77; N, 8.55. Found: C, 78.09; H, 6.69; N, 8.48%

4.6.3.2 9-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3,4,6,7-tetrahydro-3,3,6,6 -tetramethylacridine-1,8(2H,5H,9H,10H)-dione (DDK-B-02)



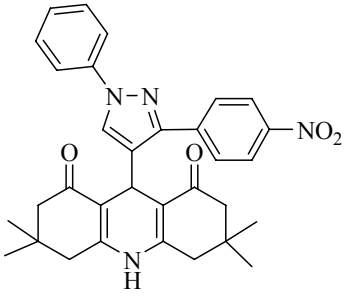
Yield: 77%; m.p. 196-198 ºC; IR (cm^{-1}): 3288 (N-H stretching of secondary amine), 3070 (C-H stretching of aromatic ring), 2956 (C-H asymmetrical stretching of CH_3 group), 2874 (C-H symmetrical stretching of CH_3 group), 1678 (C=O stretching of carbonyl group), 1629 (N-H deformation of -NH group), 1602, 1570 and 1502 (C=C stretching of aromatic ring), 1406 (C-H asymmetrical deformation of CH_3 group), 1359 (C-H symmetrical deformation of CH_3 group), 1003 (C-H in plane bending for aromatic ring), 756 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 0.96 (s, 6H, $H_{a, a'}$), 1.04 (s, 6H, $H_{b, b'}$), 2.02-2.16 (m, 4H, $H_{c, c'}$), 2.29-2.38 (m, 4H, $H_{d, d'}$), 5.01 (s, 1H, H_e), 7.20-7.24 (m, 1H, H_f), 7.39-7.43 (m, 2H, $H_{g, g'}$), 7.64-7.67 (m, 2H, $H_{h, h'}$), 7.13-7.15 (d, 2H, $H_{i, i'}$, J = 8.8 Hz), 8.12-8.14(d, 2H, $H_{j, j'}$, J = 7.88 Hz), 7.83 (s, 1H, H_k), 9.10 (s, 1H, H_l); MS: *m/z* 523; Anal. Calcd. for $C_{32}H_{32}ClN_3O_2$: C, 73.06; H, 6.13; N, 7.99. Found: C, 72.97; H, 6.05; N, 7.90%.

4.6.3.3 3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-9-(3-(3-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)acridine-1,8(2H,5H,9H,10H)-dione (DDK-B-03)



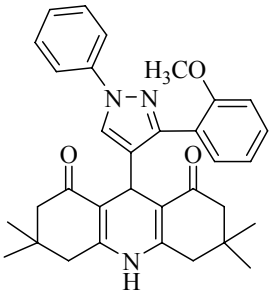
Yield: 71%; m.p. 216-218 ºC; MS: *m/z* 536; Anal. Calcd. for $C_{32}H_{32}N_4O_4$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.54; H, 5.95; N, 10.37%.

4.6.3.4 3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-9-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)acridine-1,8(2H,5H,9H,10H)-dione (DDK-B-04)



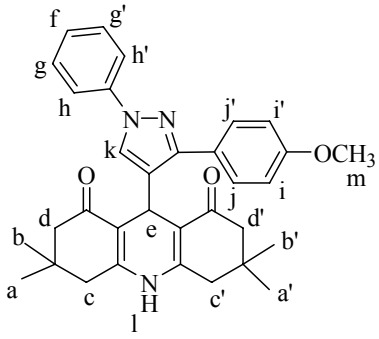
Yield: 77%; m.p. 233-235 ºC; MS: *m/z* 536; Anal. Calcd. for $C_{32}H_{32}N_4O_4$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.55; H, 5.94; N, 10.36%.

4.6.3.5 3,4,6,7-tetrahydro-9-(3-(2-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-3,3,6,6-tetramethylacridine-1,8(2H,5H,9H,10H)-dione (DDK-B-05)



Yield: 70%; m.p. 191-193 ºC; MS: *m/z* 521; Anal. Calcd. for $C_{33}H_{35}N_3O_3$: C, 75.98; H, 6.76; N, 8.06. Found: C, 75.91; H, 6.69; N, 7.97%.

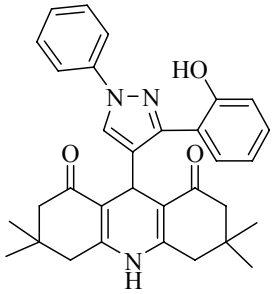
4.6.3.6 3,4,6,7-tetrahydro-9-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-3,3,6,6-tetramethylacridine-1,8(2H,5H,9H,10H)-dione (DDK-B-06)



Yield: 83%; m.p. 218-220 ºC; IR (cm^{-1}): 3265 (N-H stretching of secondary amine), 3067 (C-H stretching of aromatic ring), 2958 (C-H asymmetrical stretching of CH_3 group), 2877 (C-H symmetrical stretching of CH_3 group), 1680 (C=O stretching of carbonyl group), 1639 (N-H deformation of -NH group), 1597 and 1487 (C=C stretching of aromatic ring),

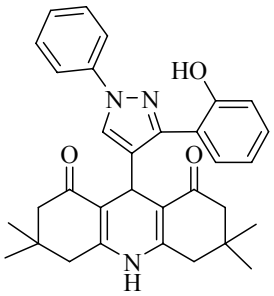
1487 (C-H asymmetrical deformation of CH_3 group), 1367 (C-H symmetrical deformation of CH_3 group), 1222 (C-O-C asymmetrical stretching of OCH_3 group), 1062 (C-O-C symmetrical stretching of OCH_3 group), 995 (C-H in plane bending for aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 0.94 (s, 6H, $H_{a, a'}$), 1.00 (s, 6H, $H_{b, b'}$), 2.01-2.17 (m, 4H, $H_{c, c'}$), 2.29-2.38 (m, 4H, $H_{d, d'}$), 5.01 (s, 1H, H_e), 7.37-7.40 (m, 1H, H_f), 7.50-7.54 (m, 2H, $H_{g, g'}$), 7.87-7.93 (m, 2H, $H_{h, h'}$), 7.00-7.02 (d, 2H, $H_{i, i'}$, J = 8.92 Hz), 7.93-7.95 (d, 2H, $H_{j, j'}$, J = 8.8 Hz), 8.13 (s, 1H, H_k), 9.11 (s, 1H, H_l), 3.85 (s, 3H, H_m); MS: *m/z* 521; Anal. Calcd. for $C_{33}H_{35}N_3O_3$: C, 75.98; H, 6.76; N, 8.06. Found: C, 75.90; H, 6.70; N, 7.97%.

4.6.3.7 3,4,6,7-tetrahydro-9-(3-(2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-3,3,6,6-tetramethylacridine-1,8(2H,5H,9H,10H)-dione (DDK-B-07)



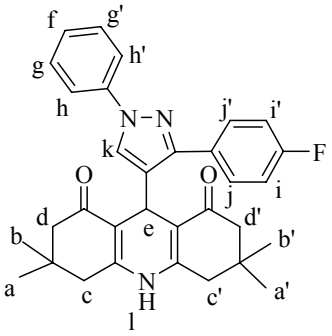
Yield: 68%; m.p. 179-181 ºC; MS: *m/z* 507; Anal. Calcd. for $C_{32}H_{33}N_3O_3$: C, 75.71; H, 6.55; N, 8.28. Found: C, 75.63; H, 6.49; N, 8.22%.

4.6.3.8 3,4,6,7-tetrahydro-9-(3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-3,3,6,6-tetramethylacridine-1,8(2H,5H,9H,10H)-dione (DDK-B-08)



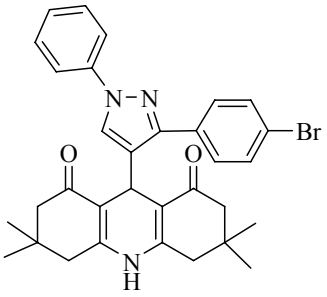
Yield: 69%; m.p. 193-195 ºC; MS: *m/z* 507; Anal. Calcd. for $C_{32}H_{33}N_3O_3$: C, 75.71; H, 6.55; N, 8.28. Found: C, 75.64; H, 6.49; N, 8.20%.

4.6.3.9 9-(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3,4,6,7-tetrahydro-



3,3,6,6-tetramethylacridine-1,8(2H,5H,9H,10H)-dione (DDK-B-09) Yield: 82%; m.p. 254-256 ºC; IR (cm^{-1}): 3282 (N-H stretching of secondary amine), 3074 (C-H stretching of aromatic ring), 2955 (C-H asymmetrical stretching of CH_3 group), 2875 (C-H symmetrical stretching of CH_3 group), 1629 (C=O stretching of carbonyl group), 1616 (N-H deformation of -NH group), 1599, 1491 and 1454 (C=C stretching of aromatic ring), 1454 (C-H asymmetrical deformation of CH_3 group), 1359 (C-H symmetrical deformation of CH_3 group), 1066 (C-H in plane bending for aromatic ring), 1018 (C-F stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 0.96 (s, 6H, $H_{a, a'}$), 1.04 (s, 6H, $H_{b, b'}$), 2.02-2.17 (m, 4H, $H_{c, c'}$), 2.29-2.38 (m, 4H, $H_{d, d'}$), 5.02 (s, 1H, H_e), 7.39-7.43 (m, 2H, $H_{g, g'}$), 7.63-8.66 (m, 2H, $H_{h, h'}$), 7.11-7.15 (t, 2H, $H_{i, i'}$), 8.10-7.11 (t, 2H, $H_{j, j'}$), 7.92 (s, 1H, H_k), 9.07 (s, 1H, H_l); MS: *m/z* 509; Anal. Calcd. for $C_{32}H_{32}FN_3O_2$: C, 75.42; H, 6.33; N, 8.25. Found: C, 75.34; H, 6.27; N, 8.19%.

4.6.3.10 9-(3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-3,4,6,7-tetrahydro-



3,3,6,6-tetramethylacridine-1,8(2H,5H,9H,10H)-dione (DDK-B-10)Yield: 79%; m.p. 244-246 ºC; MS: *m/z* 570; Anal. Calcd. for $C_{32}H_{32}BrN_3O_2$: C, 67.37; H, 5.65; N, 7.37, Found: C, 67.28; H, 5.57; N, 7.28%.

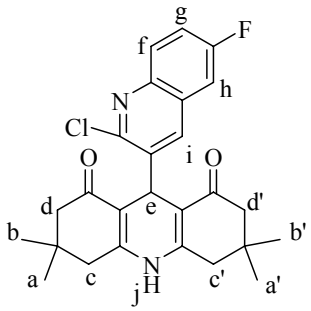
4.6.4 Synthesis of substituted 2-chloro-quinoline-3-carbaldehydes

Synthesis of substituted 2-chloro-quinoline-3-carbaldehydes was achieved using previously published methods [73].

4.6.5 General procedure for the synthesis of 9-(2-chloro-6-substituted-quinolin-3-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethylacridine-1,8(2H,5H,9H,10H)-dione (DDK-B-11 to DDK-B-20)

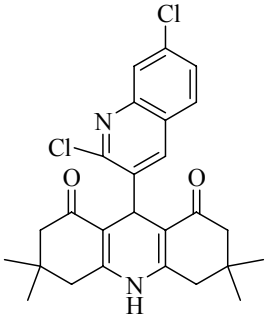
A mixture of the 5,5-dimethylcyclohexane-1,3-dione (dimedone) (0.01 mol), substituted 2-chloro-quinoline-3-carbaldehyde (0.005 mol) and ammonium acetate (0.08 mol) was irradiated under microwave irradiation at 120 °C for 2-3 min. The microwave irradiation was operated in 30-second cycles. The progress of the reaction was monitored by TLC. Upon completion of the reaction the reaction mass was poured into ice-cold water, the product was filtered, washed with water, dried and crystallized from ethanol-DMF (9:1) mixture.

4.6.5.1 9-(2-chloro-6-fluoroquinolin-3-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl acridine-1,8(2H,5H,9H,10H)-dione (DDK-B-11)

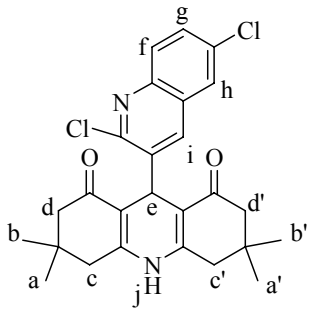


Yield: 75%; m.p. 209-211 ºC; IR (cm^{-1}): 3282 (N-H stretching of secondary amine), 3074 (C-H stretching of aromatic ring), 2955 (C-H asymmetrical stretching of CH_3 group), 2875 (C-H symmetrical stretching of CH_3 group), 1629 (C=O stretching of carbonyl group), 1599, 1491 and 1454 (C=C stretching of aromatic ring), 1454 (C-H asymmetrical deformation of CH_3 group), 1359 (C-H symmetrical deformation of CH_3 group), 1018 (C-F stretching), 958 (C-H in plane bending for aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 0.98 (s, 6H, $H_{a, a'}$), 1.08 (s, 6H, $H_{b, b'}$), 2.07-2.24 (m, 4H, $H_{c, c'}$), 2.41-2.51 (m, 4H, $H_{d, d'}$), 4.62 (s, 1H, H_e), 7.74-7.77 (m, 1H, H_f), 7.11-7.16 (m, 1H, H_g), 7.30-7.33 (m, 1H, H_h), 8.00 (s, 1H, H_i), 11.43 (s, 1H, H_j); MS: *m/z* 452; Anal. Calcd. for $C_{26}H_{26}ClFN_2O_2$: C, 68.94; H, 5.79; N, 6.18. Found: C, 68.86; H, 5.72; N, 6.11%

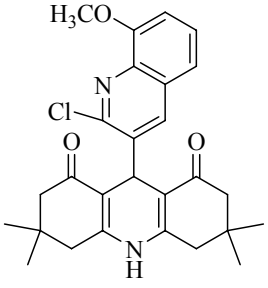
4.6.5.2 9-(2,7-dichloroquinolin-3-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethylacridine-1,8(2H,5H,9H,10H)-dione (DDK-B-12) Yield: 72%; m.p. 237-239 ºC; MS: *m/z* 469; Anal. Calcd. for $C_{26}H_{26}Cl_2N_2O_2$: C, 66.53; H, 5.58; N, 5.97. Found: C, 66.46; H, 5.50; N, 5.88%



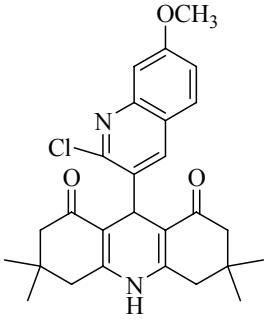
4.6.5.3 9-(2,6-dichloroquinolin-3-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethylacridine-1,8(2H,5H,9H,10H)-dione (DDK-B-13) Yield: 79%; m.p. 243-245 ºC; ; IR (cm^{-1}): 3290 (N-H stretching of secondary amine), 3079 (C-H stretching of aromatic ring), 2958 (C-H asymmetrical stretching of CH_3 group), 2872 (C-H symmetrical stretching of CH_3 group), 1653 (C=O stretching of carbonyl group), 1614 (N-H deformation of -NH group), 1577 and 1450 (C=C stretching of aromatic ring), 1425 (C-H asymmetrical deformation of CH_3 group), 1363 (C-H symmetrical deformation of CH_3 group), 1004 (C-H in plane bending for aromatic ring), 735 (C-Cl stretching)); ^{1}H NMR (DMSO-d_6) δ ppm: 0.87 (s, 6H, $H_{a, a'}$), 0.99 (s, 6H, $H_{b, b'}$), 1.98-2.53 (m, 8H, $H_{c, c', d, d'}$), 4.49 (s, 1H, H_e), 7.79-7.83 (d, 2H, $H_{f, i}$), 7.40-7.42 (m, 1H, H_g), 7.17-7.19 (d, 1H, H_h), 11.59 (s, 1H, H_j); MS: *m/z* 469; Anal. Calcd. for $C_{26}H_{26}Cl_2N_2O_2$: C, 66.53; H, 5.58; N, 5.97. Found: C, 66.47; H, 5.49; N, 5.89%



4.6.5.4 9-(2-chloro-8-methoxyquinolin-3-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl acridine-1,8(2H,5H,9H,10H)-dione (DDK-B-14) Yield: 69%; m.p. 228-230 ºC; MS: *m/z* 464; Anal. Calcd. for $C_{27}H_{29}ClN_2O_3$: C, 69.74; H, 6.29; N, 6.02. Found: C, 69.67; H, 6.21; N, 5.95%

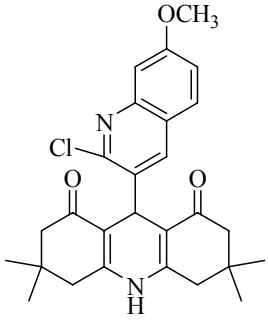


4.6.5.5 9-(2-chloro-7-methoxyquinolin-3-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl acridine-1,8(2H,5H,9H,10H)-dione (DDK-B-15)



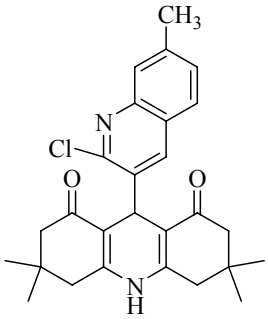
Yield: 71%; m.p. 249-251 ºC; MS: *m/z* 464; Anal. Calcd. for $C_{27}H_{29}ClN_2O_3$: C, 69.74; H, 6.29; N, 6.02. Found: C, 69.66; H, 6.21; N, 5.96%

4.6.5.6 9-(2-chloro-6-methoxyquinolin-3-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl acridine-1,8(2H,5H,9H,10H)-dione (DDK-B-16)



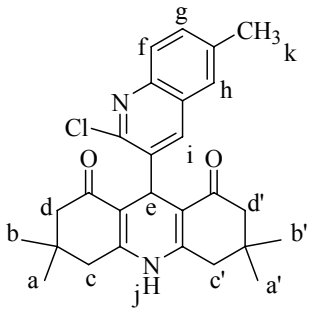
Yield: 78%; m.p. 238-240 ºC; MS: *m/z* 464; Anal. Calcd. for $C_{27}H_{29}ClN_2O_3$: C, 69.74; H, 6.29; N, 6.02. Found: C, 69.67; H, 6.20; N, 5.94%

4.6.5.7 9-(2-chloro-7-methylquinolin-3-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl acridine-1,8(2H,5H,9H,10H)-dione (DDK-B-17)



Yield: 70%; m.p. 182-184 ºC; MS: *m/z* 448; Anal. Calcd. for $C_{27}H_{29}ClN_2O_2$: C, 72.23; H, 6.51; N, 6.24. Found: C, 72.16; H, 6.43; N, 6.17%

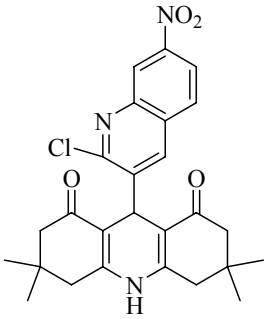
4.6.5.8 9-(2-chloro-6-methylquinolin-3-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl acridine-1,8(2H,5H,9H,10H)-dione (DDK-B-18)



Yield: 76%; m.p. 217-219 ºC; ; IR (cm^{-1}): 3252 (N-H stretching of secondary amine), 3045 (C-H stretching of aromatic ring), 2955 (C-H asymmetrical stretching of CH_3 group), 2879 (C-H symmetrical stretching of CH_3 group), 1660 (C=O stretching of carbonyl group), 1566, 1500 and 1467 (C=C stretching of aromatic ring), 1427 (C-H asymmetrical deformation of CH_3

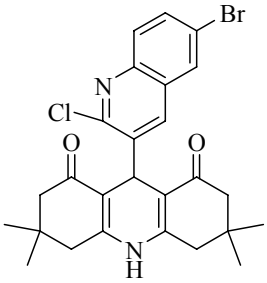
group), 1365 (C-H symmetrical deformation of CH_3 group), 1066 (C-H in plane bending for aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 0.93 (s, 6H, $H_{a, a'}$), 1.03 (s, 6H, $H_{b, b'}$), 2.01-2.19 (m, 4H, $H_{c, c'}$), 2.36-2.50 (m, 4H, $H_{d, d'}$), 4.55 (s, 1H, H_e), 7.90-7.91 (d, 1H, H_f), 7.09-7.15 (m, 2H, $H_{g, h}$), 8.13 (s, 1H, H_i), 11.25 (s, 1H, H_j), 2.31 (s, 3H, H_k); MS: *m/z* 448; Anal. Calcd. for $C_{27}H_{29}ClN_2O_2$: C, 72.23; H, 6.51; N, 6.24. Found: C, 72.15; H, 6.44; N, 6.15%

4.6.5.9 9-(2-chloro-7-nitroquinolin-3-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl acridine-1,8(2H,5H,9H,10H)-dione (DDK-B-19)



Yield: 72%; m.p. 226-228 ºC; MS: *m/z* 479; Anal. Calcd. for $C_{26}H_{26}ClN_3O_4$: C, 65.06; H, 5.46; N, 8.76. Found: C, 64.97; H, 5.39; N, 8.70%

4.6.5.10 9-(6-bromo-2-chloroquinolin-3-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethyl acridine-1,8(2H,5H,9H,10H)-dione (DDK-B-20)



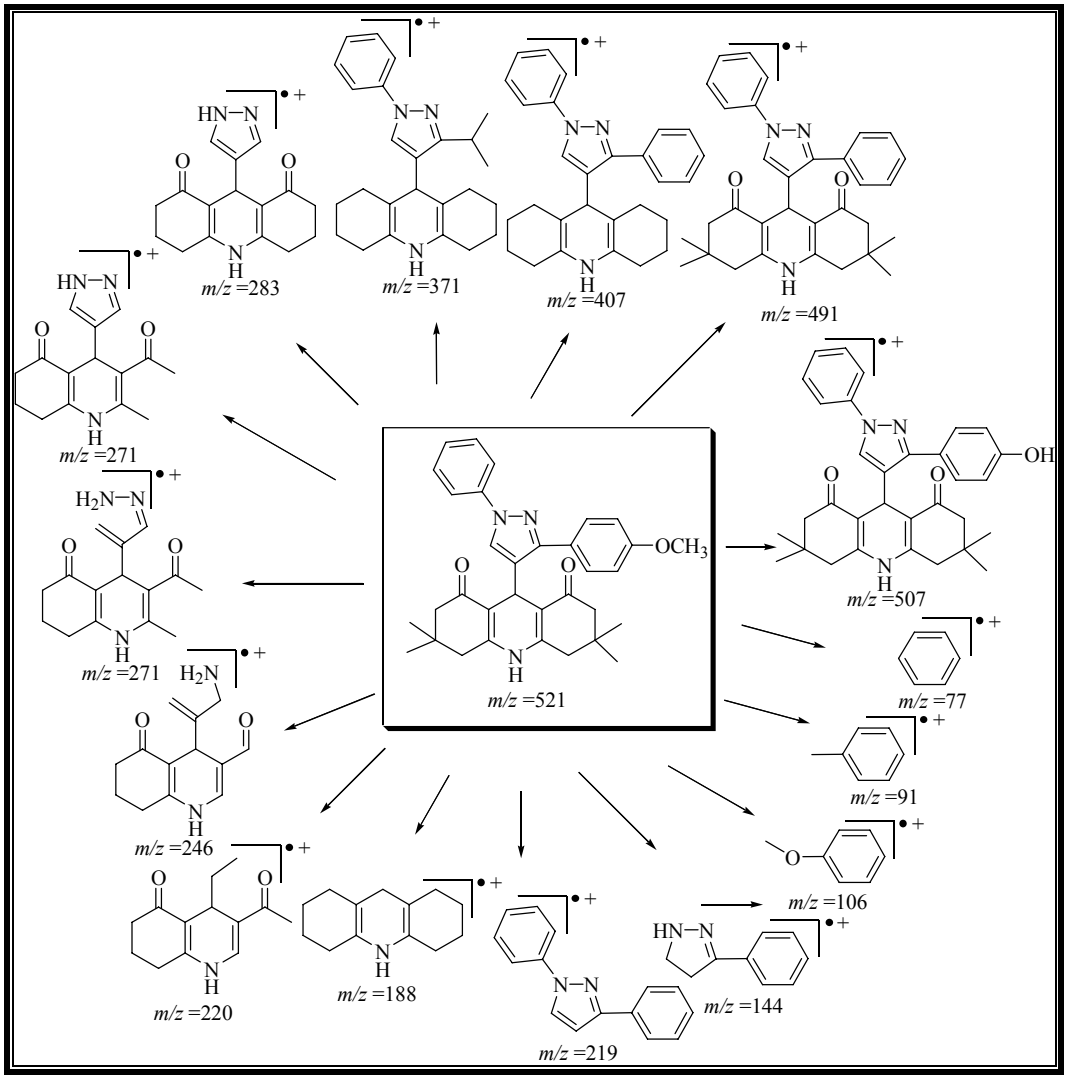
Yield: 74%; m.p. 247-249 ºC; MS: *m/z* 513; Anal. Calcd. for $C_{26}H_{26}BrClN_2O_2$: C, 60.77; H, 5.10; N, 5.45. Found: C, 60.69; H, 5.03; N, 5.38%

4.7 Spectral discussion

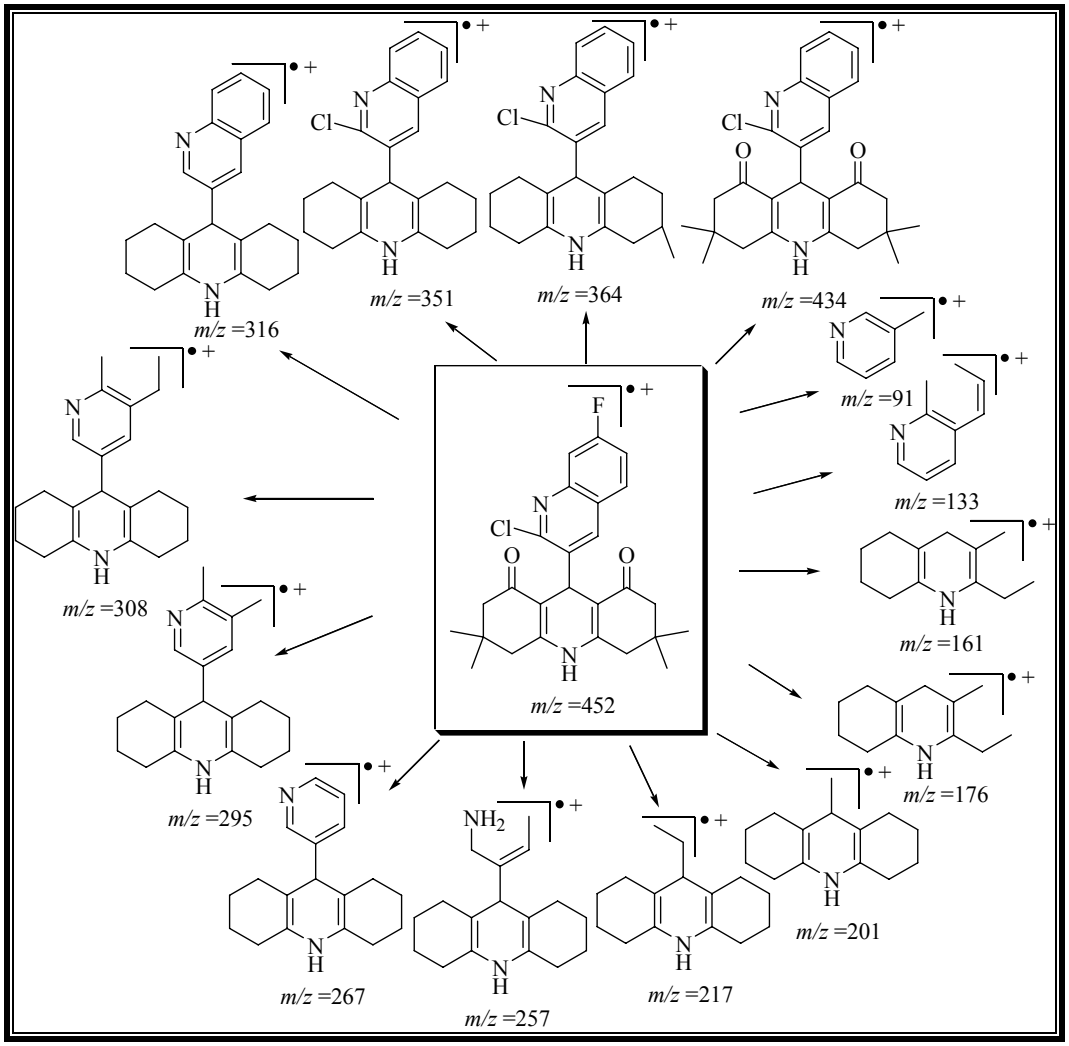
4.7.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of respective compound. Mass fragmentation pattern for a representative compound of each series is depicted below.

4.7.1.1 Mass fragmentation pattern for DDK-B-06



4.7.1.2 Mass fragmentation pattern for DDK-B-11



4.7.2 IR spectral study

IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For acridines (**DDK-B-01 to DDK-B-20**), a characteristic band of carbonyl group was observed in the range of 1629-1680 cm^{-1}. Another characteristic C-H asymmetrical and symmetrical stretching bands of methyl groups were observed at 2955-2958 cm^{-1} and 2835-2877 cm^{-1} respectively. Also, N-H stretching band of secondary amine was observed at 3252-3282 cm^{-1} suggesting formation of desired products (**DDK-B-01 to DDK-B-20**).

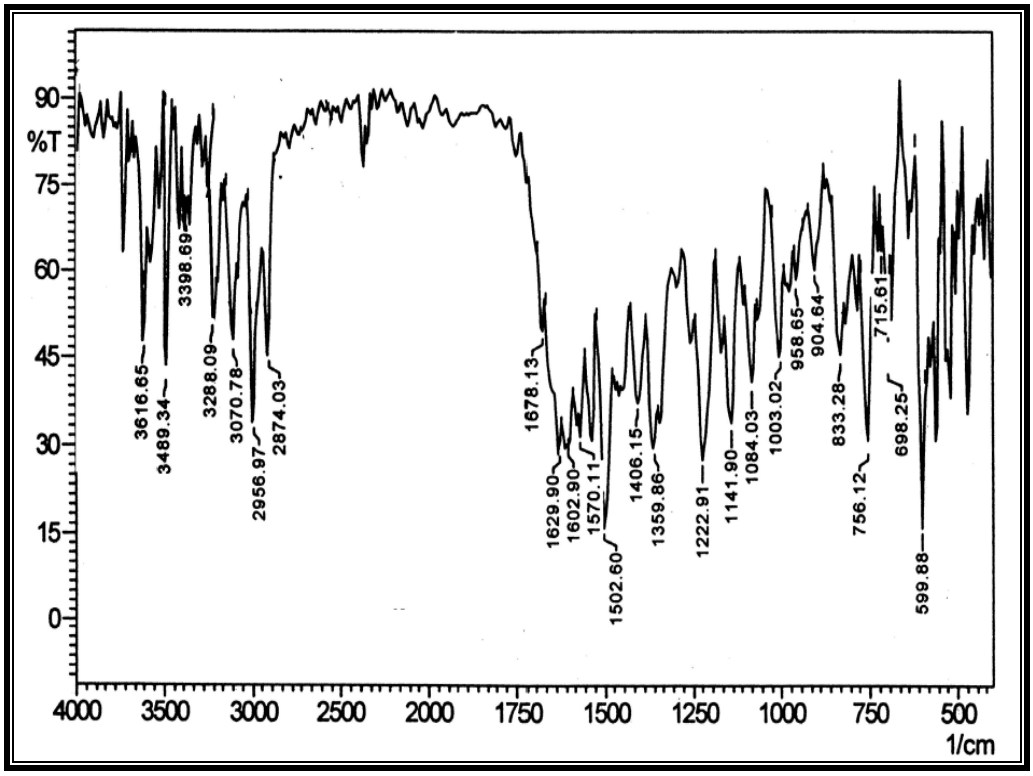
4.7.3 ^{1}H NMR spectral study

^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

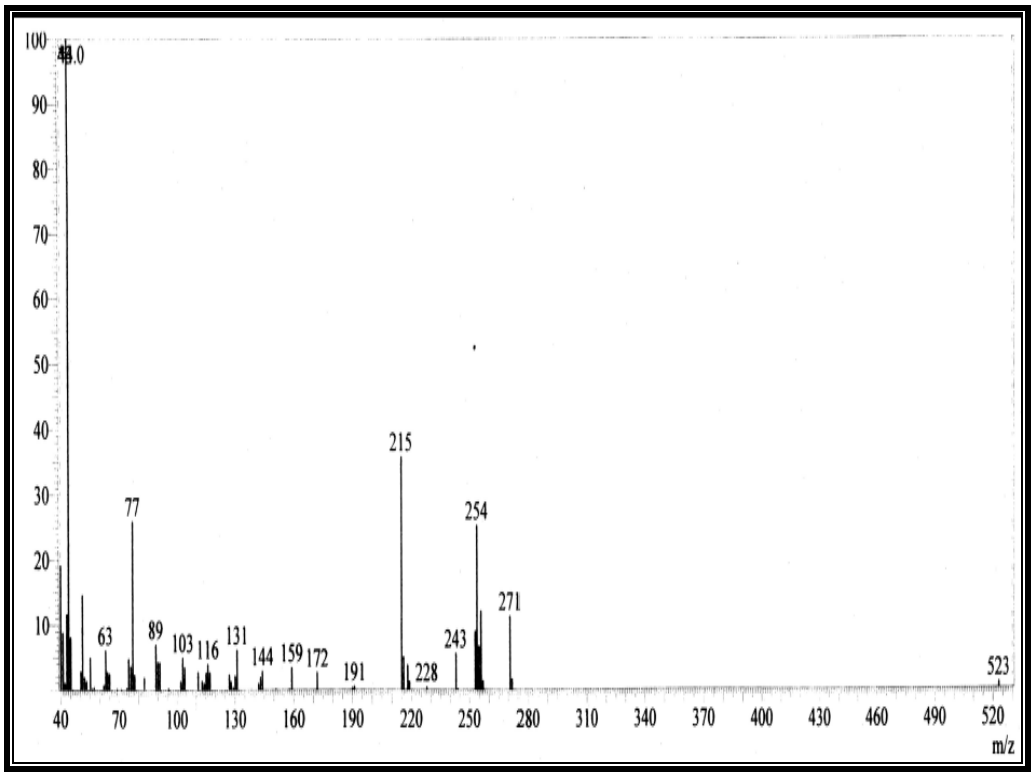
For acridines (**DDK-B-01 to DDK-B-10**), characteristic singlets were observed for methyl and methylene groups at 0.94-1.04 δ ppm and 2.01-2.38 δ ppm respectively. Confirmatory signal of methine proton was observed at 5.01-5.02 δ ppm. The aromatic ring protons were observed at 7.00-8.15 δ ppm and *J* value were found to be in accordance with substitution pattern on phenyl ring. The singlet for secondary amine (-NH) proton was observed at 9.07-9.11 δ ppm.

While, for acridines (**DDK-B-11 to DDK-B-20**), characteristic singlets were observed for methyl and methylene groups at 0.87-1.08 δ ppm and 1.98-2.53 δ ppm respectively. Confirmatory signal of methine proton was observed at 4.49-4.62 δ ppm. The aromatic ring protons were observed at 7.09-8.13 δ ppm and *J* value were found to be in accordance with substitution pattern on phenyl ring. The singlet for secondary amine (-NH) proton was observed at 11.25-11.59 δ ppm.

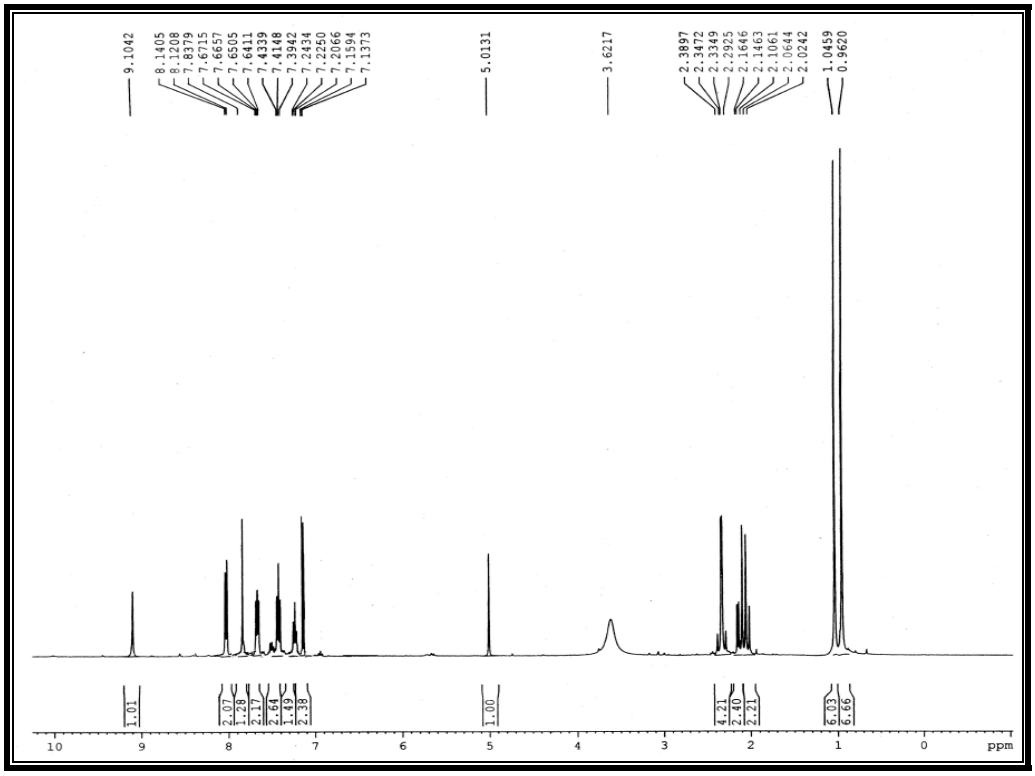
IR spectrum of DDK-B-02



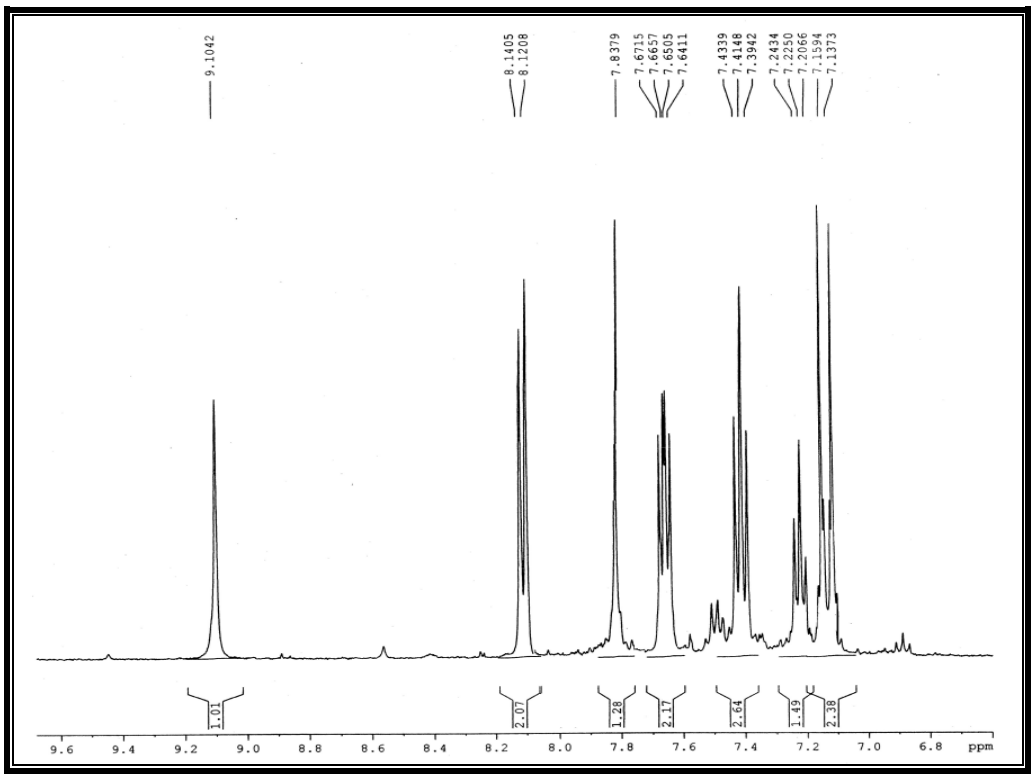
Mass spectrum of DDK-B-02



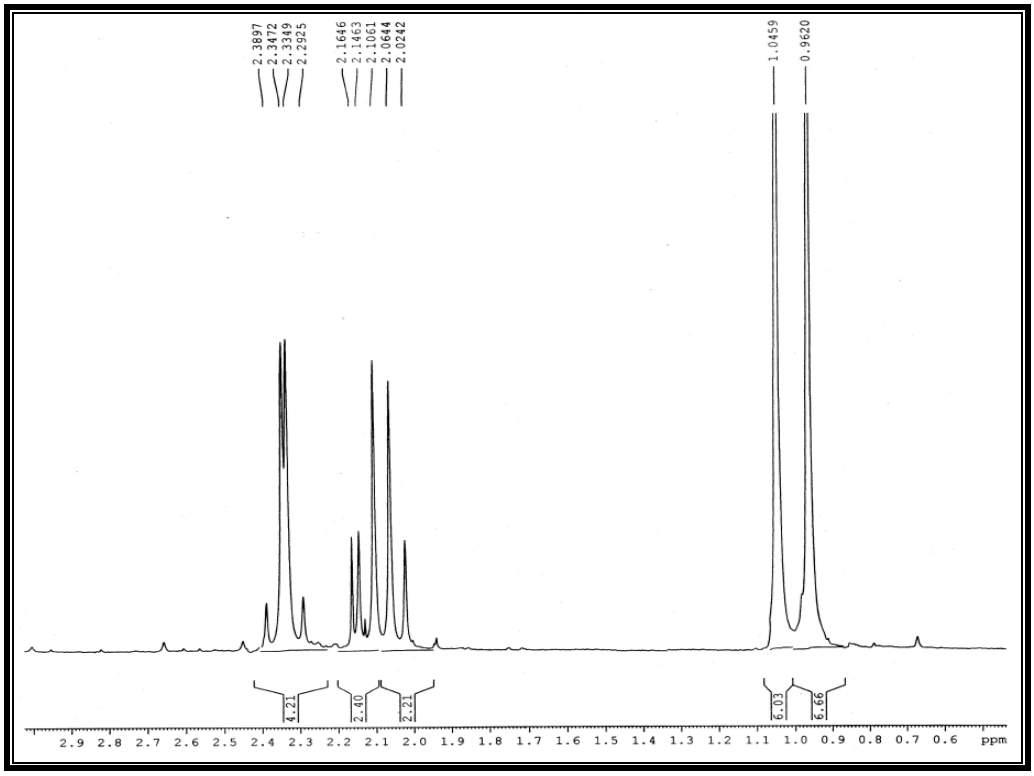
^{1}H NMR spectrum of DDK-B-02



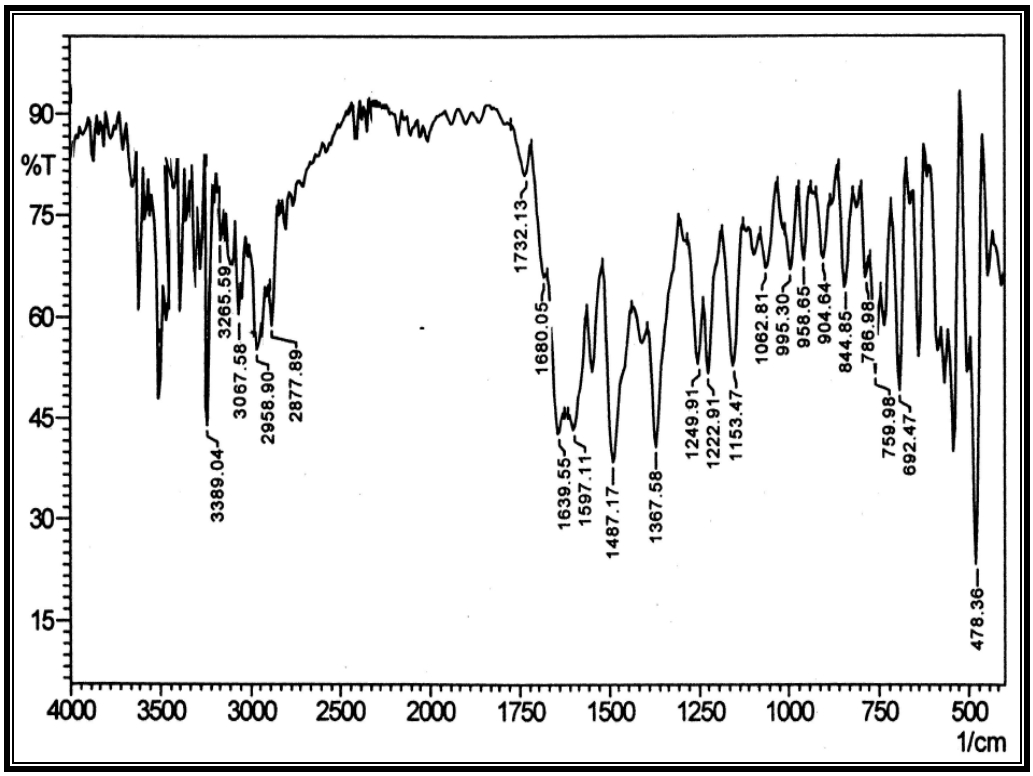
Expanded ^{1}H NMR spectrum of DDK-B-02



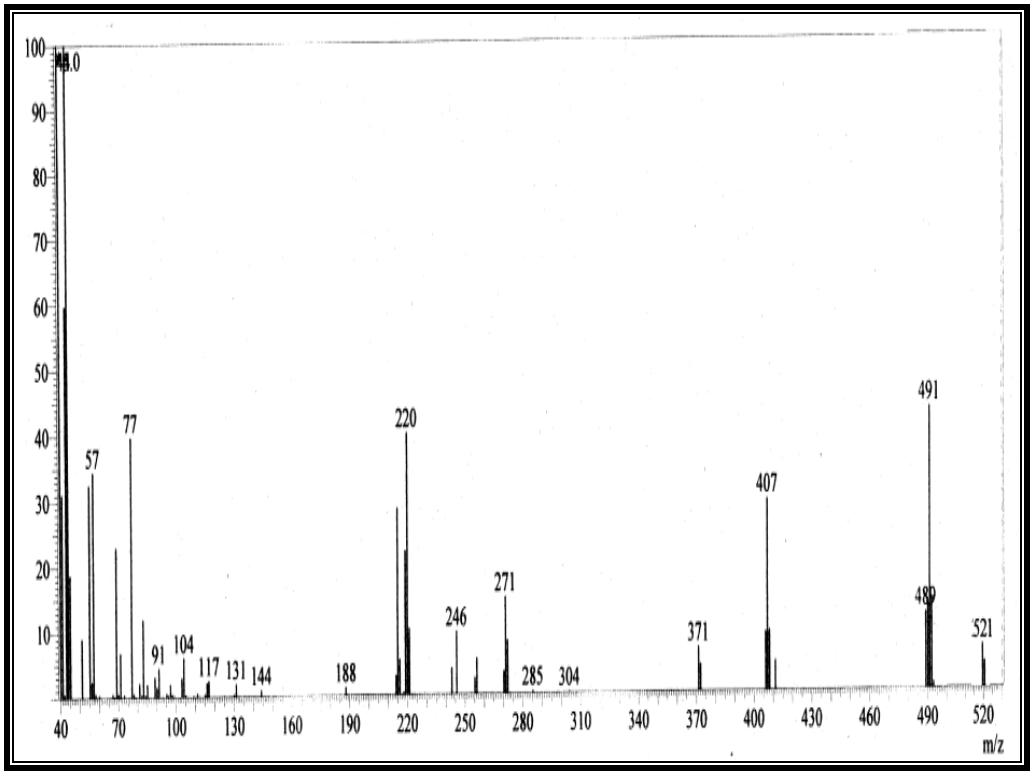
Expanded ^{1}H NMR spectrum of DDK-B-02



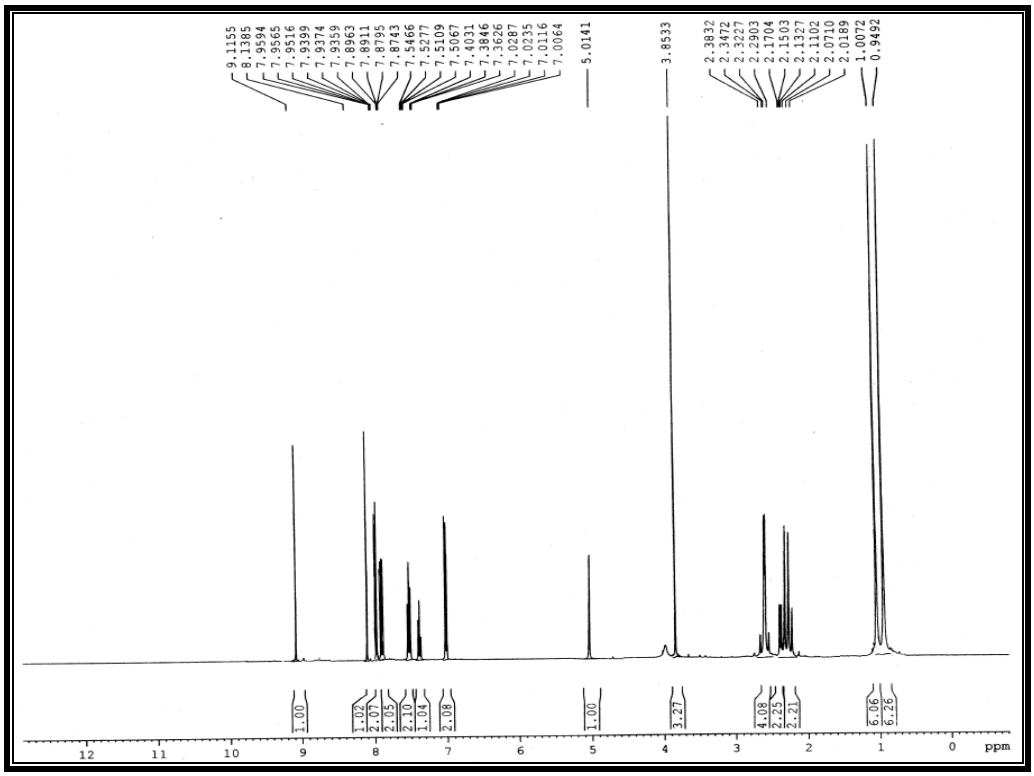
IR spectrum of DDK-B-06



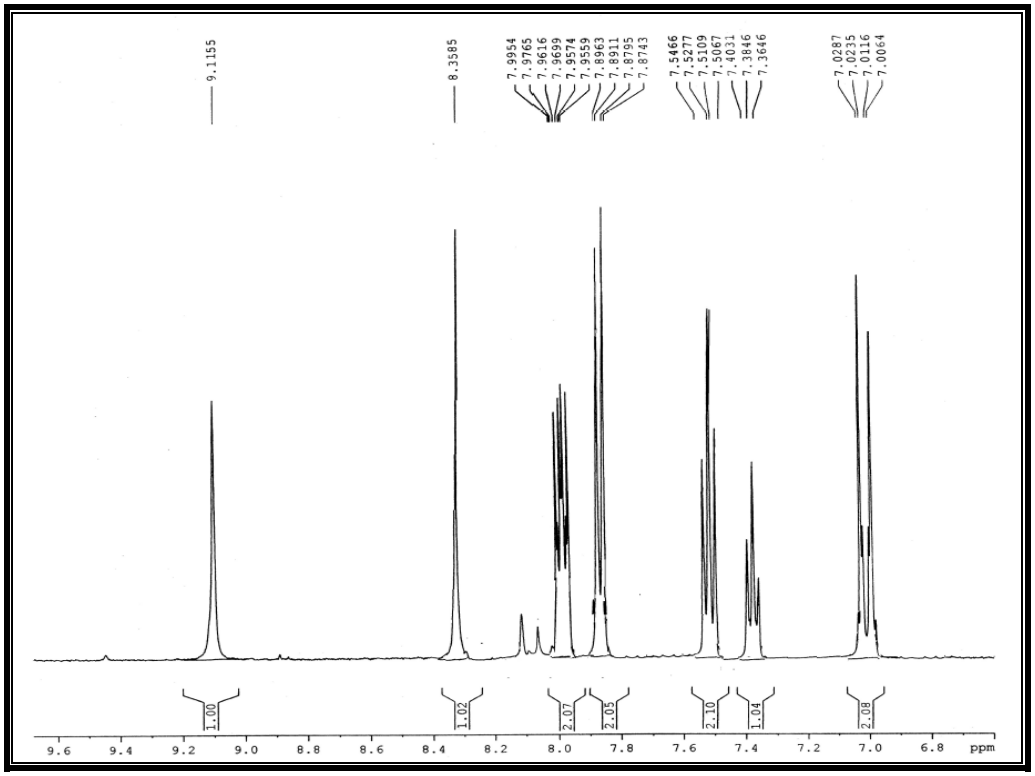
Mass spectrum of DDK-B-06



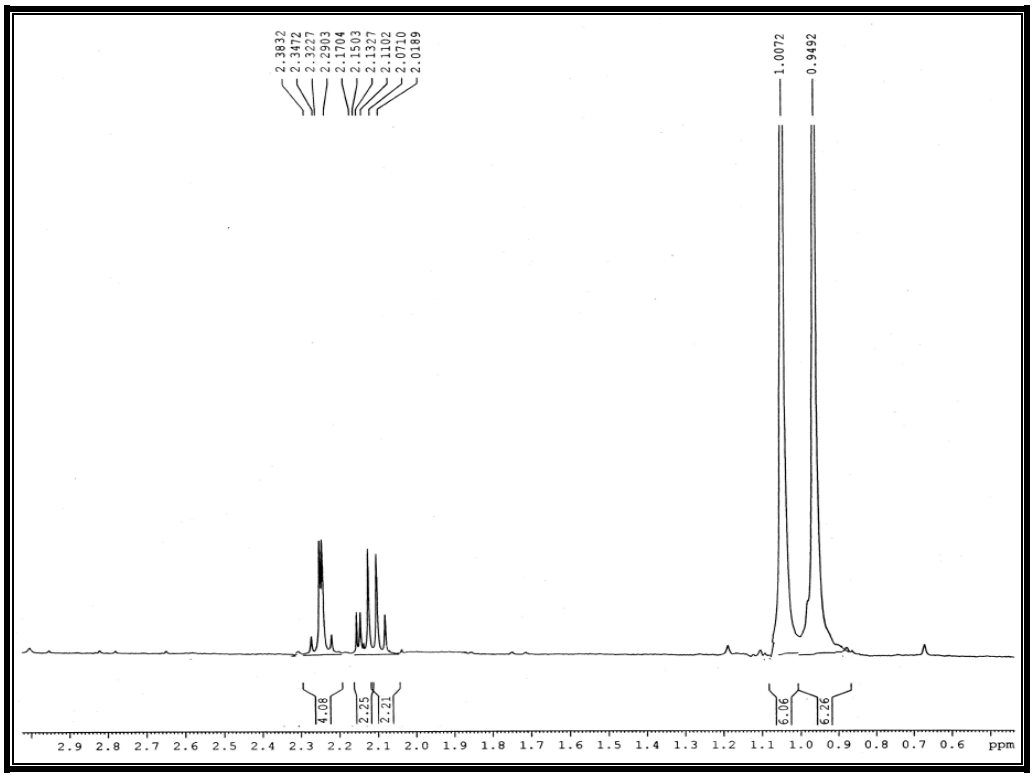
^{1}H NMR spectrum of DDK-B-06



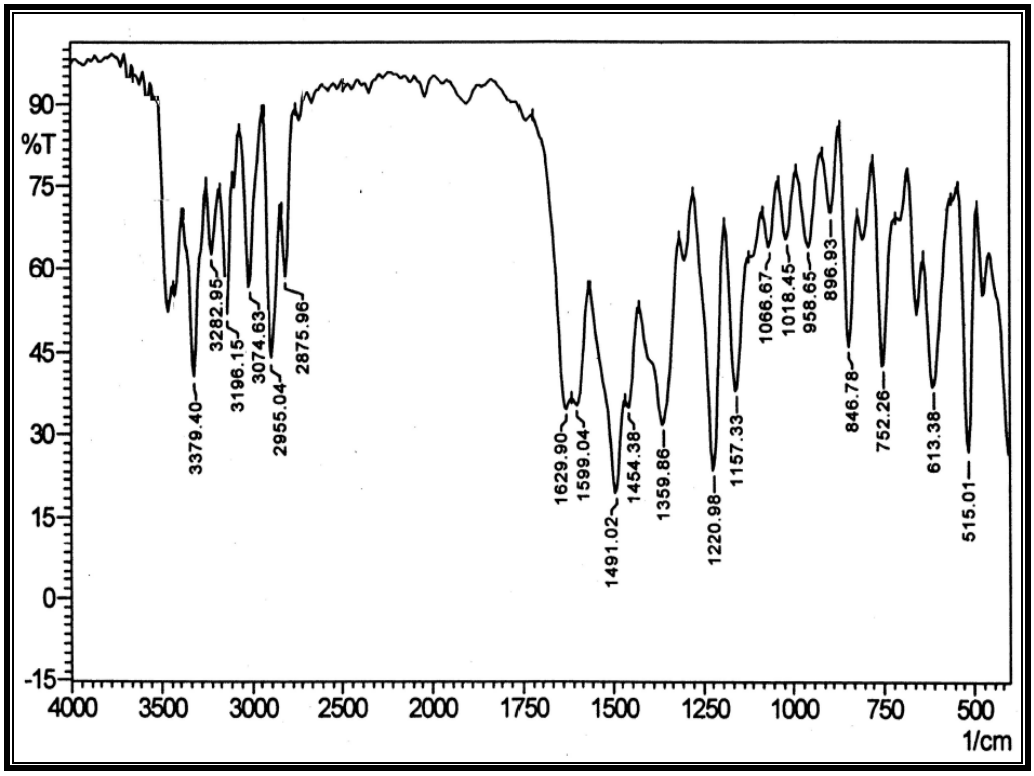
Expanded ^{1}H NMR spectrum of DDK-B-06



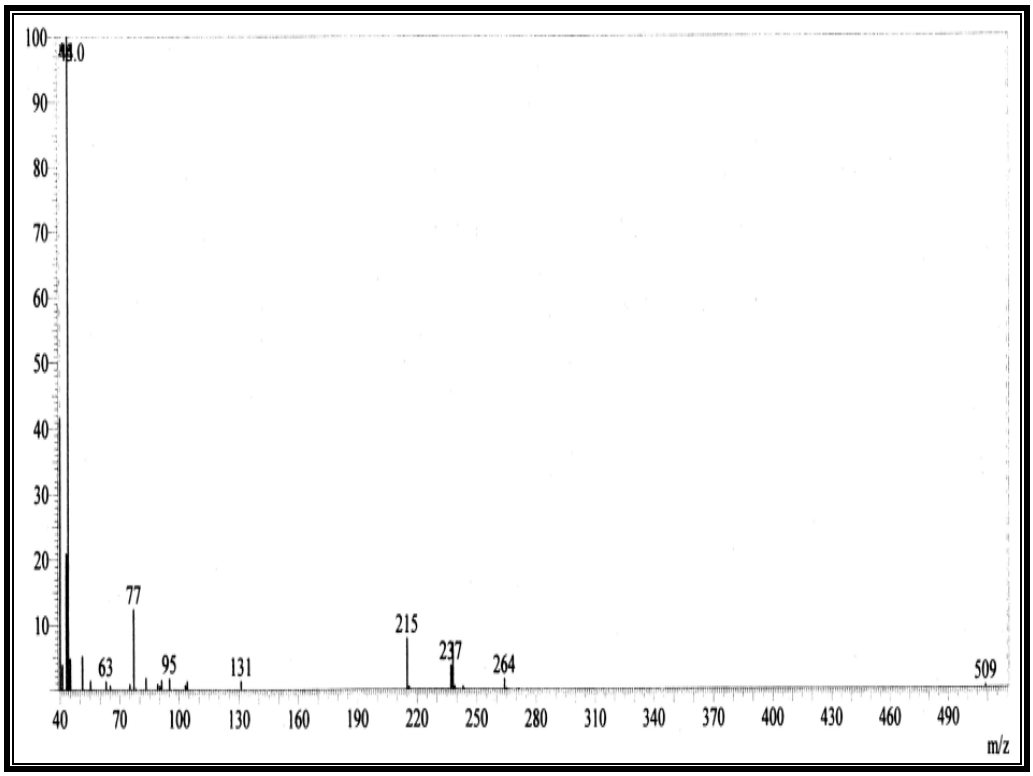
Expanded ^{1}H NMR spectrum of DDK-B-06



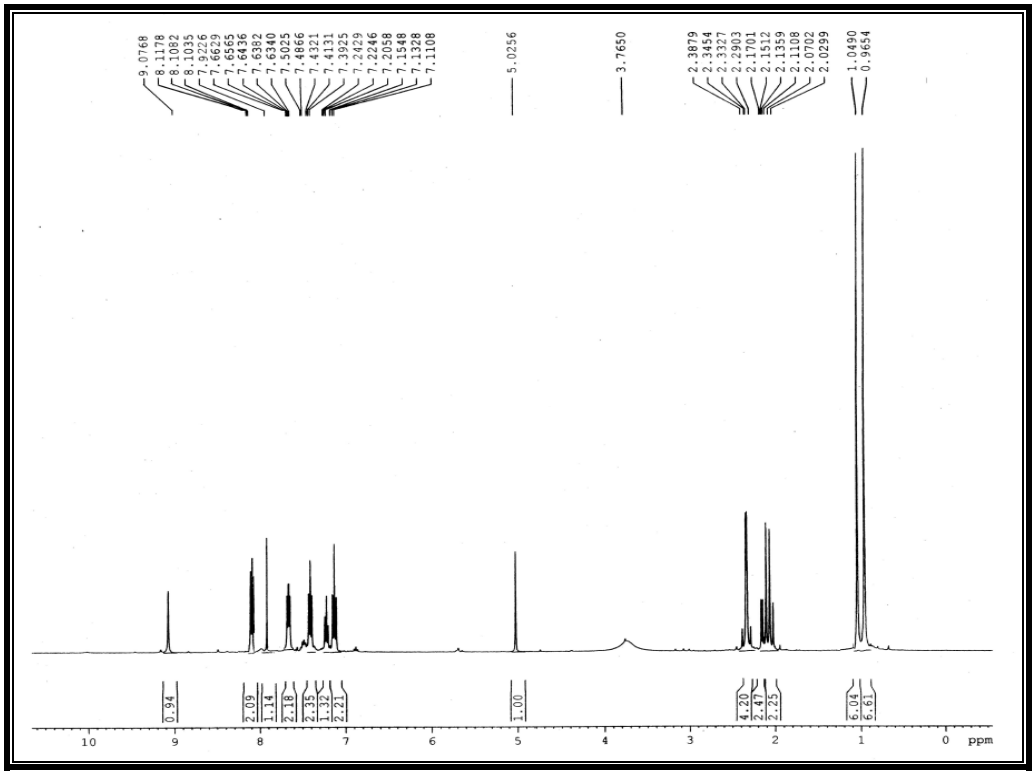
IR spectrum of DDK-B-09



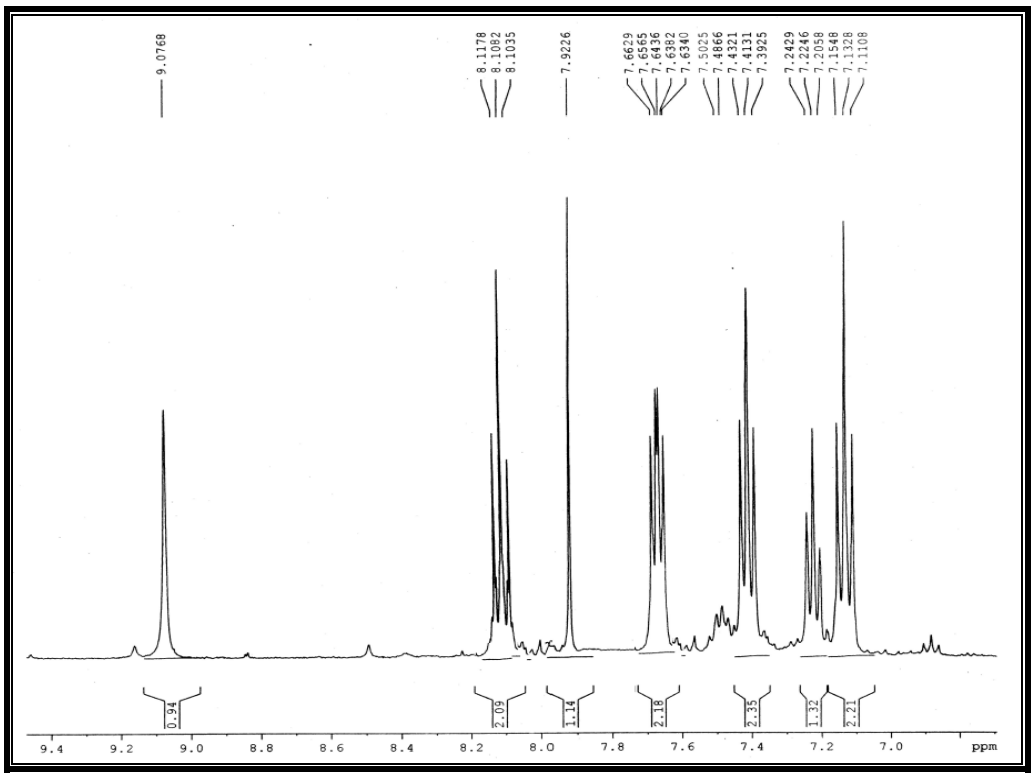
Mass spectrum of DDK-B-09



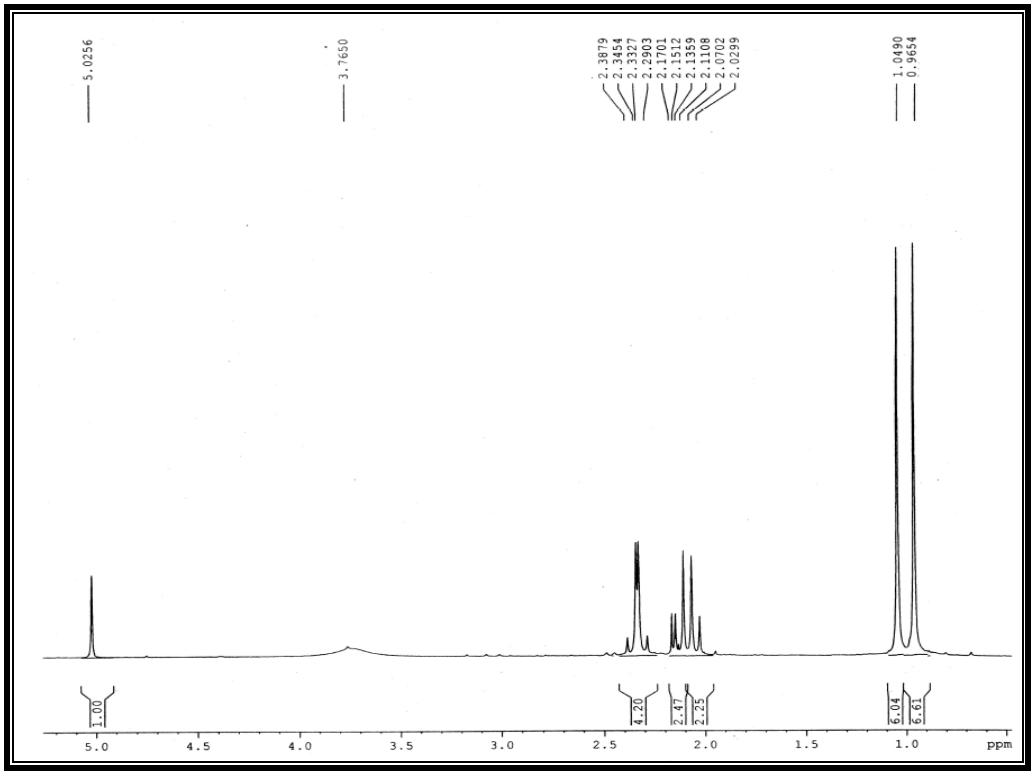
^{1}H NMR spectrum of DDK-B-09



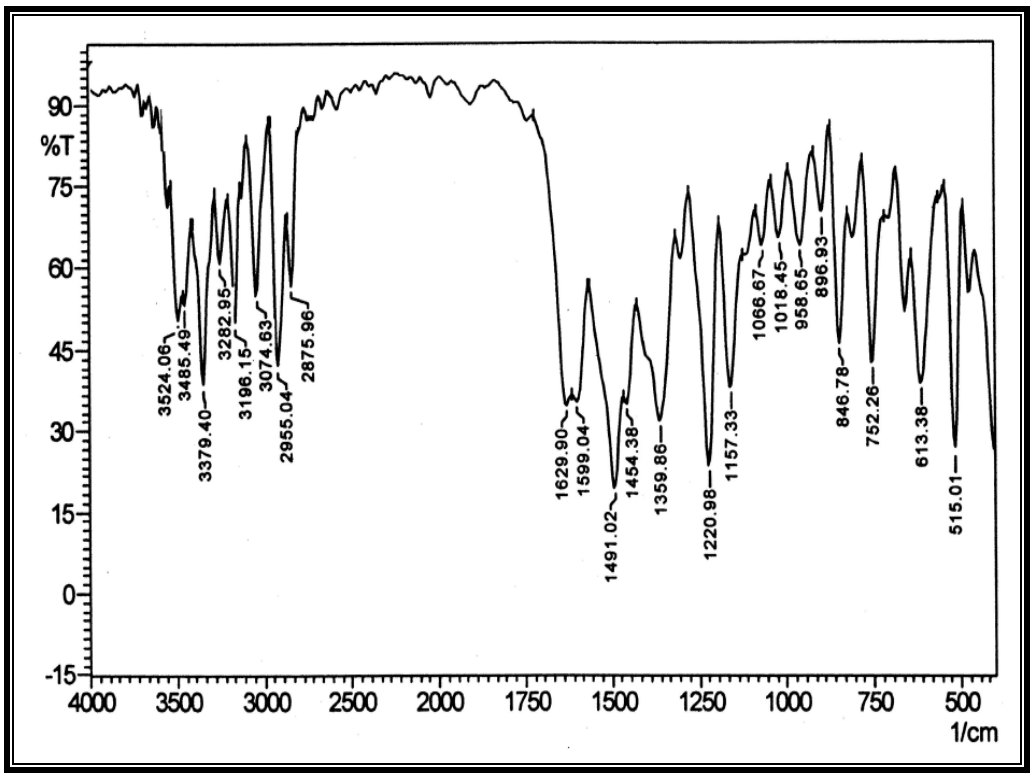
Expanded ^{1}H NMR spectrum of DDK-B-09



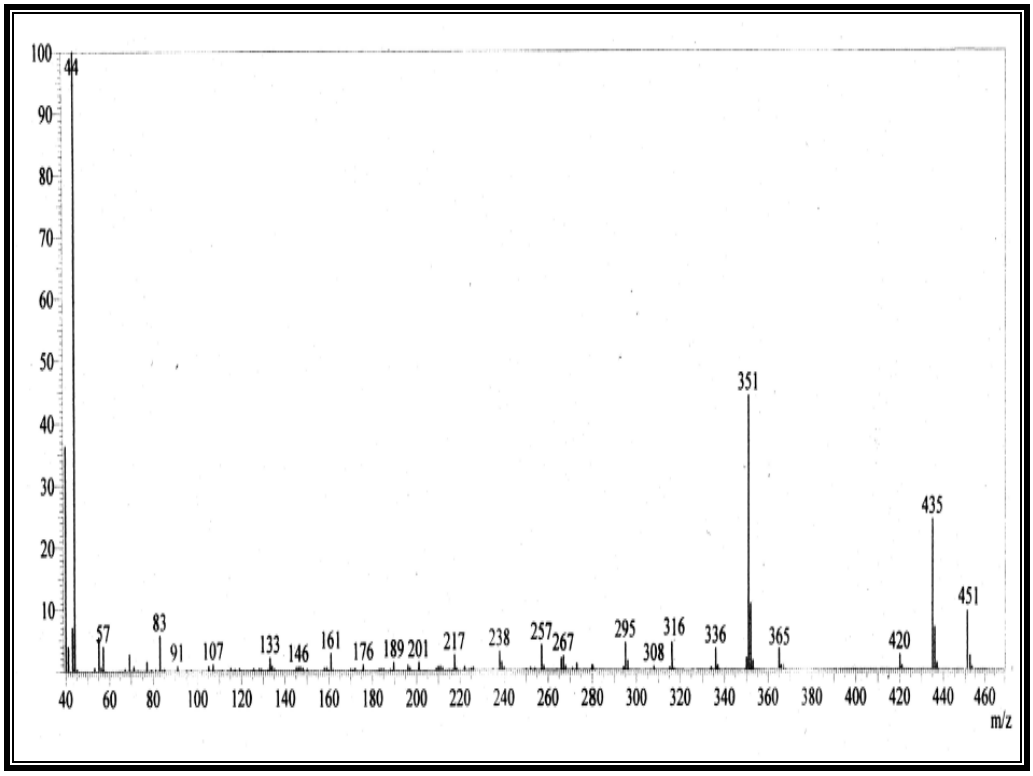
Expanded ^{1}H NMR spectrum of DDK-B-09



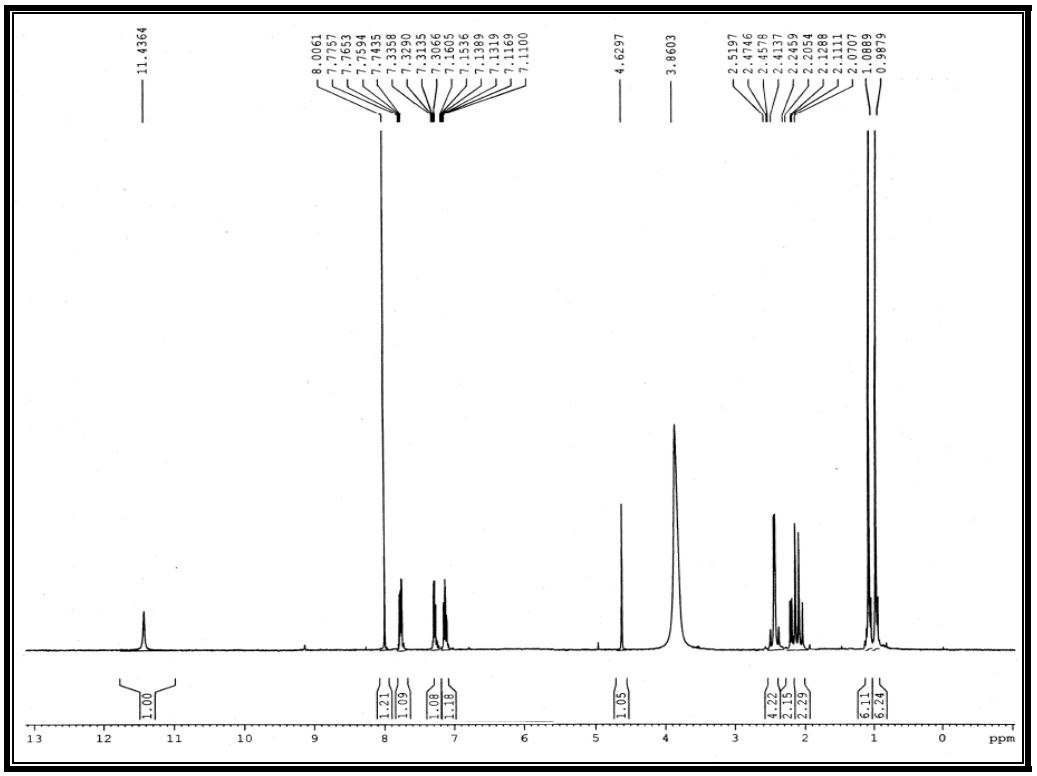
IR spectrum of DDK-B-11



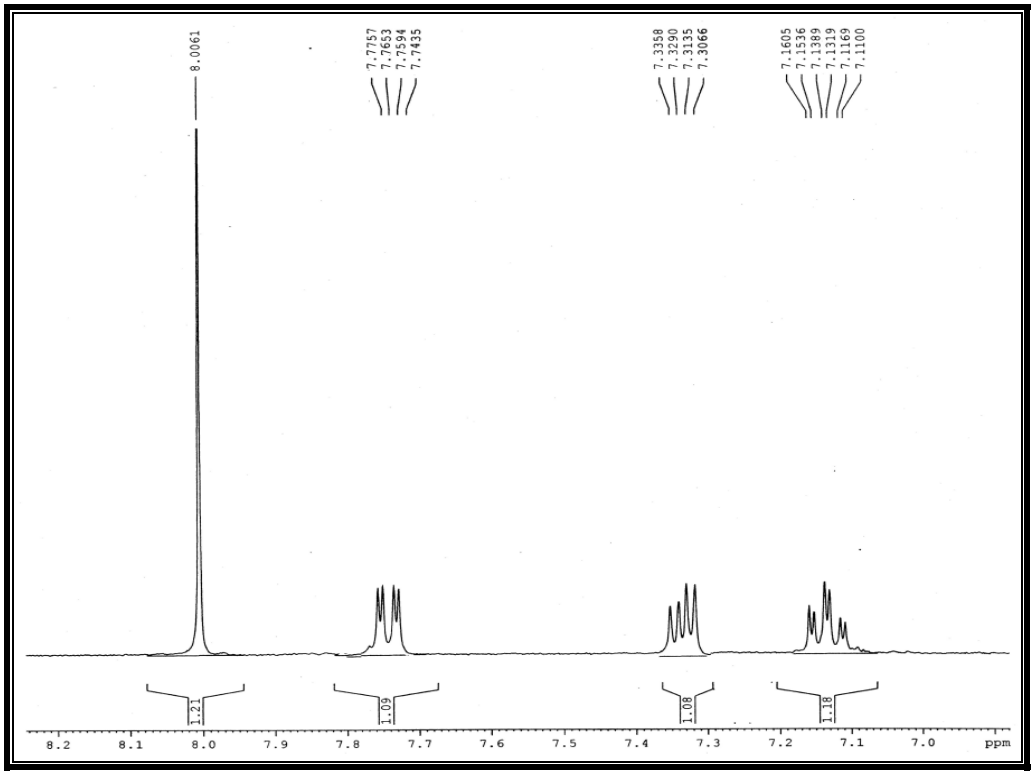
Mass spectrum of DDK-B-11



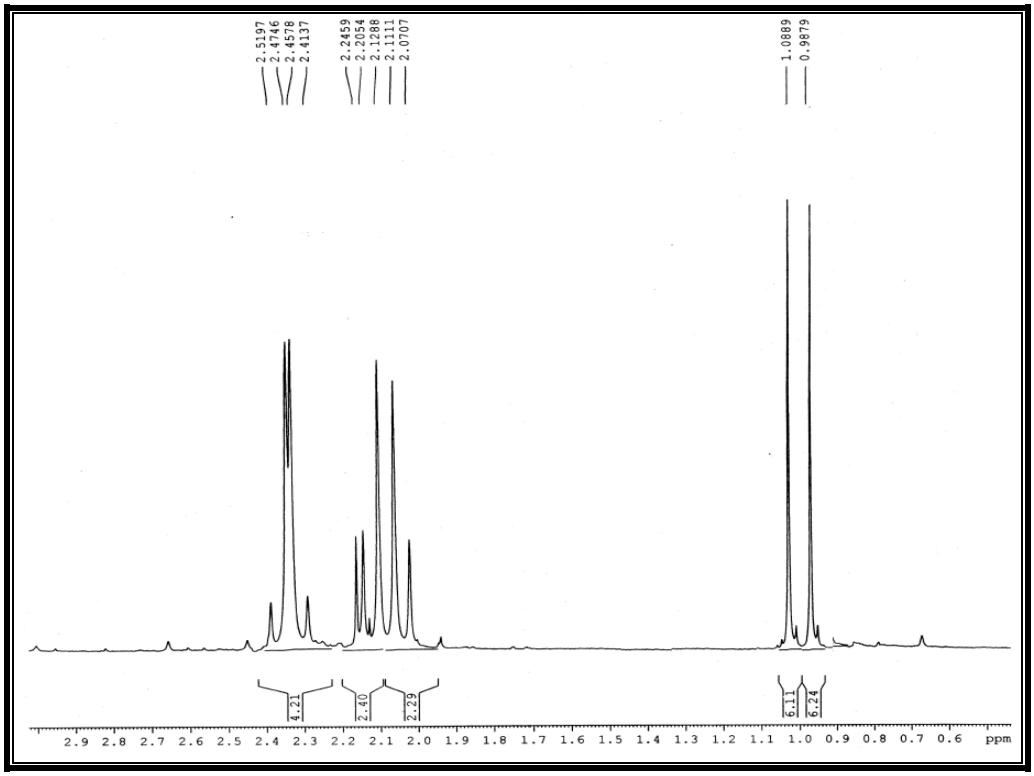
^{1}H NMR spectrum of DDK-B-11



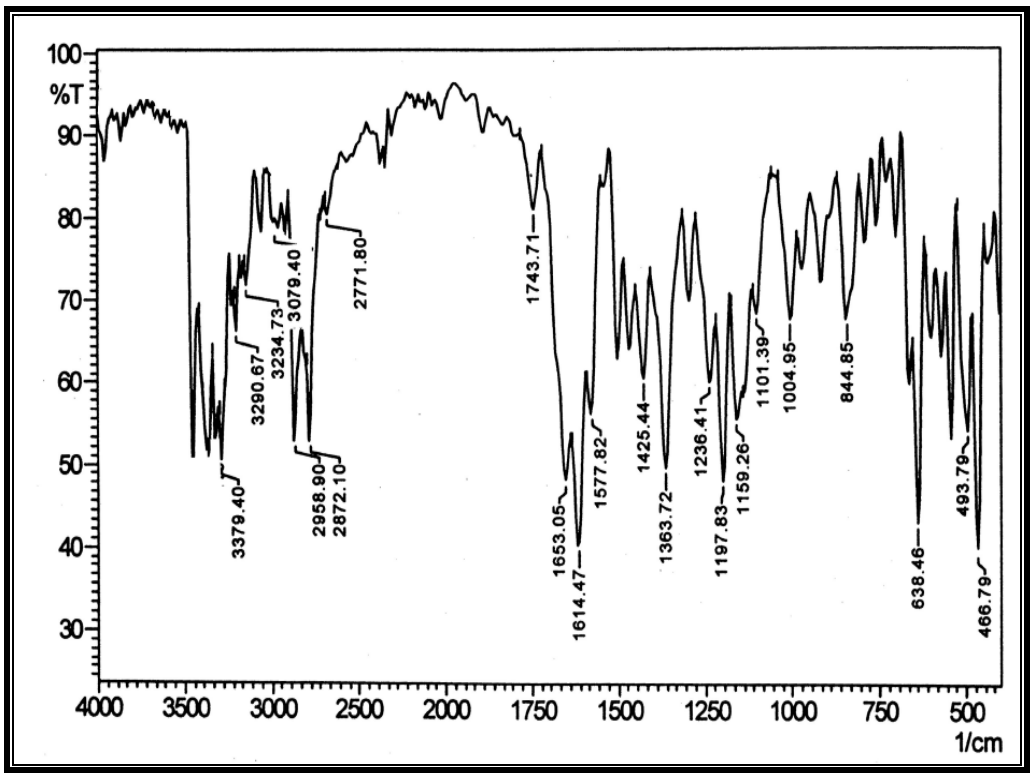
Expanded ^{1}H NMR spectrum of DDK-B-11



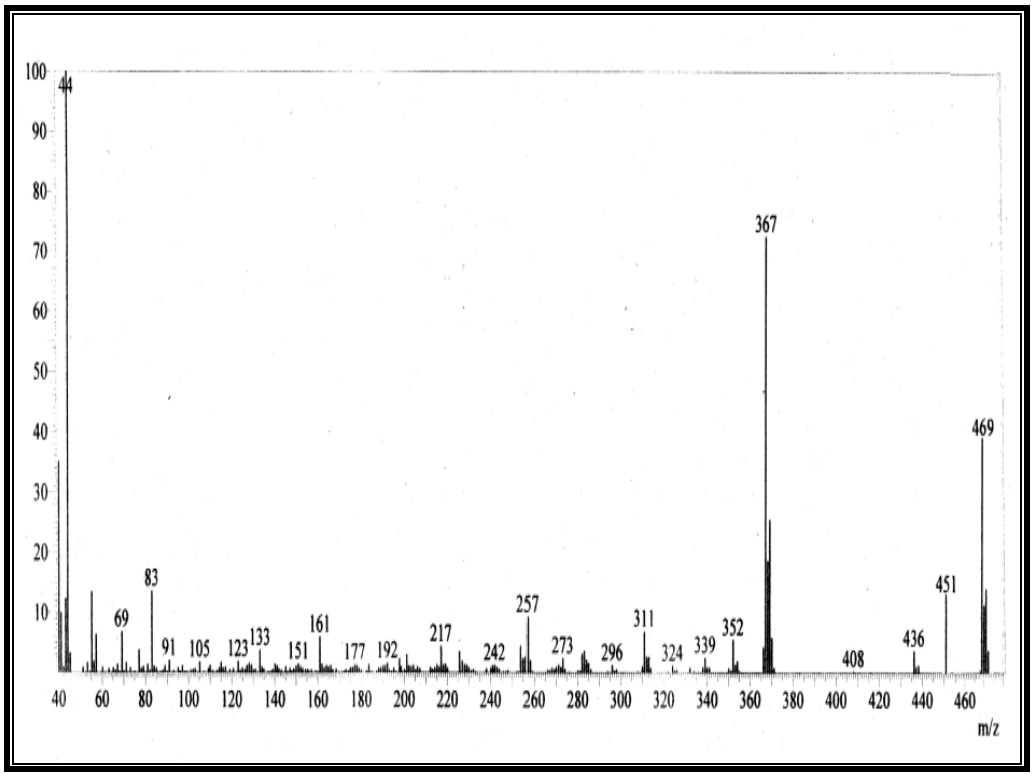
Expanded ^{1}H NMR spectrum of DDK-B-11



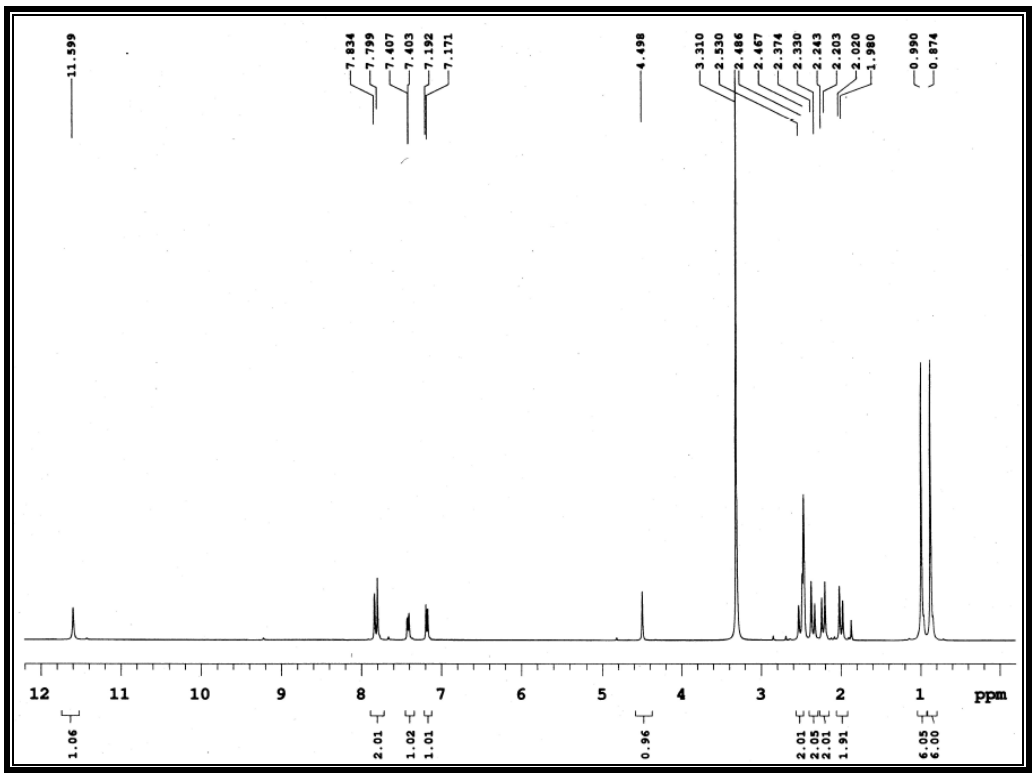
IR spectrum of DDK-B-13



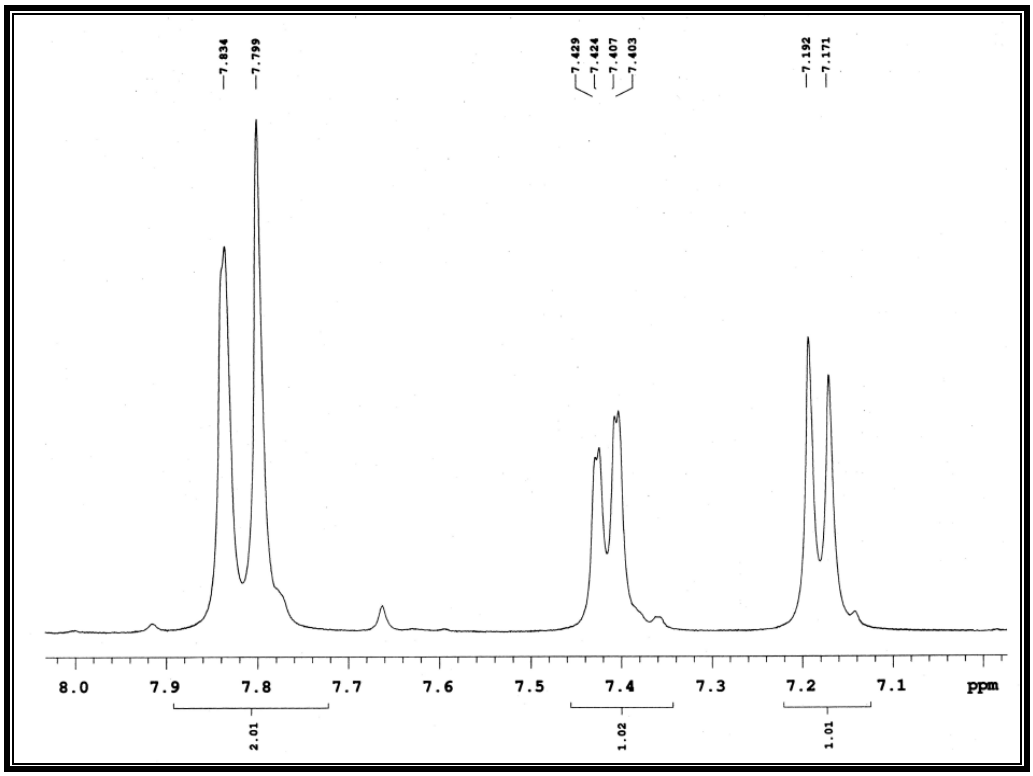
Mass spectrum of DDK-B-13



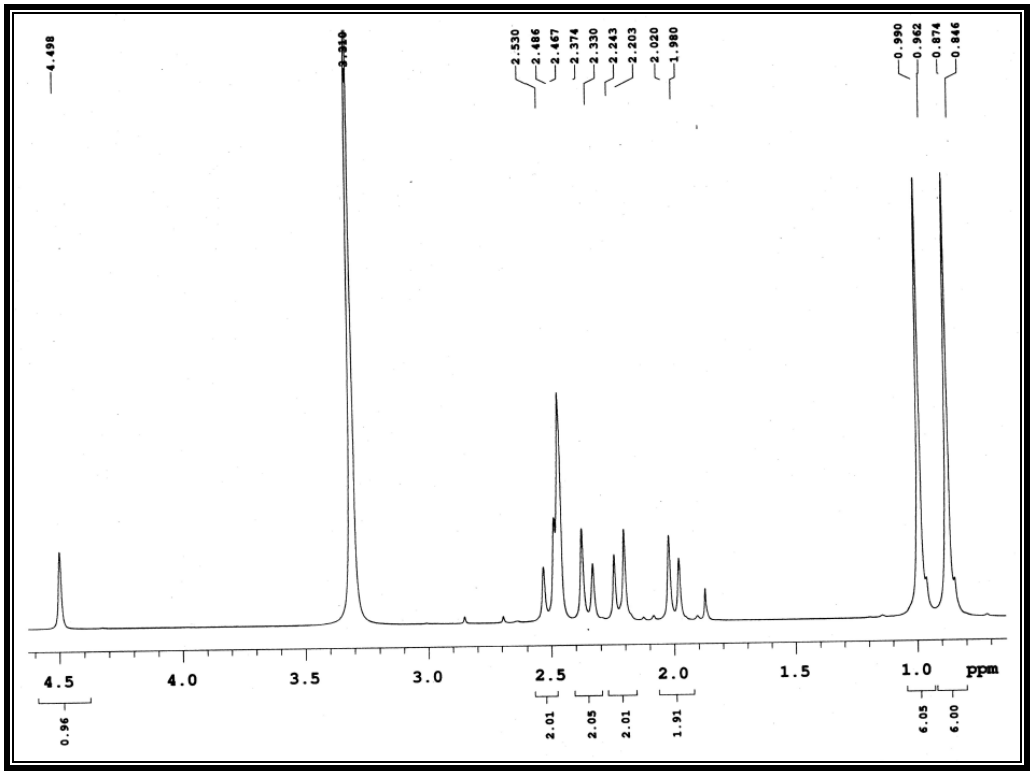
^{1}H NMR spectrum of DDK-B-13



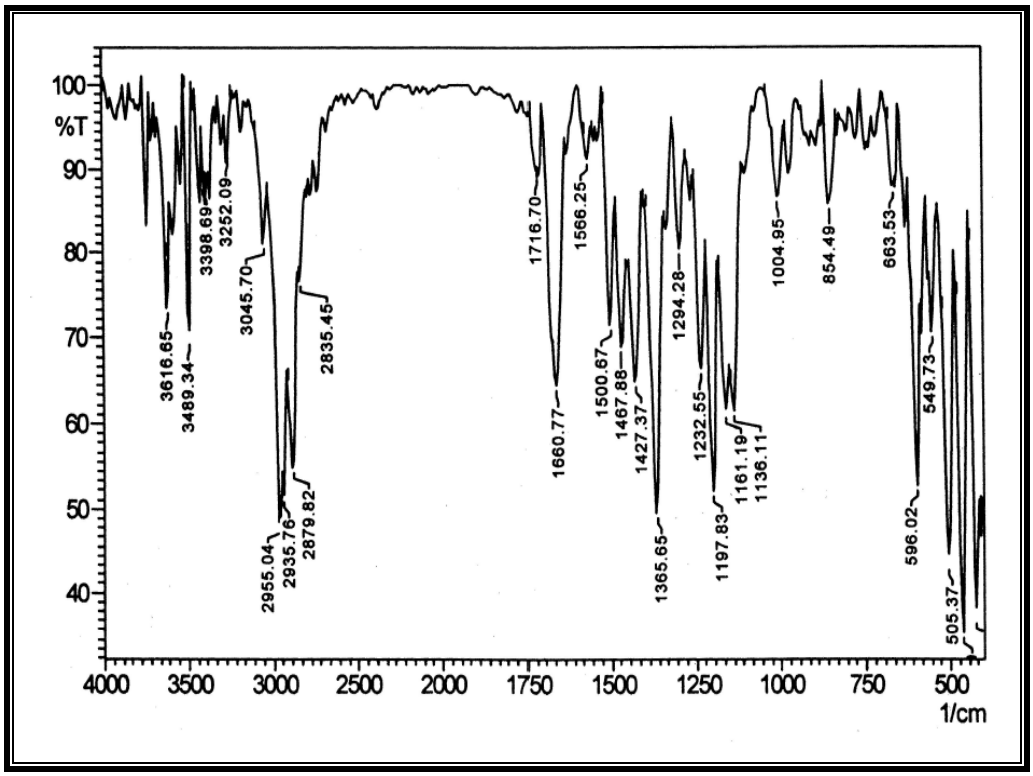
Expanded ^{1}H NMR spectrum of DDK-B-13



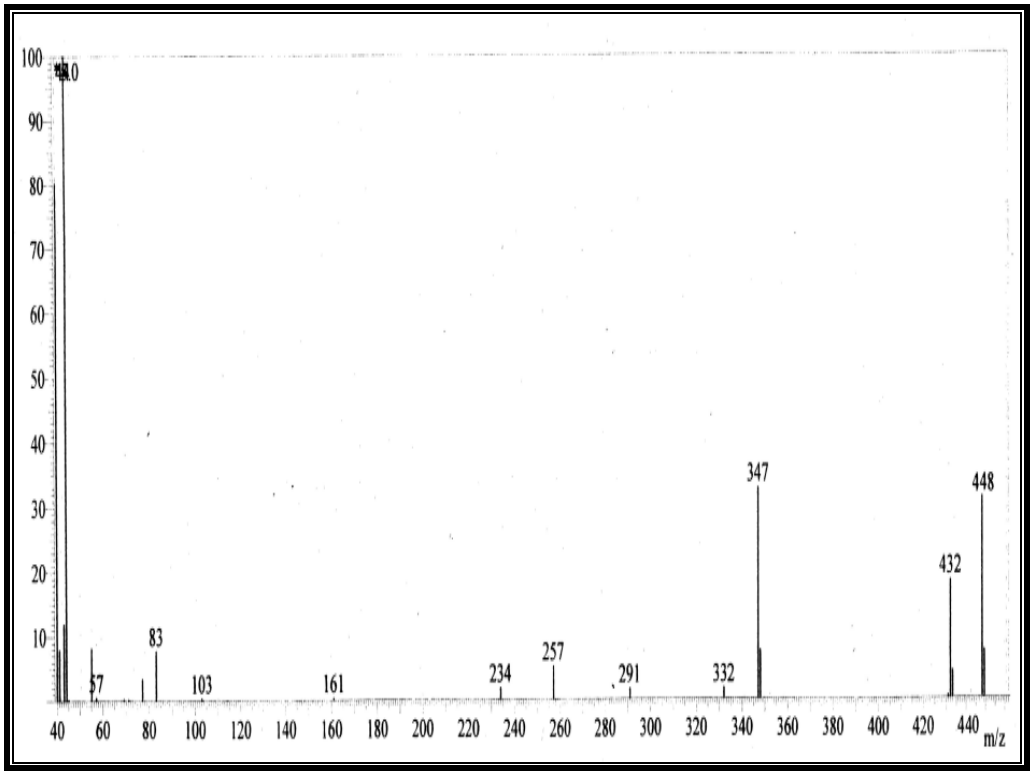
Expanded ^{1}H NMR spectrum of DDK-B-13



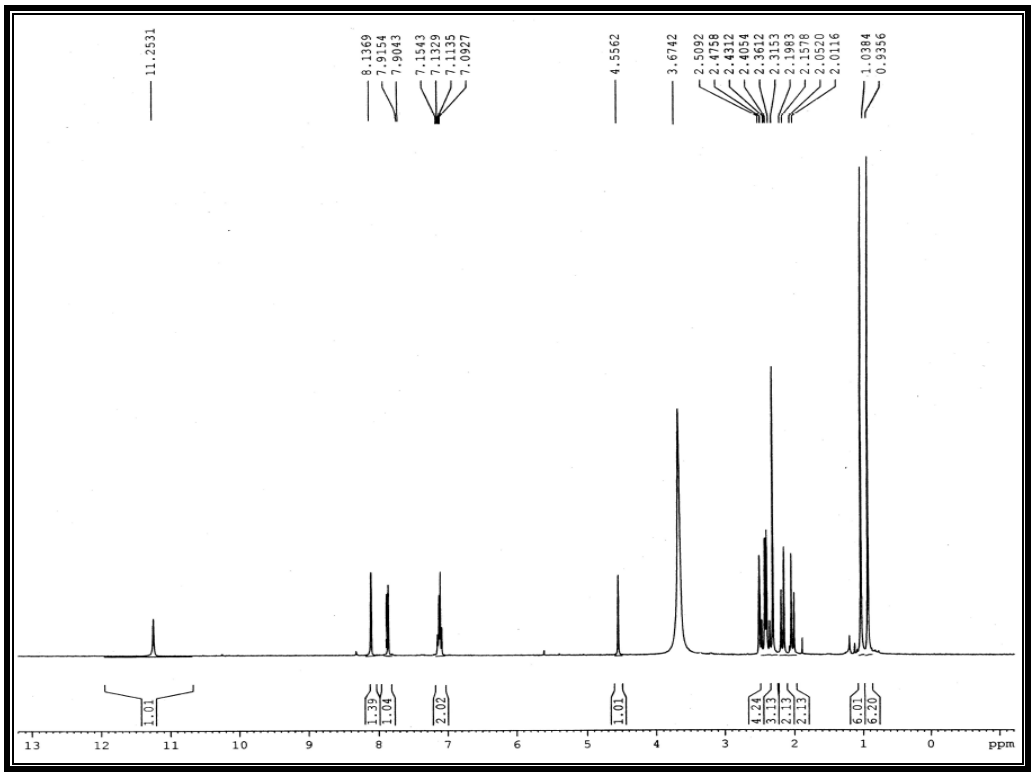
IR spectrum of DDK-B-18



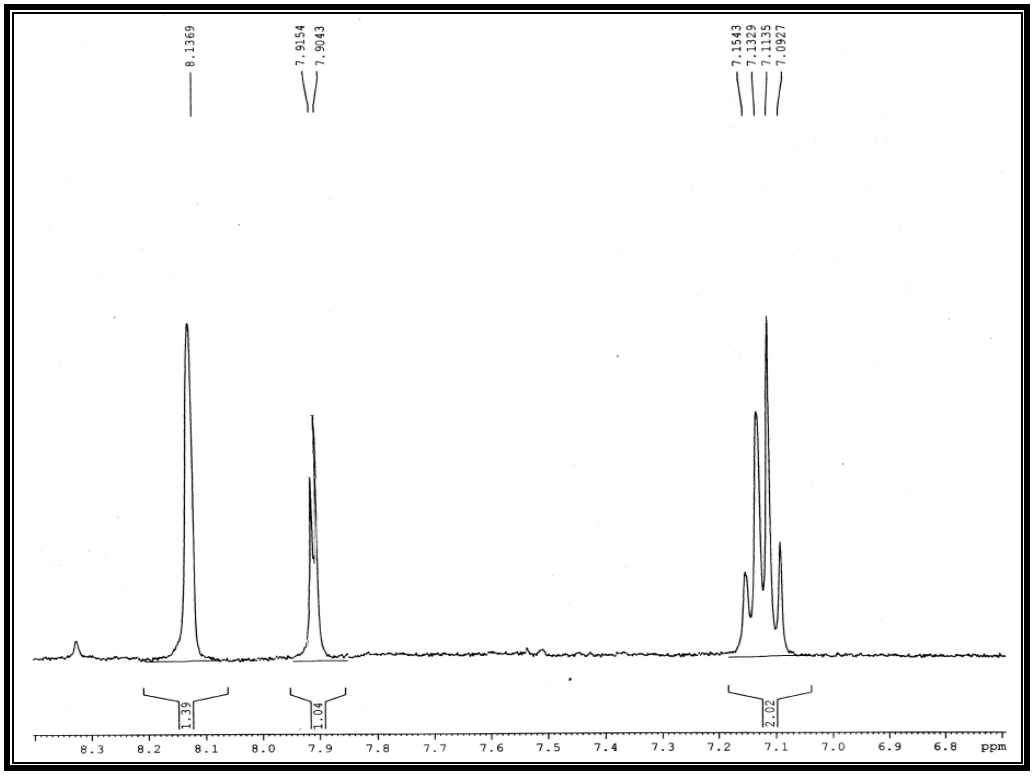
Mass spectrum of DDK-B-18



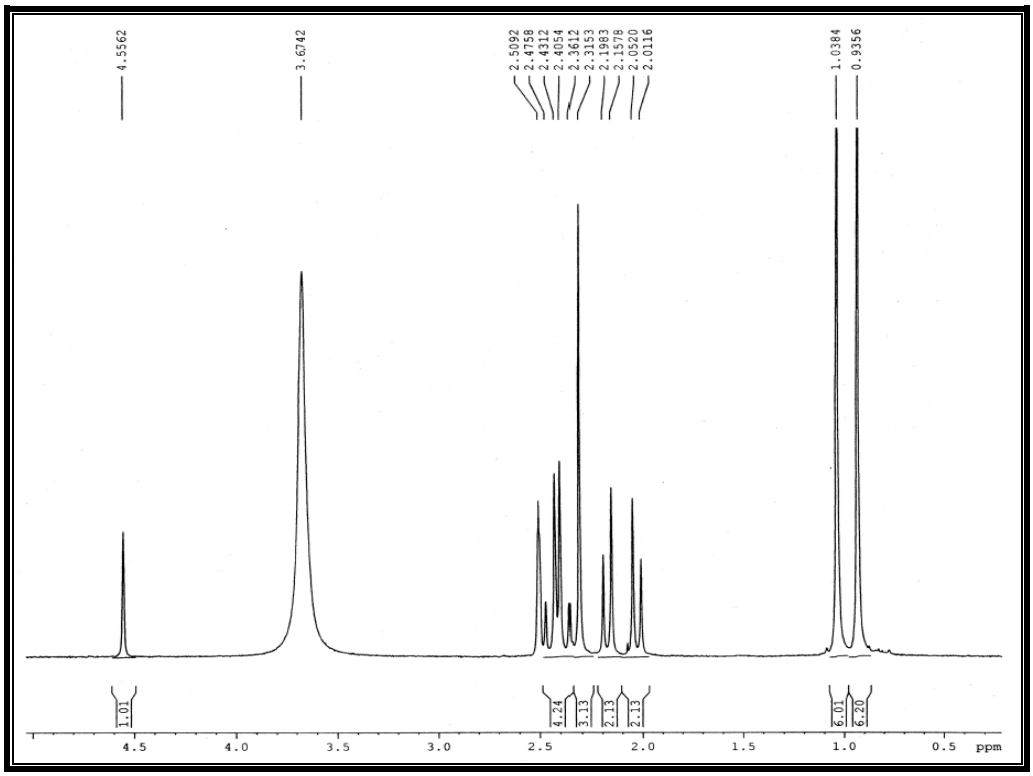
^{1}H NMR spectrum of DDK-B-18



Expanded ^{1}H NMR spectrum of DDK-B-18



Expanded ^{1}H NMR spectrum of DDK-B-18



4.8 Biological evaluation

4.8.1 Antimicrobial evaluation

All of the synthesized compounds (**DDK-B-01 to DDK-B-20**) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [74] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin, and Griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using microdilution broth method according to NCCLS standards [74(a)]. Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations. In primary screening 1000 μg mL^{-1}, 500 μg mL^{-1} and 250 μg mL^{-1} concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution at 125 μg mL^{-1}, 62.5 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1}, and 6.250 μg mL^{-1} concentration against all microorganisms. The tubes were inoculated with 10^8 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table 1.

Table 1. Antibacterial and antifungal activity of synthesized compounds DDK-B-01 to DDK-B-20

Code	Minimum inhibition concentration (μg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	***S. p.***	***E.c.***	***P.a.***	***C. a.***	***A. n.***	***A.c.***
DDK-B-01	500	1000	500	100	1000	500	500
DDK-B-02	1000	500	1000	1000	500	500	1000
DDK-B-03	500	500	250	500	>1000	1000	500
DDK-B-04	250	62.5	125	250	1000	500	250
DDK-B-05	125	100	1000	500	100	1000	500
DDK-B-06	500	1000	250	1000	500	500	>1000
DDK-B-07	1000	250	500	500	500	100	250
DDK-B-08	100	125	100	62.5	>1000	1000	1000
DDK-B-09	10	25	50	25	100	100	100
DDK-B-10	25	500	250	100	500	1000	>1000
DDK-B-11	500	500	>1000	1000	500	1000	>1000
DDK-B-12	62.5	250	500	125	1000	100	500
DDK-B-13	100	500	1000	1000	500	500	100
DDK-B-14	1000	1000	250	>1000	500	250	1000
DDK-B-15	250	250	500	500	1000	100	1000
DDK-B-16	250	1000	1000	250	>1000	1000	500
DDK-B-17	125	500	>1000	1000	500	250	500
DDK-B-18	100	500	500	500	125	250	250
DDK-B-19	500	1000	250	1000	500	1000	500
DDK-B-20	250	100	500	>1000	1000	500	1000
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

4.9 References and notes

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Chapter 5
Studies on microwave assisted synthesis of polyhydroquinolines

5.0 Introduction

Quinoline is a heterocyclic scaffold of paramount importance to human race. Several quinoline derivatives isolated from natural resources or prepared synthetically are significant with respect to medicinal chemistry and biomedical use. Compounds containing quinoline motif are most widely used as antimalarials [1], antibacterials [2] antifungals [3] and anticancer agents [4]. Additionally, quinoline derivatives find use in the synthesis of fungicides, virucides, biocides, alkaloids, rubber chemicals and flavoring agents. [5]. Because of their importance as substructures in a broad range of natural and designed products, significant efforts continue to be directed into the development of new quinoline-based structures.

In view of these observations and with a view to further explore the pharmacological profile of this class of compounds, the present study includes synthesis of novel quinoline derivatives viz. 5,6,7,8-tetrahydroquinolines and 7,8-dihydro-2,4-disubstituted-quinolin-5(*1H,4H,6H*)-ones. The study is divided in two sections:

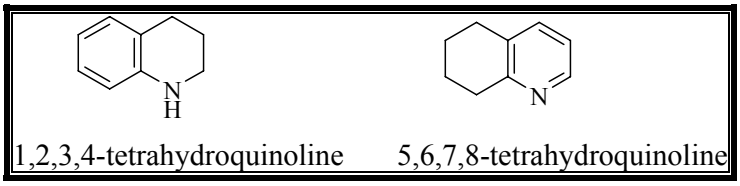
5.1 : Synthesis and biological evaluation of 2-amino-5,6,7,8-tetrahydro-4-heteroaryl- quinoline-3-carbonitriles

5.2 : Synthesis and biological evaluation of 7,8-dihydro-7,7-dimethyl-2,4-disubstituted-quinolin-5(*1H,4H,6H*)-ones

5.1: Synthesis and biological evaluation of 2-amino-5,6,7,8-tetrahydro-4-heteroaryl-quinoline-3-carbonitriles

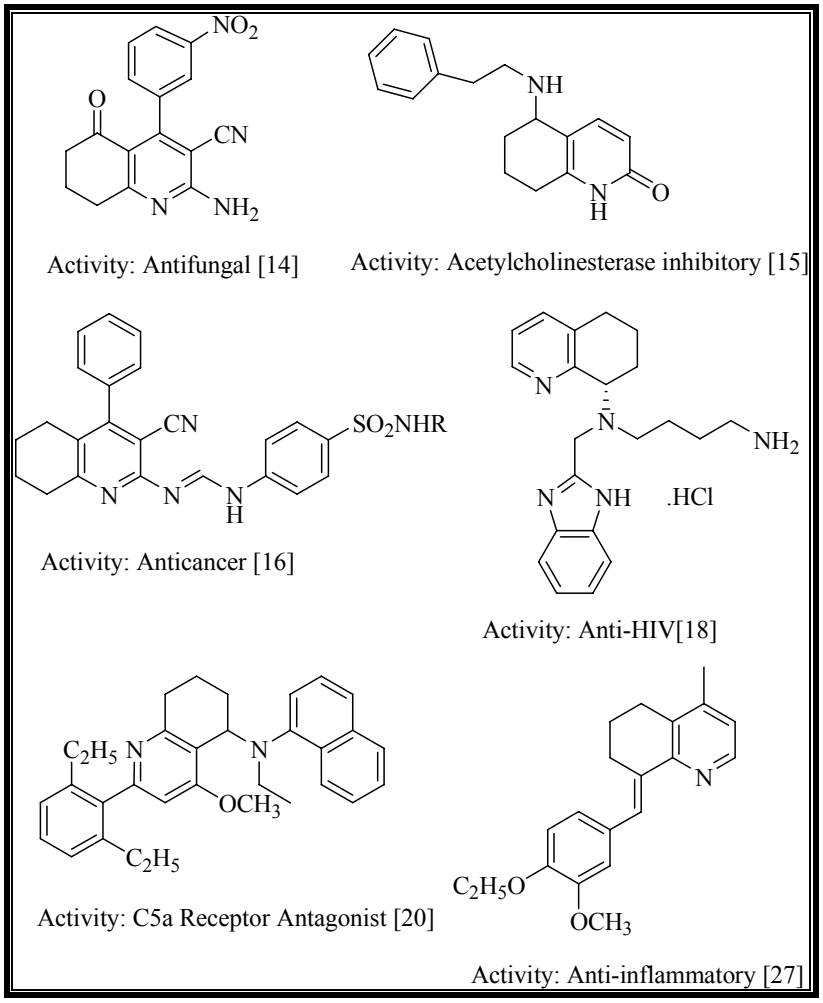
5.1.1 Introduction

Among quinoline derivatives, tetrahydroquinolines are an important structural subunit of natural products and many tetrahydroquinoline derivatives exhibit interesting biological and pharmaceutical activities [6], including anti-HIV [7], anti-cancer [8] anti-malarial [9], cholesteryl ester transfer protein inhibitors [10], anti-diabetic [11] etc. Among various tetrahydroquinolines, biological profile of 1,2,3,4-tetrahydroquinolines and 5,6,7,8-tetrahydroquinolines is extensively studied.



1,2,3,4-tetrahydroquinoline 5,6,7,8-tetrahydroquinoline

Recently, the *5,6,7,8*-tetrahydroquinolines have drawn considerable attention due to their interesting pharmacological applications as RET tyrosine kinase inhibitors [12], antimicrobial [13], anti-fungal [14], acetylcholinesterase inhibitory [15], anti-cancer [16, 17], anti-HIV [18, 19], C5a receptor antagonists agents [20, 21], anti-ulcer [22-24], anti-depressant [25, 26], anti-inflammatory [27, 28], antimalarial [29], Na+/H+ exchange inhibitors [30], tachykinin receptor antagonist [31], modulators of chemokine receptors [32], alpha-2B-adrenergic receptor antagonist [33] etc. Some examples of published derivatives of *5,6,7,8*-tetrahydroquinolines with their biological activities are shown in the following figure.

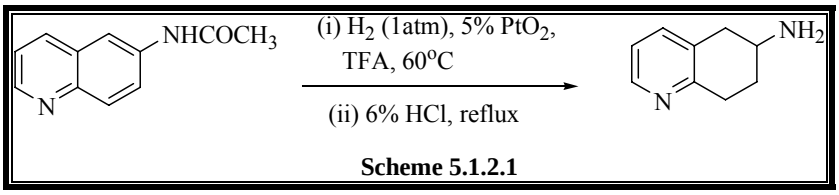


5.1.2 Reported synthetic strategies

Synthetic methodologies for preparing tetrahydroquinoline derivatives have attracted considerable interest and several methods offering good results have been reported. However, most of them describe the synthesis of *1,2,3,4*-tetrahydroquinoline nucleus and concise methods to access usefully functionalized *5,6,7,8*-tetrahydroquinolines are scarce in the literature [34].

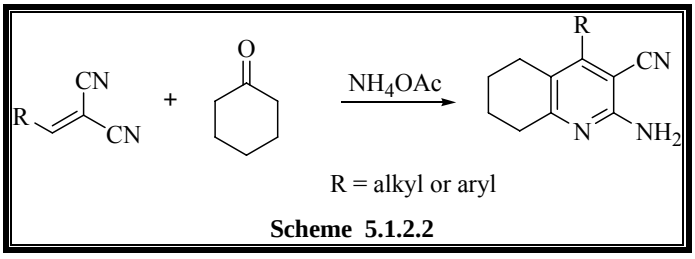
Few researchers have reported hydrogenation of quinolines to *5,6,7,8*-tetrahydroquinolines. Skupinska et al. have reported concise preparation of amino-5,6,7,8-tetrahydroquinolines and amino-5,6,7,8-tetrahydroisoquinolines via catalytic hydrogenation of acetamidoquinolines and acetamidoisoquinolines [34] (Scheme 5.1.2.1). Different researchers have reported hydrogenation under variety of

conditions, viz. using Ru/ZrO2·xH2O [35] or polymer-supported Rhodium(I)-1,3-Bis(diphenylphosphino)propane moieties [36] as catalysts, and hydrogenation over raney-nickel and ruthenium/carbon [37]. Huck et al. have reported hydrogenation of cinchona alkaloids and cincholidine over 5 wt% Pt/Al_2O_3 in acetic acid [38].



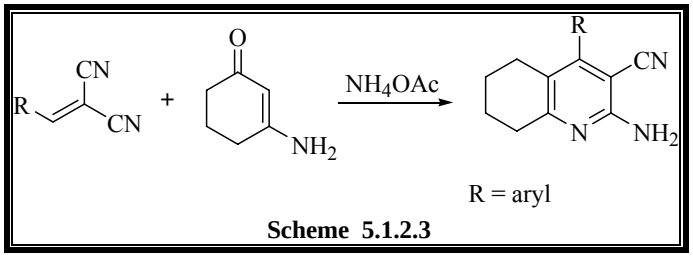
Scheme 5.1.2.1

Literature survey revealed number of reports describing synthesis of 5,6,7,8-tetrahydroquinoline-3-carbonitriles. Several reports describe the synthesis of 2-amino-5,6,7,8-tetrahydroquinoline-3-carbonitriles by the reaction of arylidene malononitrile with cyclohexanone and ammonium acetate [28, 39, 40] (Scheme 5.1.2.2).



Scheme 5.1.2.2

The same compounds were obtained by the reaction of 3-amino-2-cyclohexen-1-one (enaminone) with arylidene malononitriles in better yields [14] (Scheme 5.1.2.3).

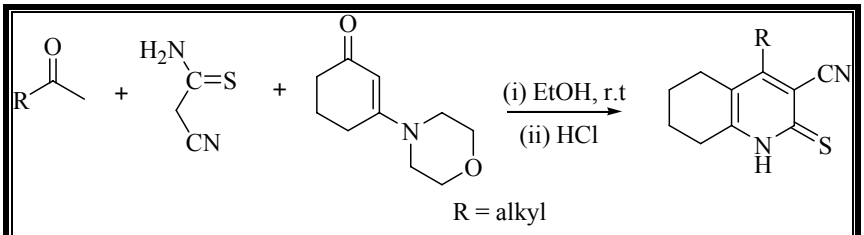


Scheme 5.1.2.3

Reaction of arylidene cyanoacetate with cyclohexanone in excess of ammonium acetate yielded 2-oxo-5,6,7,8-tetrahydroquinoline-3-carbonitriles [41]. One pot synthesis from aldehyde, ethyl cyanacetate, cyclohexanone and ammonium

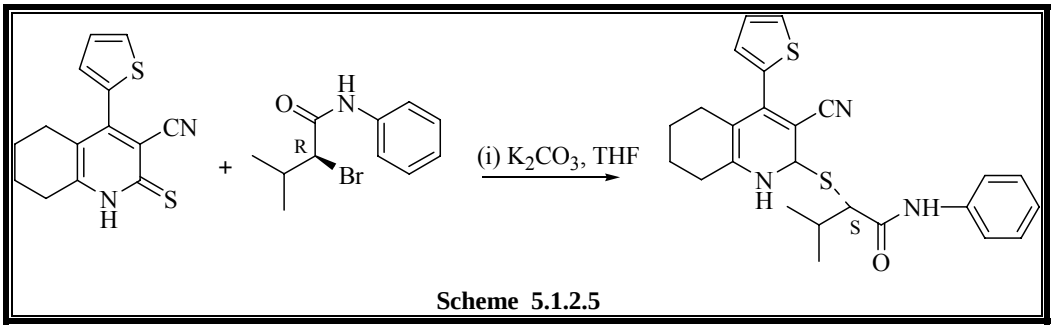
acetate is also reported yielding same compounds [42].

Synthesis of 4-alkyl/aryl-3-cyano-5,6,7,8-tetrahydroquinoline-2(1H)-thiones is also reported using different methods viz. cyclocondensation of arylmethylene cyclohexanones with cyanothioacetamide [13, 43], reaction of cyclohexanone [12, 44, 47] or its enamine [45] with arylmethylenecyanothioacetamides. Three component condensation of cyanothioacetamide with aliphatic aldehydes and enamine is reported to give 4-alkyl-3-cyano-5,6,7,8-tetrahydroquinoline-2(1*H*)-thiones [46] (Scheme 5.1.2.4).



Scheme 5.1.2.4

Yao et al. have reported synthesis and structures of (S)- and (R)-2-[3-cyano-4-(2-thienyl)-5,6,7,8-tetrahydroquinolin-2-ylsulfanyl]-3-methyl-N-phenylbutyramide by the reaction of 4-(2-thienyl)-3-cyano-5,6,7,8-tetrahydroquinoline-2(1*H*)-thione with (R) and (S) 2-Bromo-3-methyl-1-phenyl-butan-1-one respectively [47] (Scheme 5.1.2.5).

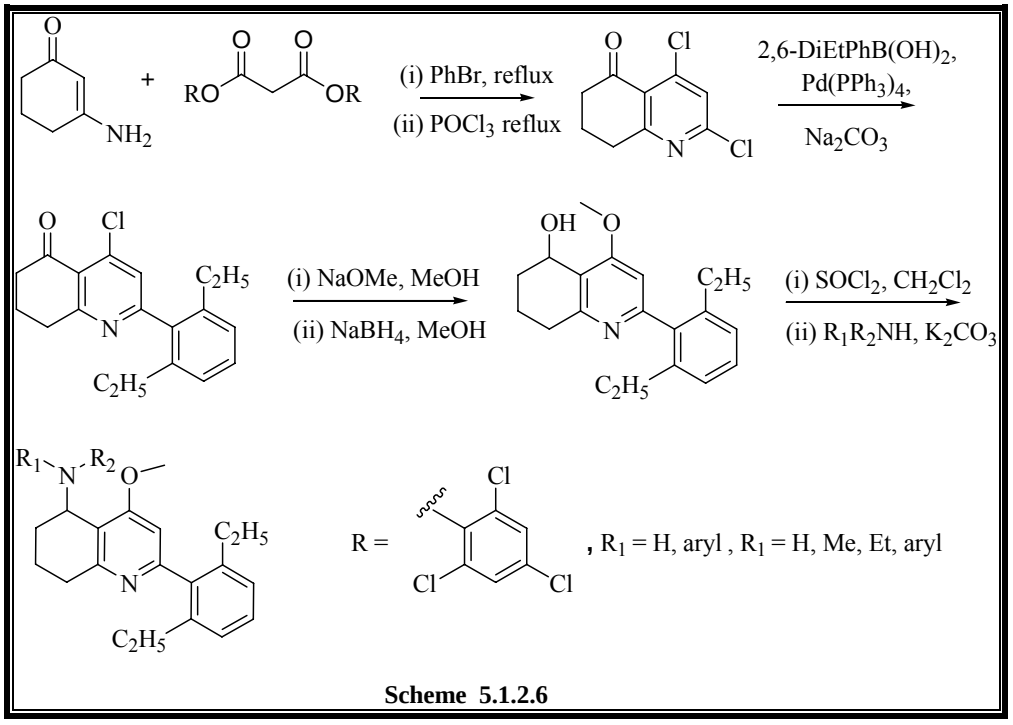


Scheme 5.1.2.5

Literature survey also revealed few reports on the synthesis of chiral 5,6,7,8-tetrahydroquinolines. Recently, Hapke et al. have reported asymmetric synthesis of axially chiral 1-Aryl-5,6,7,8-tetrahydroquinolines by cobalt-catalyzed [2+2+2] cycloaddition reaction of 1-aryl-1,7-octadiynes and nitriles [48]. Synthesis of chiral ethyl-5-(acetoxyimino)-2,7,7-trimethyl-4-(1-naphthyl)-5,6,7,8-tetrahydroquinoline-3-

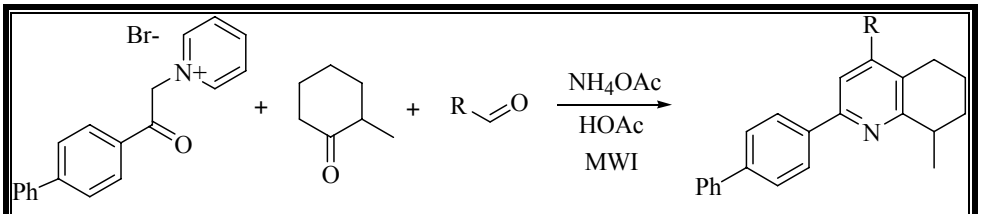
carboxylate via lipase-catalyzed hydrolysis was reported by Zhou et al. [49]. Chelucci et al. have reported synthesis of chiral 2-methyl-5,6,7,8-tetrahydroquinolines from naturally occurring monoterpenes [50]. Regioselective syntheses of optically active (*R*)-5-methyl- and (*R*)-7-methyl-5,6,7,8-tetrahydroquinolines was reported by Chelucci et al. earlier [51].

Cyclocondensation of 3-amino-2-cyclohexen-1-one with malonic acid bis(2,4,6-trichlorophenyl) ester yielded 2,4-Dichloro-7,8-dihydro-6H-quinolin-5-one, which upon Suzuki cross-coupling with 2,6-diethylphenylboronic acid followed by reaction with sodium methoxide, sodium borohydride, thionyl chloride and secondary amines yielded [2-(2,6-Diethyl-phenyl)-4-methoxy-5,6,7,8-tetrahydro-quinolin-5-yl]-dialkyl/aryl-amine [20] (Scheme 5.1.2.6).



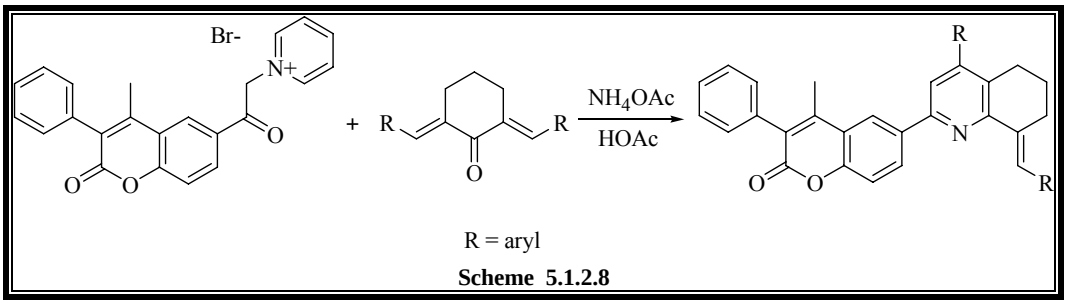
Scheme 5.1.2.6

Recently, few approaches involving Kronhke pyridine ssynthesis for the synthesis of 5,6,7,8-tetrahydroquinolines have been reported. Yan and co-workers have reported a one pot approach using modified two-step synthesis via Krohnke pyridine synthesis involving a three-component tandem reaction of N-phenacylpyridinium bromide, aromatic aldehydes and substituted cyclic ketones in ammonium acetate-acetic acid under microwave irradiation [52] (Scheme 5.1.2.7).



Scheme 5.1.2.7

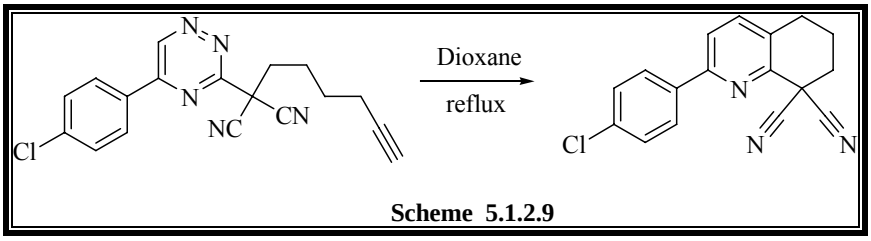
While Brahmbhatt et al. have reported synthesis of 6-(4-aryl-8-aryledine-5, 6,7,8-tetrahydroquinolin-2-yl)coumarins from 3-phenyl-4-methyl-6-coumarinoyl methylpyridinium bromide and 2,6-dibenzylidene-cyclohexanone using Krohnke's reaction condition under conventional heating [53] (Scheme 5.1.2.8).



R = aryl

Scheme 5.1.2.8

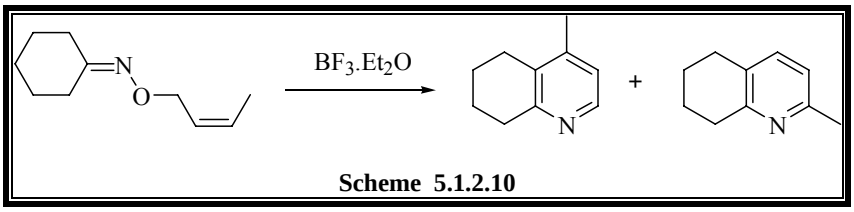
Taylor et al. have reported the synthesis of 5,6,7,8-tetrahydroquinolines by the intramolecular Diels-Alder Reactions of 1,2,4-Triazines [54] (Scheme 5.1.2.9).



Scheme 5.1.2.9

A highly selective transformation of 5,6,7,8-tetrahydro-2H-1-benzopyran-2,5-diones with hydrazides, arylhydrazines and heterocyclic hydrazines as nitrogen-contg. nucleophiles into the corresponding 1-amino-5,6,7,8-tetrahydroquinoline-2,5-diones was investigated by Trebse et al. [55]. Koyama et al. have reported synthesis of 5,6,7,8-tetrahydroquinolines by thermolysis of oxime *O*-allyl ethers in presence of

boron trifluoride etherate [56] (Scheme 5.1.2.10).



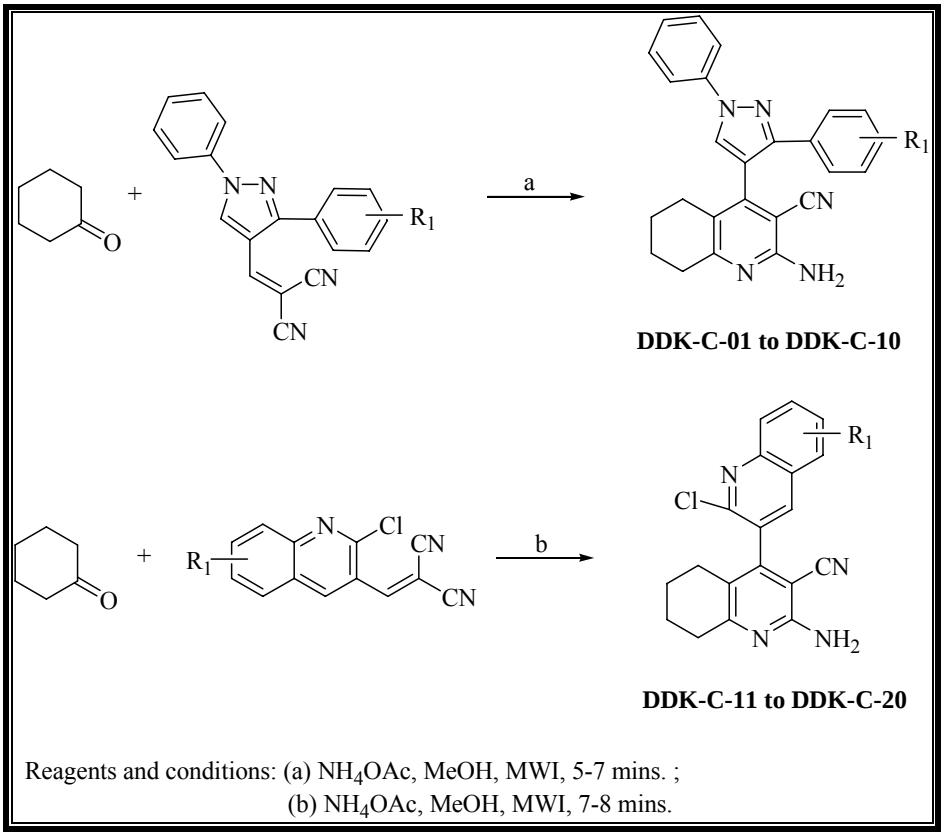
Scheme 5.1.2.10

5.1.3 Current Work

The chemistry of quinoline and its derivatives has been studied for over a century due to their diverse biological activities. Among various quinoline derivatives, 5,6,7,8-tetrahydroquinoline derivatives draw a special attention for their wide spectrum biological activities viz. anti-microbial, anti-cancer, anti-HIV, anti-inflammatory, antimalarial and antidepressant etc.

Keeping in mind various biomedical applications and with a view to further assess the pharmacological profile of this class of compounds, two novel series of 5,6,7,8-tetrahydroquinolines (**DDK-C-01 to DDK-C-20**) have been synthesized. The synthesis of 5,6,7,8-tetrahydroquinolines (**DDK-C-01 to DDK-C-10**) and (**DDK-C-11 to DDK-C-20**) was achieved by the one pot microwave-assisted reaction of cyclohexanone with 2-((3-(aryl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)malononitrile & 2-((2-chloro-substitutedquinolin-3-yl)methylene)malononitrile respectively and excess of ammonium acetate. The products were characterized by FT-IR, mass, ^{1}H NMR spectroscopy and elemental analyses. The newly synthesized compounds were subjected to antimicrobial activity.

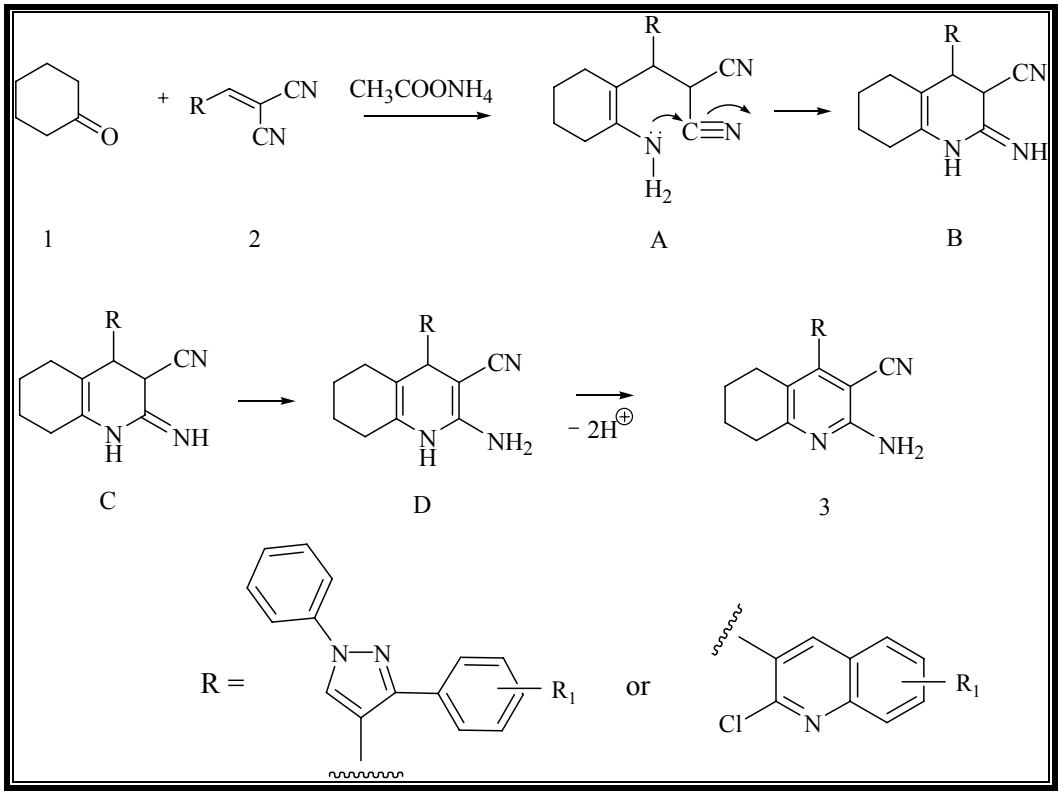
5.1.4 Reaction scheme



Code	R_1	M.F.	M.W.	M.P. ºC	Yield %	R_{f1}	R_{f2}
DDK-C-01	H	$C_{25}H_{21}N_5$	391	237-239	73	0.48	0.62
DDK-C-02	4-Cl	$C_{25}H_{20}ClN_5$	425	209-211	78	0.47	0.65
DDK-C-03	3-NO_2	$C_{25}H_{20}N_6O_2$	436	199-201	74	0.51	0.69
DDK-C-04	4-NO_2	$C_{25}H_{20}N_6O_2$	436	213-215	75	0.50	0.64
DDK-C-05	2-OCH_3	$C_{26}H_{23}N_5O$	421	237-239	61	0.48	0.63
DDK-C-06	4-OCH_3	$C_{26}H_{23}N_5O$	421	207-209	81	0.55	0.69
DDK-C-07	2-OH	$C_{25}H_{21}N_5O$	407	223-225	62	0.52	0.68
DDK-C-08	4-OH	$C_{25}H_{21}N_5O$	407	201-203	72	0.51	0.66
DDK-C-09	4-F	$C_{25}H_{20}FN_5$	409	207-209	81	0.52	0.67
DDK-C-10	4-Br	$C_{25}H_{20}Br\ N_5$	470	233-235	78	0.52	0.69
DDK-C-11	4-F	$C_{19}H_{14}ClFN_4$	352	196-198	78	0.52	0.68
DDK-C-12	3-Cl	$C_{19}H_{14}Cl_2N_4$	369	219-221	77	0.53	0.63
DDK-C-13	4-Cl	$C_{26}H_{26}Cl_2N_2O_2$	369	195-197	80	0.51	0.56
DDK-C-14	2-OCH_3	$C_{20}H_{17}ClN_4O$	364	214-216	59	0.53	0.60
DDK-C-15	3-OCH_3	$C_{20}H_{17}ClN_4O$	364	203-205	71	0.59	0.70
DDK-C-16	4-OCH_3	$C_{20}H_{17}ClN_4O$	364	238-240	80	0.54	0.61
DDK-C-17	3-CH_3	$C_{20}H_{17}ClN_4$	348	229-231	69	0.51	0.62
DDK-C-18	4-CH_3	$C_{20}H_{17}ClN_4$	348	205-207	81	0.52	0.63
DDK-C-19	4-NO_2	$C_{19}H_{14}ClN_5O_2$	379	239-241	65	0.52	0.60
DDK-C-20	4-Br	$C_{19}H_{14}BrClN_2O_2$	413	242-244	79	0.55	0.64

TLC Solvent system R_{f1}: Hexane: Ethyl acetate – 6:4,
TLC Solvent system R_{f2}: Chloroform:Methanol – 9.5:0.5.

5.1.5 Mechanism



The probable mechanism involves the synthesis of 5,6,7,8-tetrahydroquinolines via formation of the intermediate Michael type products, followed by intramolecular cyclization and aromatization as suggested by Elkholy et al. [40].

5.1.6 Experimental

5.1.6.1 Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. Microwave assisted reactions were carried out in QPro-M microwave synthesizer. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

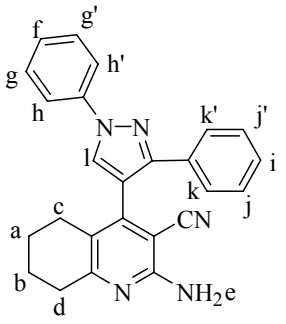
5.1.6.2 Synthesis of 2-((3-(aryl)-1-phenyl-1H-pyrazol-4-yl)methylene)malononitriles

A mixture of 3-(aryl)-1-phenyl-1H-pyrazole-4-carbaldehyde (0.01 mol) and malononitrile (0.01 mol) in ethanol (3 mL) in presence of catalytic amount of piperidine (4-5 drops) was subjected to microwave irradiation at 80 °C for 1-2 min. The microwave irradiation was operated in 30-second cycles. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the separated solid was filtered, dried and crystallized from ethanol.

5.1.6.3 General procedure for the synthesis of 2-Amino-4-[3-(aryl)-1-phenyl-1H-pyrazol-4-yl]-5,6,7,8-tetrahydro-quinoline-3-carbonitriles (DDK-C-01 to DDK-C-10)

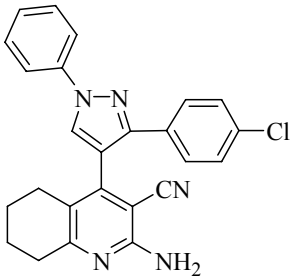
A mixture of the cyclohexanone (0.01 mol), 2-((3-(aryl)-1-phenyl-1H-pyrazol-4-yl)methylene)malononitrile (0.01 mol) and ammonium aceate (0.08 mol) in ethanol (5 mL) was irradiated under microwave irradiation at 80 °C for 5-7 min. The microwave irradiation was operated in 30-second cycles. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the separated solid was filtered, dried and crystallized from ethanol.

5.1.6.3.1 2-Amino-4-(1,3-diphenyl-1H-pyrazol-4-yl)-5,6,7,8-tetrahydro-quinoline-3-carbonitrile (DDK-C-01)



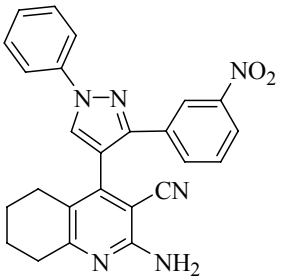
Yield: 73%; m.p. 237-239 ºC; IR (cm^{-1}): 3416 and 3286 (N-H stretching of primary amine), 3062 (C-H stretching of aromatic ring), (C-H stretching of CH_2 group of cyclohexane ring) 2214 (C≡N stretching of nitrile group), 1644 (N-H deformation of NH_2 group), 1562, 1506 and 1462 (C=C stretching of aromatic ring), 1246 (C-N stretching of primary amine), 1074 (C-H in plane bending for aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 1.54-1.81 (m, 4H, $H_{a, b}$), 2.74-2.86 (m, 2H, H_c), 2.35-2.42 (m, 2H, H_d), 6.55 (s, 1H, H_e), 7.30-7.54 (m, 8H, $H_{f, g, g', h, h', i, i', j, j'}$), 7.87-7.90 (m, 2H, $H_{k, k'}$), 8.38 (s, 1H, H_l); MS: *m/z* 391; Anal. Calcd. for $C_{25}H_{21}N_5$: C, 76.70; H, 5.41; N, 17.89. Found: C, 76.62; H, 5.34; N, 17.81%

5.1.6.3.2 2-Amino-4-[3-(4-chloro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6,7,8-tetra hydro-quinoline-3-carbonitrile (DDK-C-02)



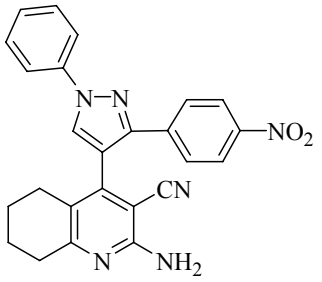
Yield: 78%; m.p. 209-211 ºC; MS: *m/z* 425; Anal. Calcd. for $C_{25}H_{20}ClN_5$: C, 70.50; H, 4.73; N, 16.44. Found: C, 70.43; H, 4.65; N, 16.36%.

5.1.6.3.3 2-amino-5,6,7,8-tetrahydro-4-(3-(3-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)quinoline-3-carbonitrile (DDK-C-03)



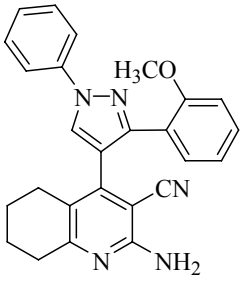
Yield: 74%; m.p. 199-201 ºC; MS: *m/z* 436; Anal. Calcd. for $C_{25}H_{20}N_6O_2$: C, 68.80; H, 4.62; N, 19.25. Found: C, 68.72; H, 4.53; N, 19.18%.

5.1.6.3.4 2-amino-5,6,7,8-tetrahydro-4-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)quinoline-3-carbonitrile (DDK-C-04)



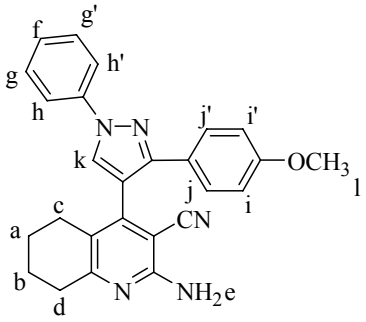
Yield: 75%; m.p. 213-215 ºC; MS: *m/z* 436; Anal. Calcd. for $C_{25}H_{20}N_6O_2$: C, 68.80; H, 4.62; N, 19.25. Found: C, 68.73; H, 4.54; N, 19.18%.

5.1.6.3.5 2-amino-5,6,7,8-tetrahydro-4-(3-(2-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)quinoline-3-carbonitrile (DDK-C-05)



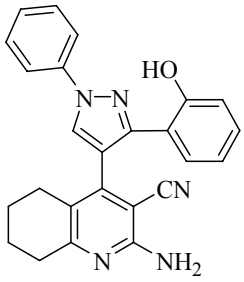
Yield: 61%; m.p. 237-239 ºC; MS: *m/z* 421; Anal. Calcd. for $C_{26}H_{23}N_5O$: C, 74.09; H, 5.50; N, 16.62. Found: C, 74.02; H, 5.43; N, 16.55%.

5.1.6.3.6 2-amino-5,6,7,8-tetrahydro-4-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)quinoline-3-carbonitrile (DDK-C-06)



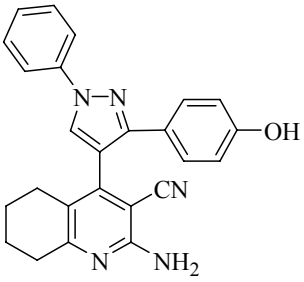
Yield: 81%; m.p. 207-209 ºC; IR (cm^{-1}): 3396 and 3381 (N-H stretching of primary amine), 3014 (C-H stretching of aromatic ring), 2981 (C-H stretching of CH_2 group of cyclohexane ring), 2214 (C≡N stretching of nitrile group), 1649 (N-H deformation of NH_2 group), 1570, 1524 and 1454 (C=C stretching of aromatic ring), 1342 (C-N stretching of primary amine), 1230 (C-O-C asymmetrical stretching of OCH_3 group), 1080 (C-O-C symmetrical stretching of OCH_3 group), 966 (C-H in plane bending for aromatic ring); 1.54-2.72 (m, 8H, $H_{a, b, c, d}$), 6.28 (s, 1H, H_e), 7.27-7.45 (m, 5H, $H_{f, g, g', h, h'}$), 6.80-6.82 (d, 2H, $H_{i, i'}$, J = 7.4 Hz), 7.83-7.85 (d, 2H, $H_{j, j'}$, J = 6.72 Hz), 8.34 (s, 1H, H_k), 3.75 (s, 3H, H_l); MS: *m/z* 421; Anal. Calcd. for $C_{26}H_{23}N_5O$: C, 74.09; H, 5.50; N, 16.62. Found: C, 74.01; H, 5.44; N, 16.54%.

5.1.6.3.7 2-amino-5,6,7,8-tetrahydro-4-(3-(2-hydroxyphenyl)-1-phenyl-1H-pyrazol -4-yl)quinoline-3-carbonitrile (DDK-C-07)



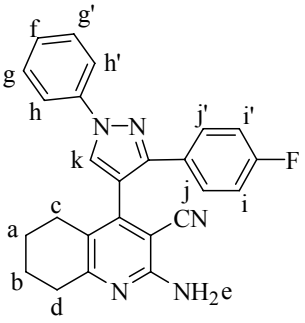
Yield: 62%; m.p. 223-225 ºC; MS: *m/z* 407; Anal. Calcd. for $C_{25}H_{21}N_5O$: C, 73.69; H, 5.19; N, 17.19. Found: C, 73.61; H, 5.12; N, 17.11%.

5.1.6.3.8 2-amino-5,6,7,8-tetrahydro-4-(3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol -4-yl)quinoline-3-carbonitrile (DDK-C-08)



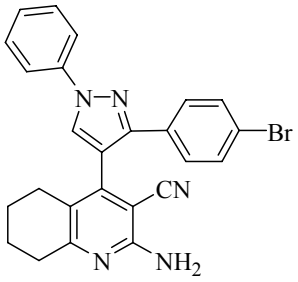
Yield: 72%; m.p. 201-203 ºC; MS: *m/z* 407; Anal. Calcd. for $C_{25}H_{21}N_5O$: C, 73.69; H, 5.19; N, 17.19. Found: C, 73.62; H, 5.12; N, 17.12%.

5.1.6.3.9 2-amino-4-(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-5,6,7,8-tetra hydroquinoline-3-carbonitrile (DDK-C-09)



Yield: 81%; m.p. 207-209 ºC; IR (cm^{-1}): 3487 and 3392 (N-H stretching of primary amine), 3045 (C-H stretching of aromatic ring), 2974 (C-H stretching of CH_2 group of cyclohexane ring), 2837 (C-H symmetrical stretching of CH_2 group), 2214 (C≡N stretching of nitrile group), 1651 (N-H deformation of NH_2 group), 1560, 1504 and 1456 (C=C stretching of aromatic ring), 1232 (C-N stretching of primary amine), 1080 (C-F stretching), 964 (C-H out of plane bending for aromatic ring). ^{1}H NMR (DMSO-d_6) δ ppm: : 1.57-1.62 (m, 2H, H_a), 1.69-1.79 (m, 2H, H_b), 2.75-2.80 (m, 2H, H_c), 2.06-2.13 (m, 1H, H_d), 2.36-2.41 (m, 1H, H_d), 6.34 (s, 1H, H_e), 7.32-7.36 (m, 1H, H_f), 7.48-7.53 (m, 4H, $H_{g, g', h, h'}$), 7.04-7.09 (t, 2H, $H_{i, i'}$), 7.87-7.90 (m, 2H, $H_{j, j'}$), 8.40 (s, 1H, H_k); MS: *m/z* 409; Anal. Calcd. for $C_{25}H_{20}FN_5$: C, 73.33; H, 4.92; N, 17.10. Found: C, 73.27; H, 4.85; N, 17.02%.

5.1.6.3.10 2-amino-4-(3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-5,6,7,8-tetra hydroquinoline-3-carbonitrile (DDK-C-10)



Yield: 78%; m.p. 233-235 ºC; MS: *m/z* 470; Anal. Calcd. for $C_{25}H_{20}BrN_5$: C, 63.84; H, 4.29; N, 14.89. Found: C, 63.76; H, 4.23; N, 14.81%.

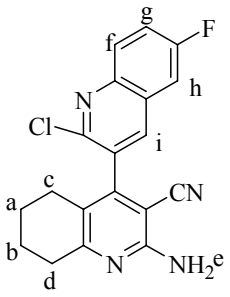
5.1.6.4 General procedure for the synthesis of 2-((2-chloro-substitutedquinolin-3-yl)methylene)malononitriles

A mixture of the substituted 2-chloroquinoline-3-carbaldehyde (0.01 mol) and malononitrile (0.01 mol) in ethanol (3 mL) in presence of catalytic amount of piperidine (4-5 drops) was subjected to microwave irradiation at 80 °C for 1-2 min. The microwave irradiation was operated in 30-second cycles. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the separated solid was filtered, dried and crystallized from ethanol.

5.1.6.5 General procedure for the synthesis of 2-Amino-5,6,7,8-tetrahydro-4-quinoline-3-carbonitriles (DDK-C-11 to DDK-C-20)

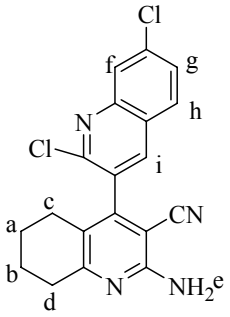
A mixture of the cyclohexanone (0.01 mol), 2-((2-chloro-substitutedquinolin-3-yl)methylene)malononitrile (0.01 mol) and ammonium aceate (0.08 mol) in ethanol (5 mL) was irradiated under microwave irradiation at 80 °C for 5-7 min. The microwave irradiation was operated in 30-second cycles. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the separated solid was filtered, dried and crystallized from ethanol.

5.2.6.5.1 2-amino-4-(2-chloro-6-fluoroquinolin-3-yl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (DDK-C-11)



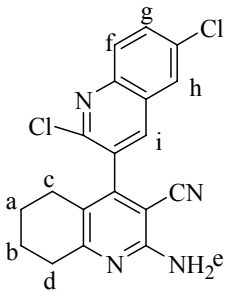
Yield: 78%; m.p. 196-198 ºC; IR (cm^{-1}): 3391 and 3296 (N-H stretching of primary amine), 3043 (C-H stretching of aromatic ring), 2922 (C-H stretching of CH_2 group of cyclohexanone ring), 2214 (C≡N stretching of nitrile group), 1645 (N-H deformation of NH_2 group), 1587, 1558 and 1446 (C=C stretching of aromatic ring), 1222 (C-N stretching of primary amine), 1062 (C-H out of plane bending for aromatic ring), 756 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 1.56-1.62 (m, 2H, H_a), 1.69-1.79 (m, 2H, H_b), 2.76-2.81 (m, 2H, H_c), 2.06-2.14 (m, 1H, H_d), 2.36-2.41 (m, 1H, H_d), 6.34 (s, 1H, H_e), 7.75-7.77 (m, 1H, H_f), 7.32-7.39 (m, 1H, H_g), 7.47-7.49 (d, 1H, H_h), 7.90 (s, 1H, H_i); MS: *m/z* 352; Anal. Calcd. for $C_{19}H_{14}ClFN_4$: C, 64.68; H, 4.00; N, 15.88. Found: C, 64.62; H, 3.93; N, 15.81%

5.2.6.5.2 2-amino-4-(2,7-dichloroquinolin-3-yl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (DDK-C-12)



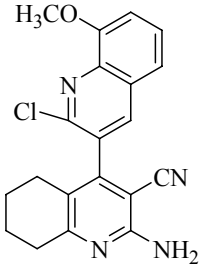
Yield: 78%; m.p. 219-221 ºC; IR (cm^{-1}): 3393 and 3360 (N-H stretching of primary amine), 3059 (C-H stretching of aromatic ring), 2952 (C-H stretching of CH_2 group of cyclohexanone ring), 2212 (C≡N stretching of nitrile group), 1646 (N-H deformation of NH_2 group), 1554, 1502 and 1444 (C=C stretching of aromatic ring), 1226 (C-N stretching of primary amine), 1010 (C-H out of plane bending for aromatic ring), 734 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 1.54-1.82 (m, 4H, $H_{a, b}$), 2.06-2.14 (m, 1H, H_d), 2.35-2.42 (m, 1H, H_d), 2.74-2.86 (m, 2H, H_c), 6.33 (s, 1H, H_e), 8.04-8.07 (d, 1H, H_f), 7.51-7.54 (m, 1H, H_g), 7.77-7.78 (d, 1H, H_h), 8.17 (s, 1H, H_i); MS: *m/z* 368; Anal. Calcd. for $C_{19}H_{14}Cl_2N_4$: C, 61.80; H, 3.82; N, 15.17. Found: C, 61.73; H, 3.74; N, 15.08%.

5.2.6.5.3 2-amino-4-(2,6-dichloroquinolin-3-yl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (DDK-C-13)



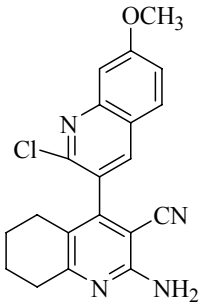
Yield: 80%; m.p. 195-197 ºC;): 3489 and 3392 (N-H stretching of primary amine), 3055 (C-H stretching of aromatic ring), 2943 (C-H stretching of CH_2 group of cyclohexane ring), 2208 (C≡N stretching of nitrile group), 1647 (N-H deformation of NH_2 group), 1543, 1506 and 1467 (C=C stretching of aromatic ring), 1236 (C-N stretching of primary amine), 1064 (C-H out of plane bending for aromatic ring), 721 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 1.61-1.79 (m, 4H, $H_{a, b}$), 2.75-2.80 (m, 2H, H_c), 2.06-2.14 (m, 1H, H_d), 2.36-2.41 (m, 1H, H_d), 6.33 (s, 1H, H_e), 7.83-7.85 (m, 2H, $H_{f, h}$), 7.68-7.71 (m, 1H, H_g), 8.10 (s, 1H, H_i); MS: *m/z* 368; Anal. Calcd. for $C_{19}H_{14}Cl_2N_4$: C, 61.80; H, 3.82; N, 15.17. Found: C, 61.73; H, 3.73; N, 15.09%.

5.1.6.5.4 2-amino-4-(2-chloro-8-methoxyquinolin-3-yl)-5,6,7,8-tetrahydro quinoline-3-carbonitrile (DDK-C-14)



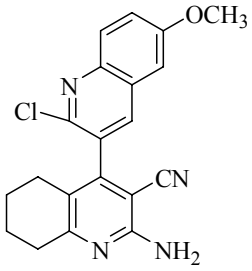
Yield: 59%; m.p. 214-216 ºC; MS: *m/z* 364; Anal. Calcd. for $C_{20}H_{17}ClN_4O$: C, 65.84; H, 4.70; N, 15.36. Found: C, 65.77; H, 4.62; N, 15.27%

5.2.6.5.5 2-amino-4-(2-chloro-7-methoxyquinolin-3-yl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (DDK-C-15)



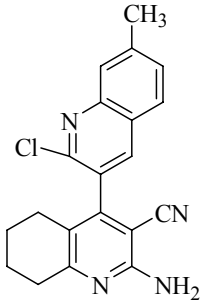
Yield: 71%; m.p. 203-205 ºC; MS: *m/z* 364; Anal. Calcd. for $C_{20}H_{17}ClN_4O$: C, 65.84; H, 4.70; N, 15.36. Found: C, 65.76; H, 4.61; N, 15.29%

5.2.6.5.6 2-amino-4-(2-chloro-6-methoxyquinolin-3-yl)-5,6,7,8-tetrahydro quinoline-3-carbonitrile (DDK-C-16)



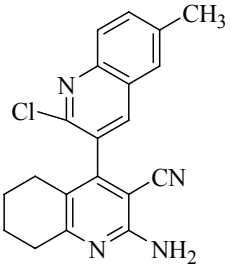
Yield: 80%; m.p. 238-240 ºC; MS: *m/z* 364; Anal. Calcd. for $C_{20}H_{17}ClN_4O$: C, 65.84; H, 4.70; N, 15.36. Found: C, 65.76; H, 4.62; N, 15.28%

5.2.6.5.7 2-amino-4-(2-chloro-7-methylquinolin-3-yl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (DDK-C-17)



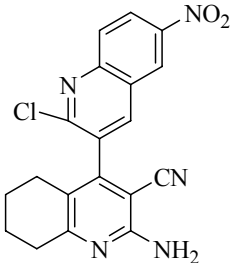
Yield: 81%; m.p. 205-207 ºC; MS: *m/z* 348; Anal. Calcd. for $C_{20}H_{17}ClN_4$: C, 68.86; H, 4.91; N, 16.06. Found: C, 68.78; H, 4.85; N, 15.00%.

5.2.6.5.8 2-amino-4-(2-chloro-6-methylquinolin-3-yl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (DDK-C-18)



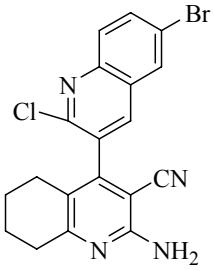
Yield: 69%; m.p. 229-231 ºC; MS: *m/z* 348; Anal. Calcd. for $C_{20}H_{17}ClN_4$: C, 68.86; H, 4.91; N, 16.06. Found: C, 68.77; H, 4.82; N, 15.99%.

5.2.6.5.9 2-amino-4-(2-chloro-6-nitroquinolin-3-yl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (DDK-C-19)



Yield: 65%; m.p. 239-241 ºC; MS: *m/z* 379; Anal. Calcd. for $C_{19}H_{14}ClN_5O_2$: C, 60.09; H, 3.72; N, 18.44. Found: C, 60.01; H, 3.64; N, 18.35%.

5.2.6.5.10 2-amino-4-(6-bromo-2-chloroquinolin-3-yl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (DDK-C-20)



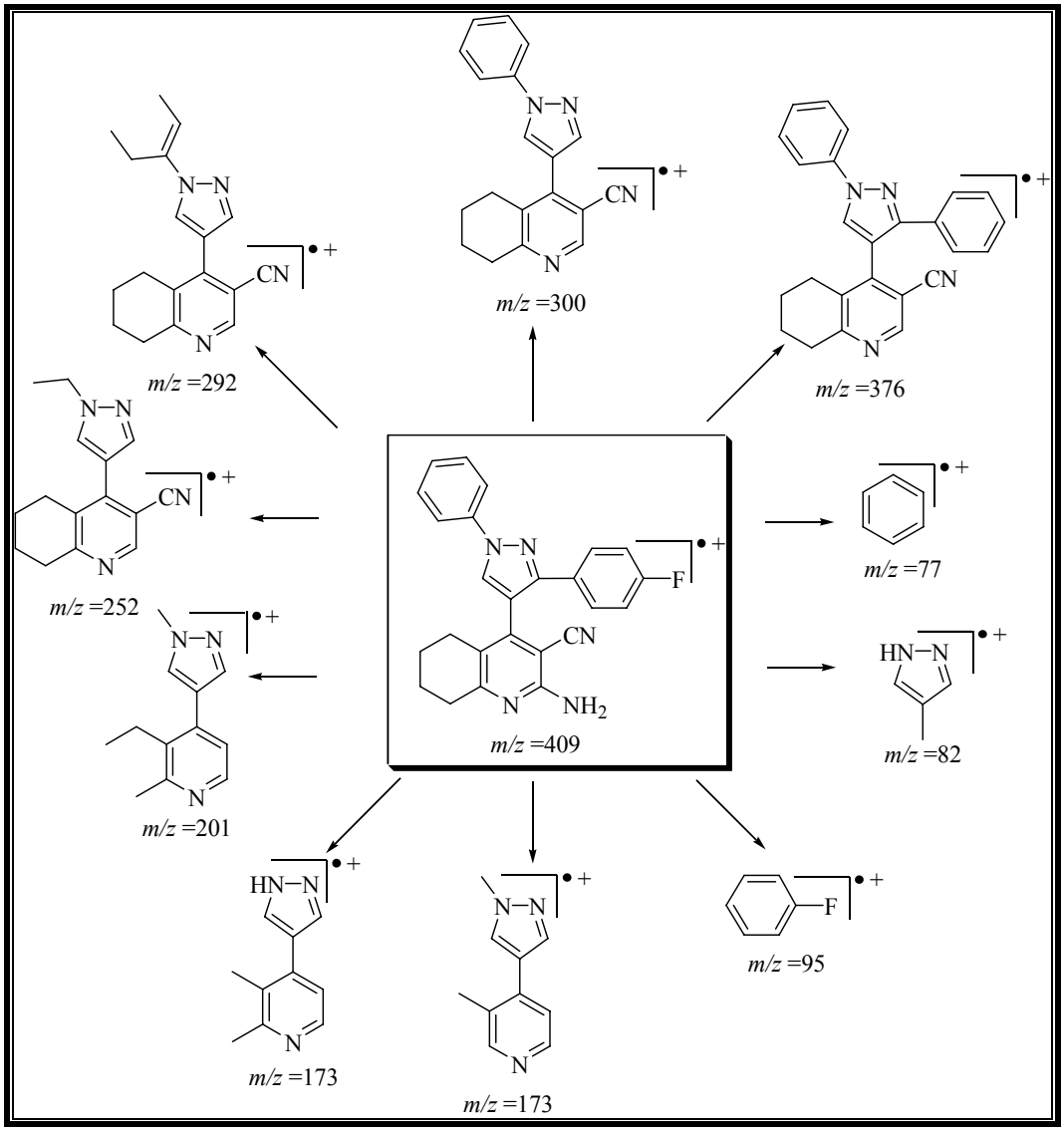
Yield: 79%; m.p. 242-244 ºC; MS: *m/z* 413; Anal. Calcd. for $C_{19}H_{14}BrClN_4$: C, 55.16; H, 3.41; N, 13.54. Found: C, 55.09; H, 3.33; N, 13.45%.

5.1.7 Spectral discussion

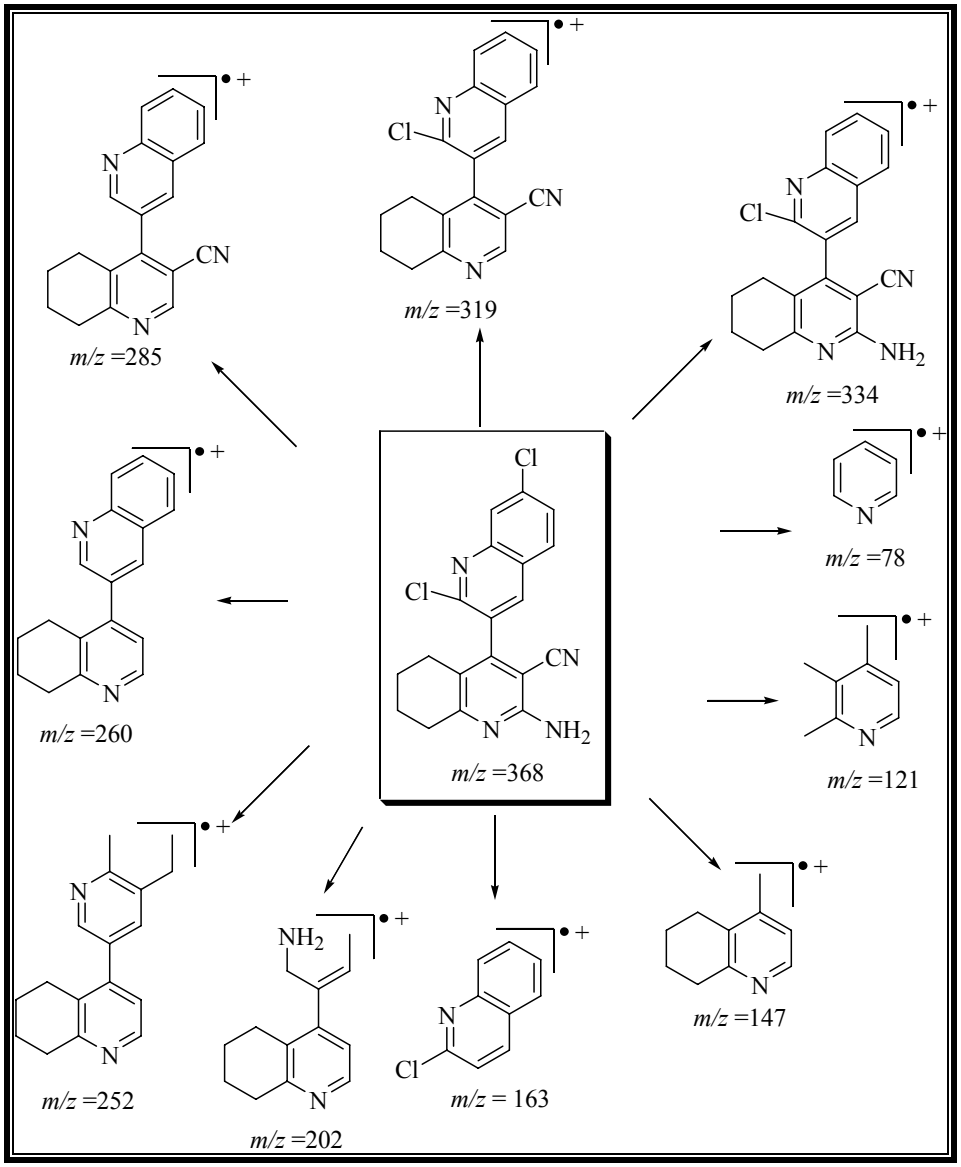
5.1.7.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of respective compound. Mass fragmentation pattern for a representative compound of each series is depicted below.

5.1.7.1.1 Mass fragmentation pattern for DDK-C-09



5.1.7.1.2 Mass fragmentation pattern for DDK-C-12



5.1.7.2 IR spectral study

IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For 5,6,7,8-tetrahydroquinolines (**DDK-C-01 to DDK-C-20**), two characteristic bands of primary amine were observed in the range of 3286-3489 cm^{-1} respectively. Another characteristic band of nitrile group was observed at 2208-2214 cm^{-1} suggesting formation of desired products (**DDK-C-01 to DDK-C-20**).

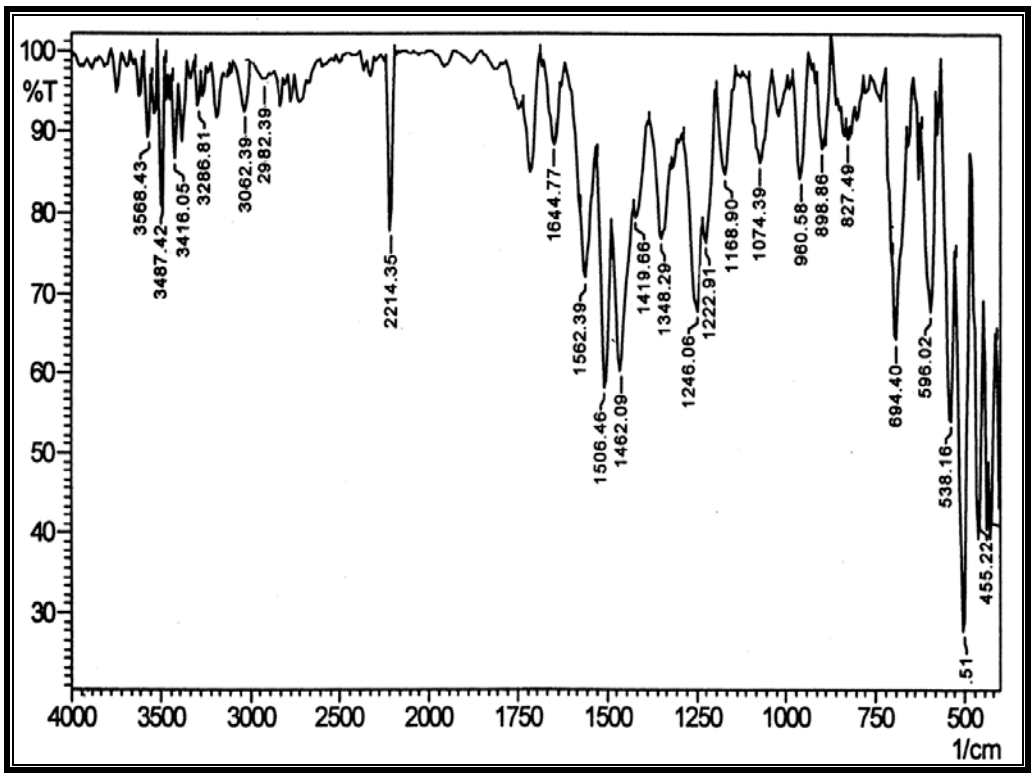
5.1.7.3 ^{1}H NMR spectral study

^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

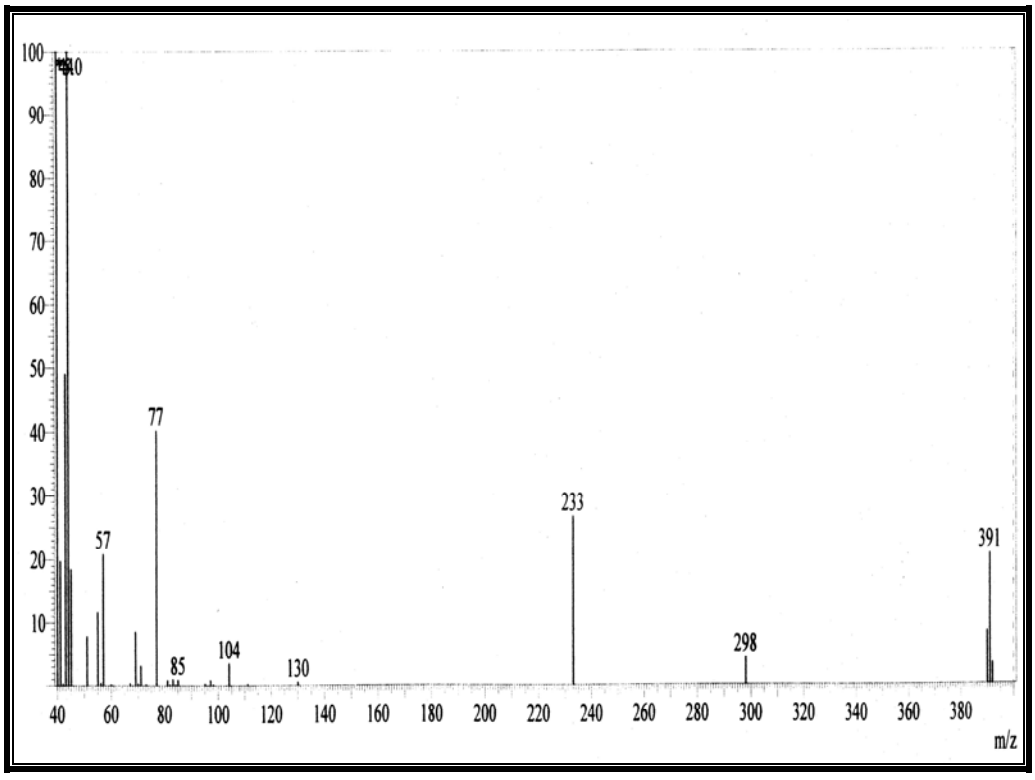
For 5,6,7,8-tetrahydroquinolines (**DDK-C-01 to DDK-C-10**), characteristic multiplets were observed for methylene groups of cyclohexane ring at 1.54-2.86 δ ppm. The aromatic ring protons were observed at 6.80-8.40 δ ppm and J value were found to be in accordance with substitution pattern on phenyl ring. The singlet for primary amine (-NH_2) proton was observed at 6.28-6.55 δ ppm.

While, for 5,6,7,8-tetrahydroquinolines (**DDK-C-11 to DDK-C-20**), characteristic multiplets were observed for methylene groups of cyclohexane ring at 1.56-2.81 δ ppm. The aromatic ring protons were observed at 7.32-8.17 δ ppm and J value were found to be in accordance with substitution pattern on phenyl ring. The singlet for primary amine (-NH_2) proton was observed at 6.33-6.34 δ ppm.

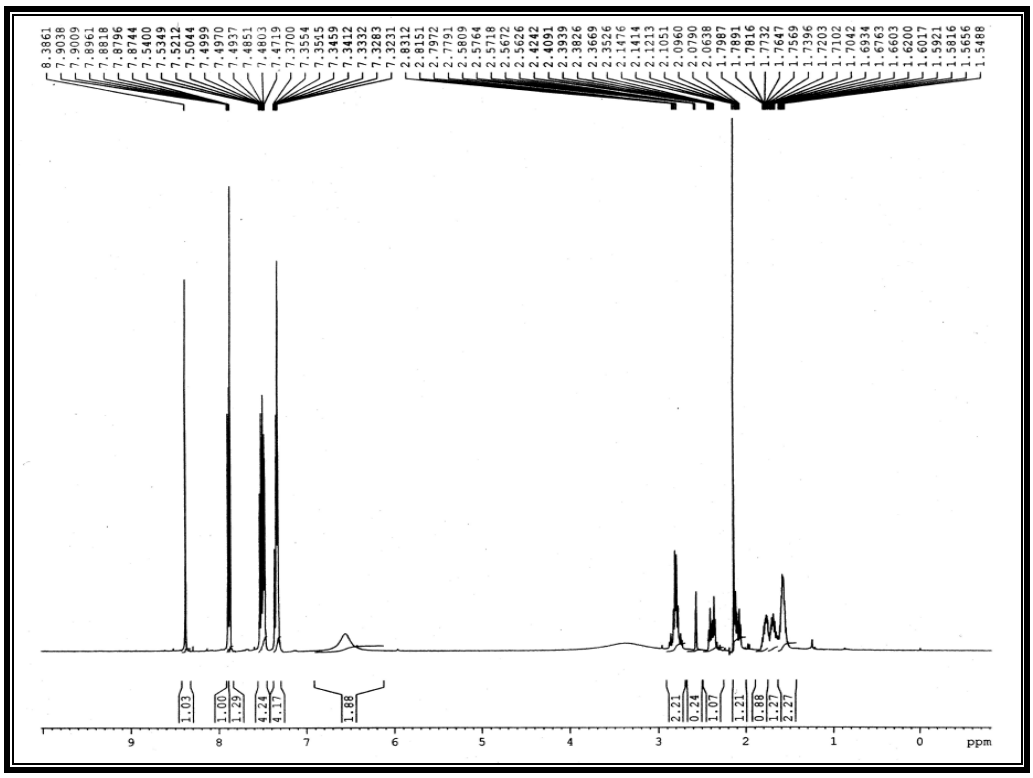
IR spectrum of DDK-C-01



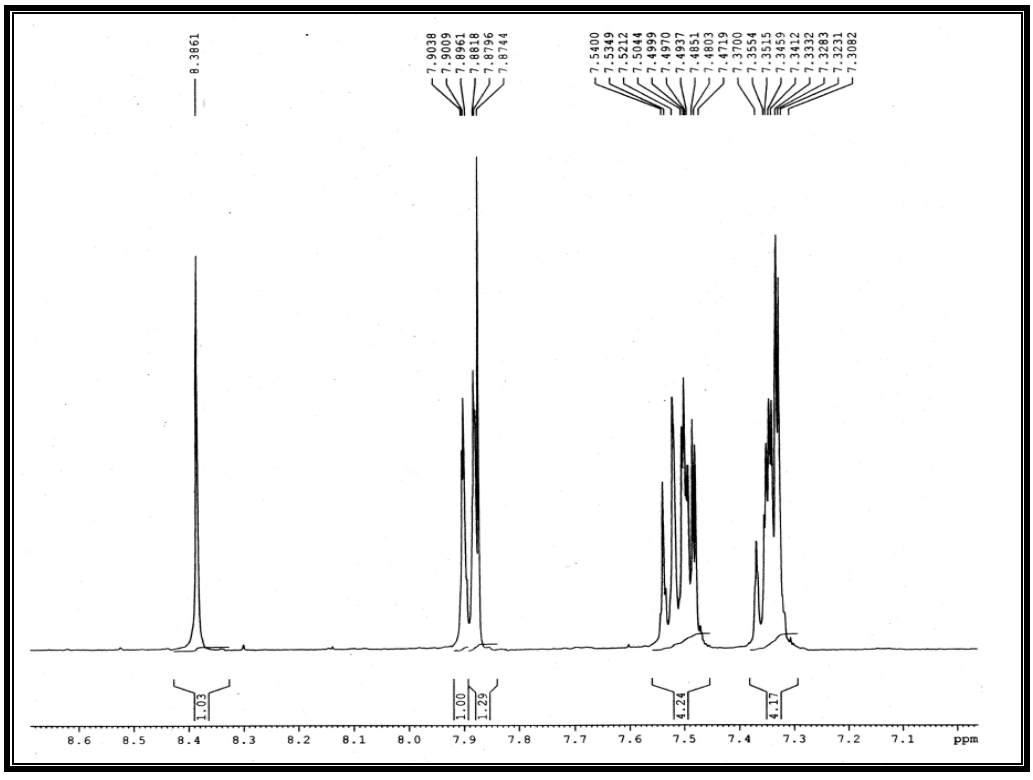
Mass spectrum of DDK-C-01



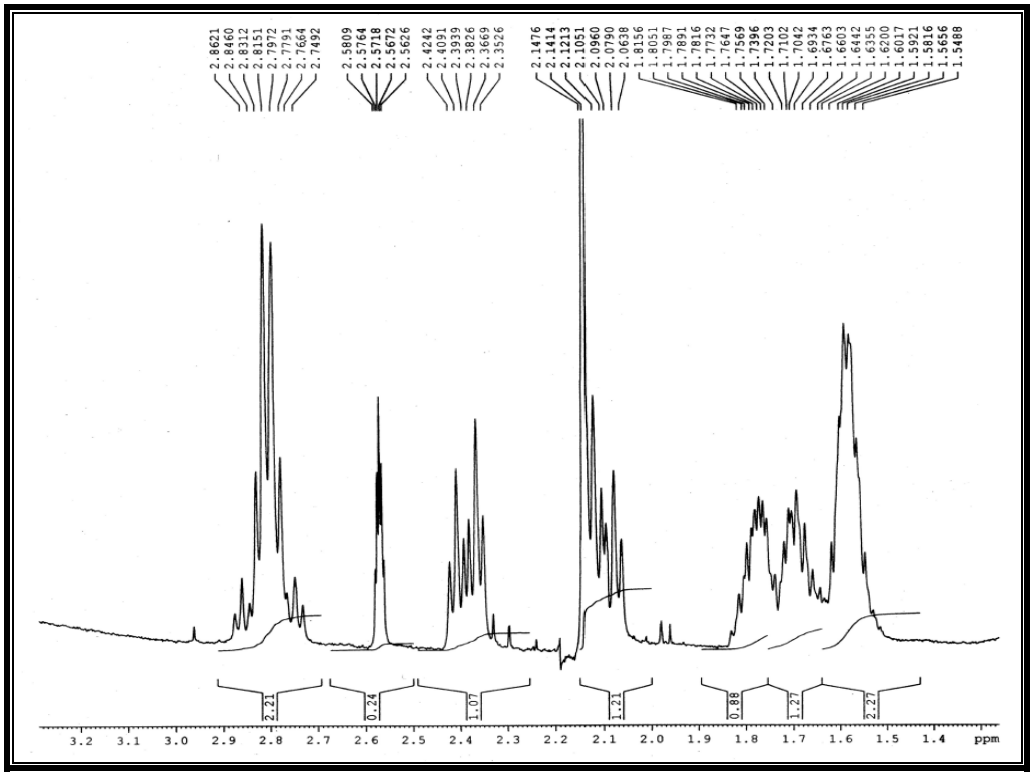
^{1}H NMR spectrum of DDK-C-01



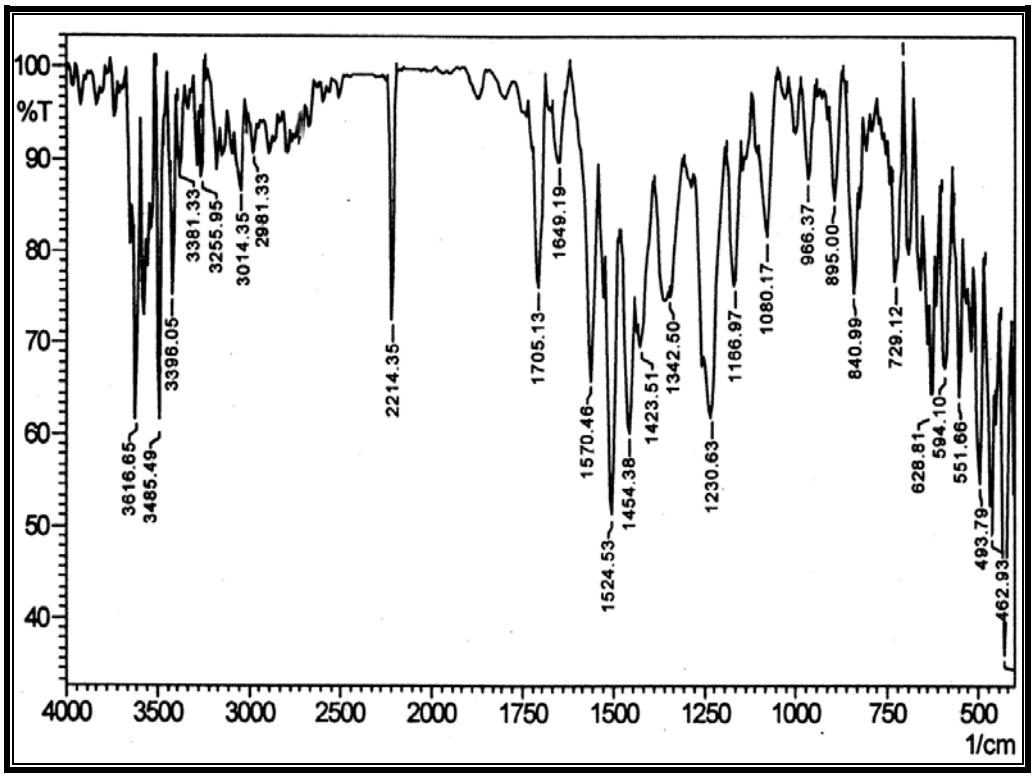
Expanded ^{1}H NMR spectrum of DDK-C-01



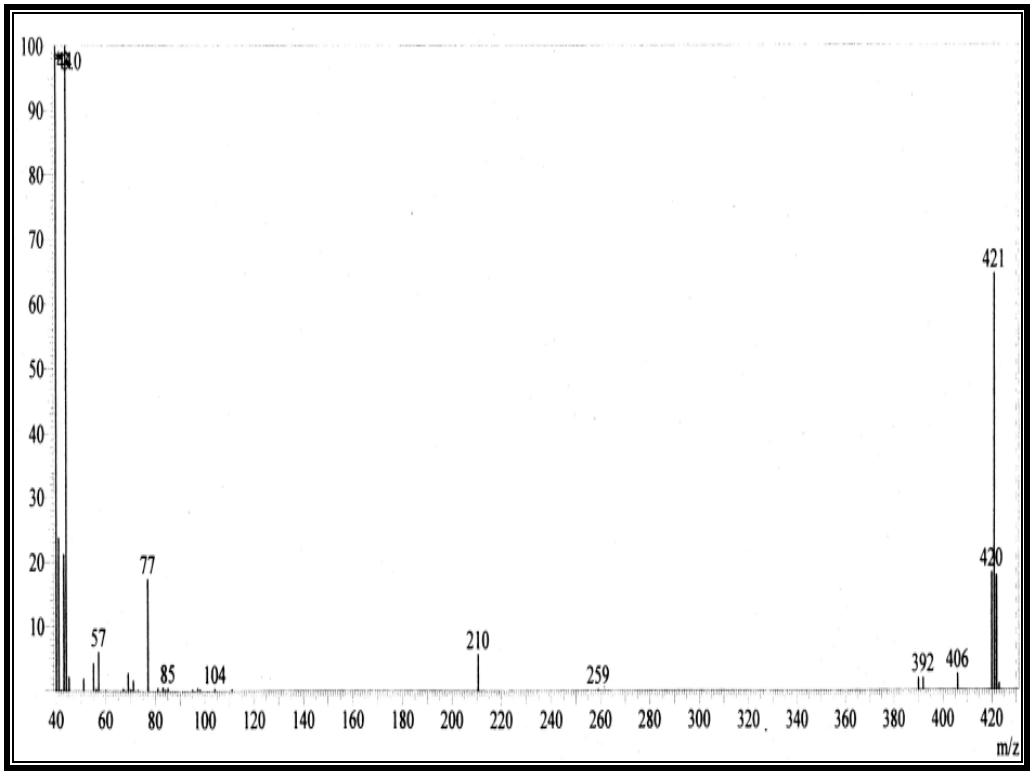
Expanded ^{1}H NMR spectrum of DDK-C-01



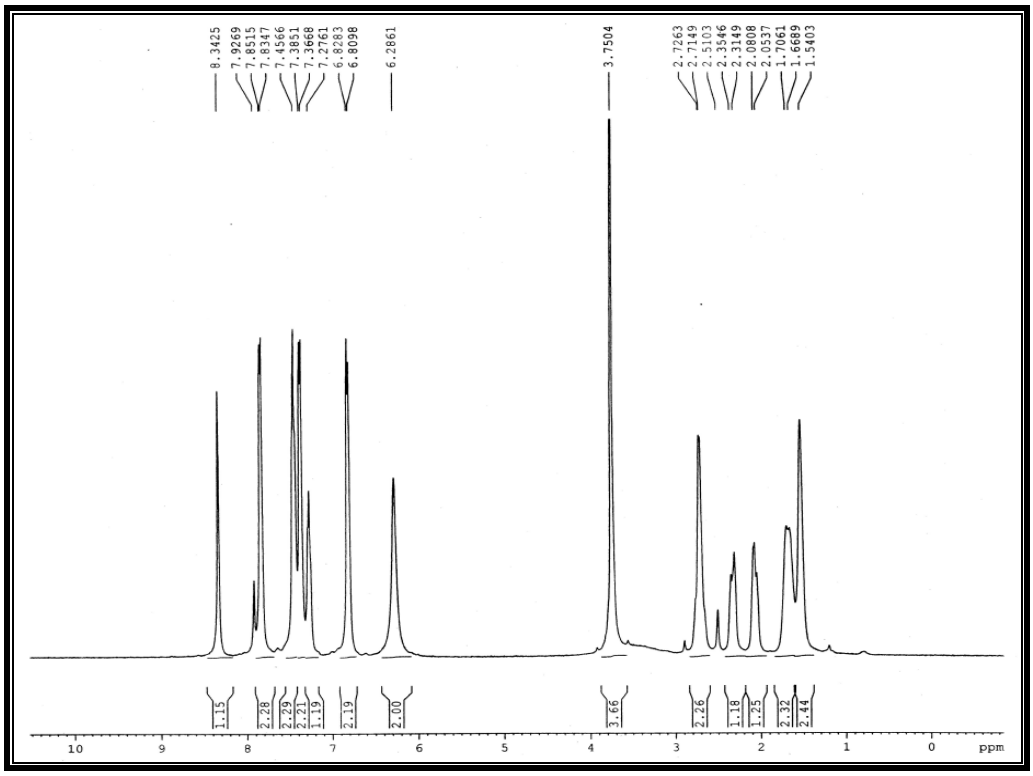
IR spectrum of DDK-C-06



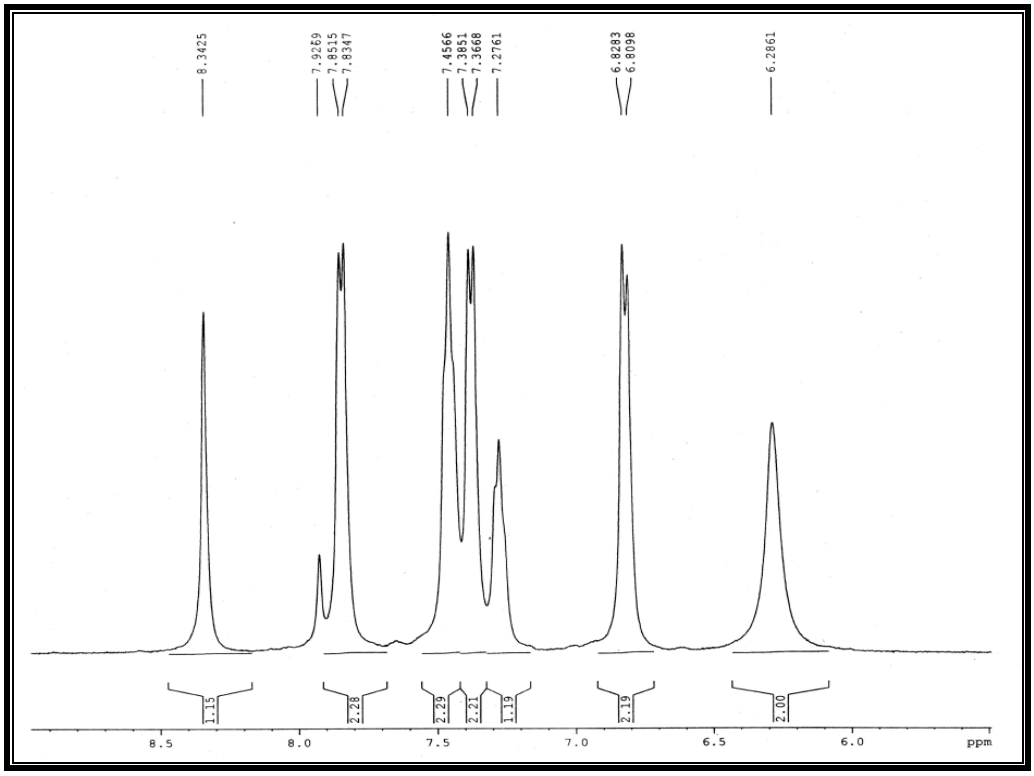
Mass spectrum of DDK-C-06



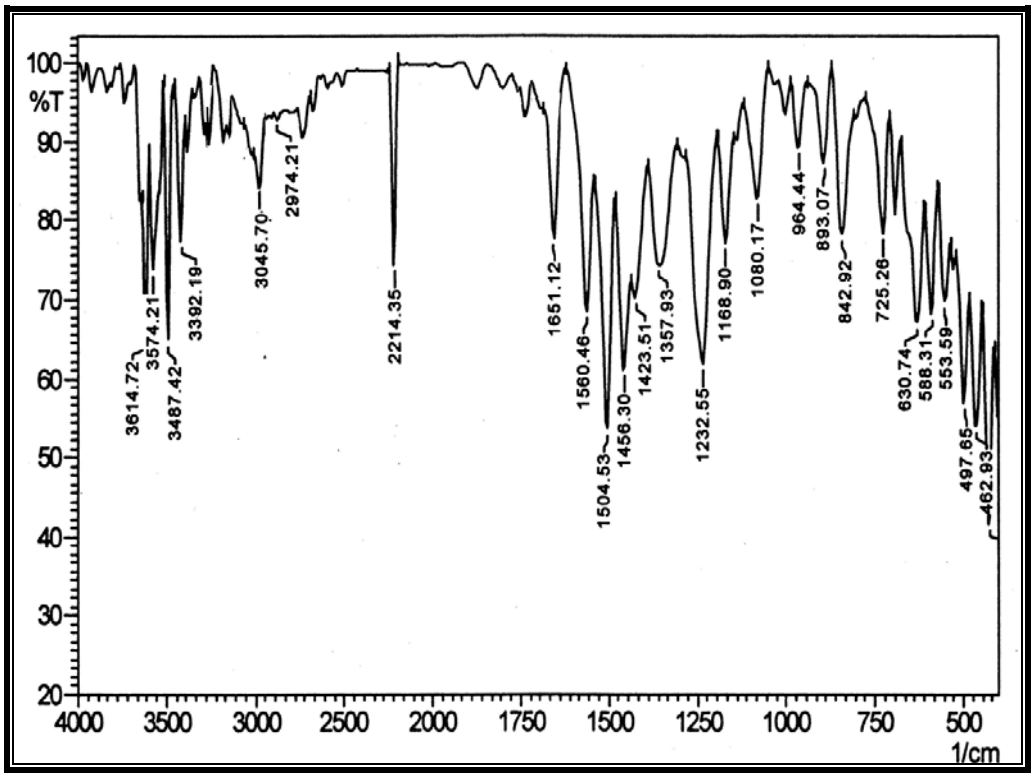
^{1}H NMR spectrum of DDK-C-06



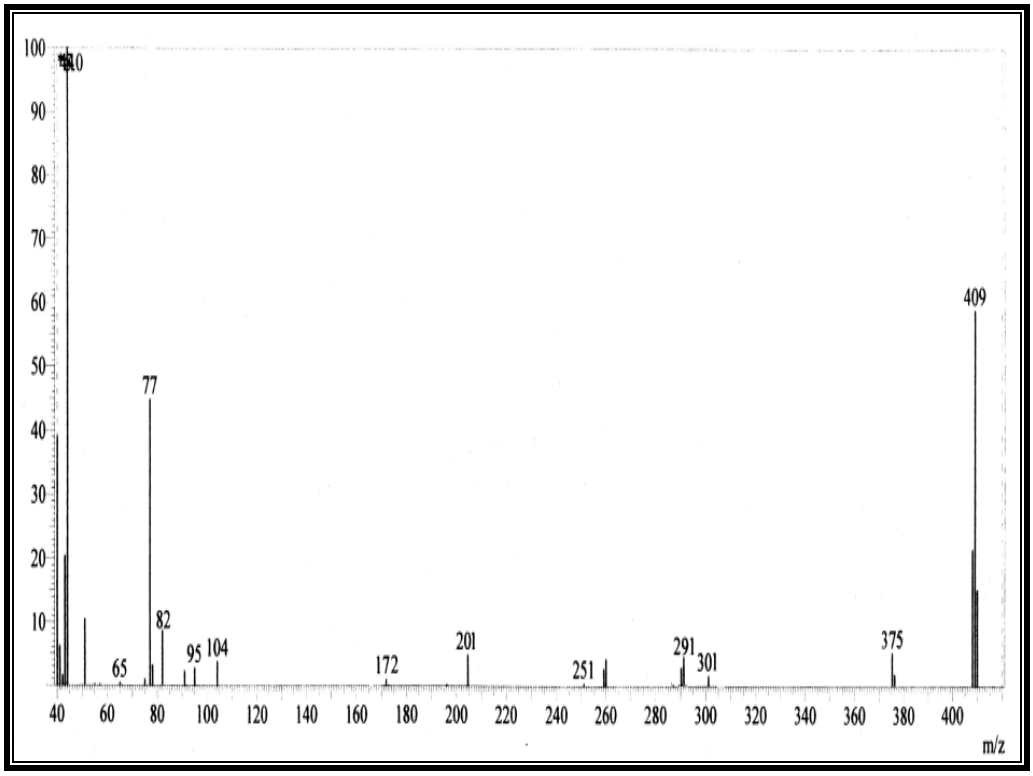
Expanded ^{1}H NMR spectrum of DDK-C-06



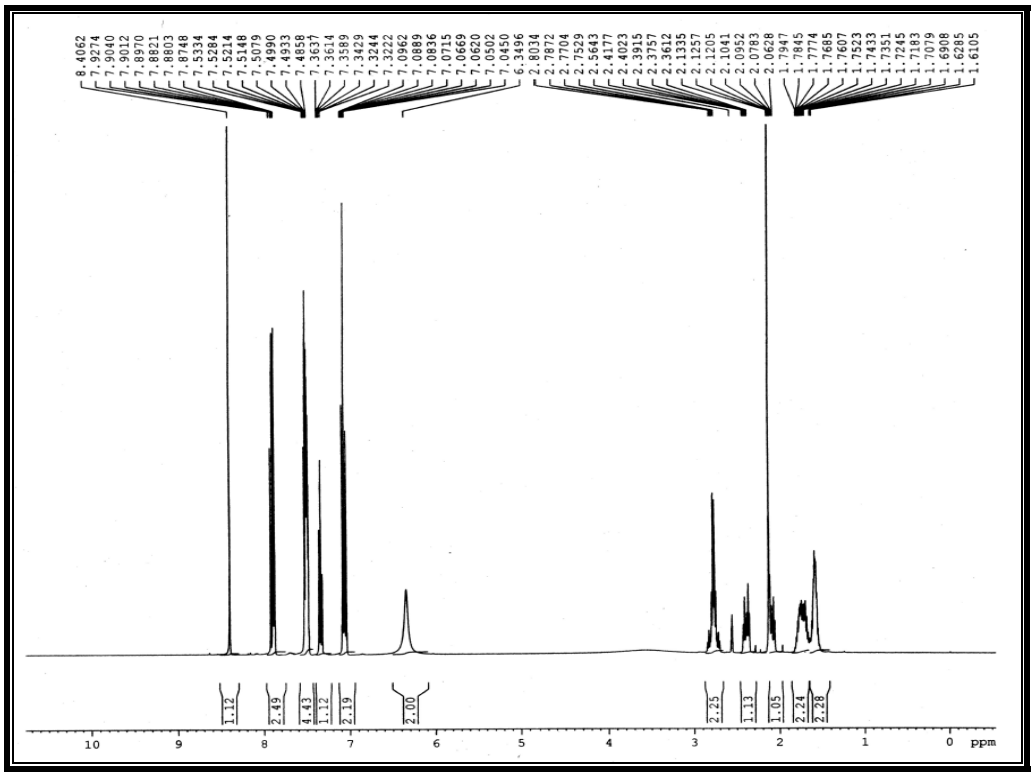
IR spectrum of DDK-C-09



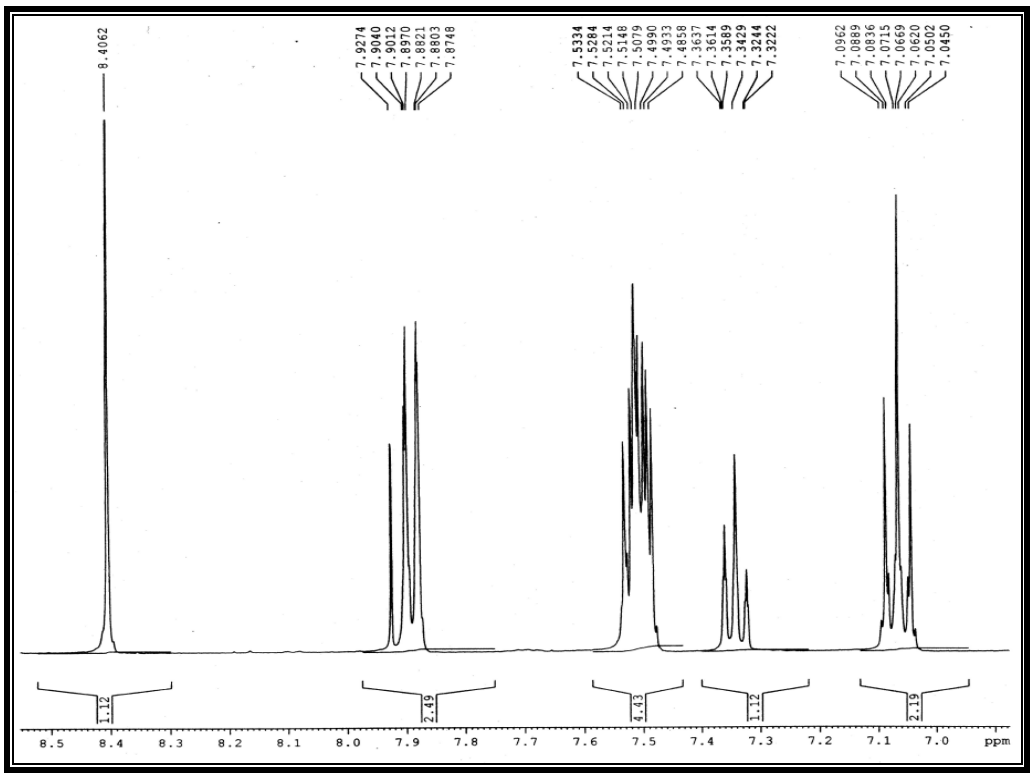
Mass spectrum of DDK-C-09



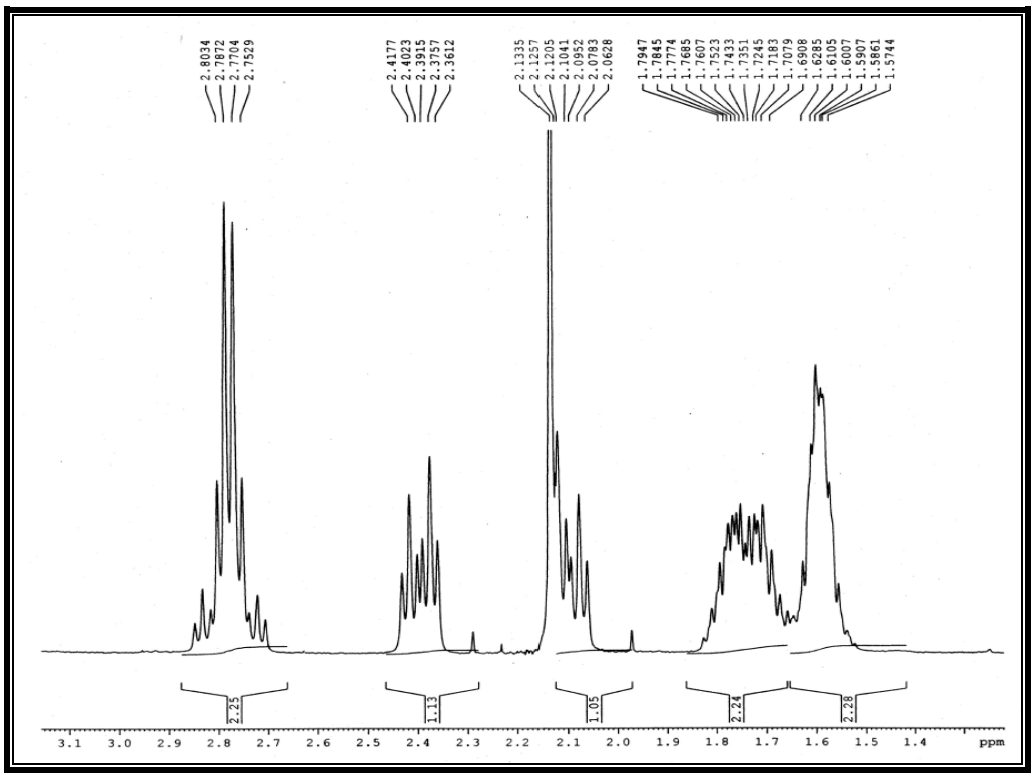
^{1}H NMR spectrum of DDK-C-09



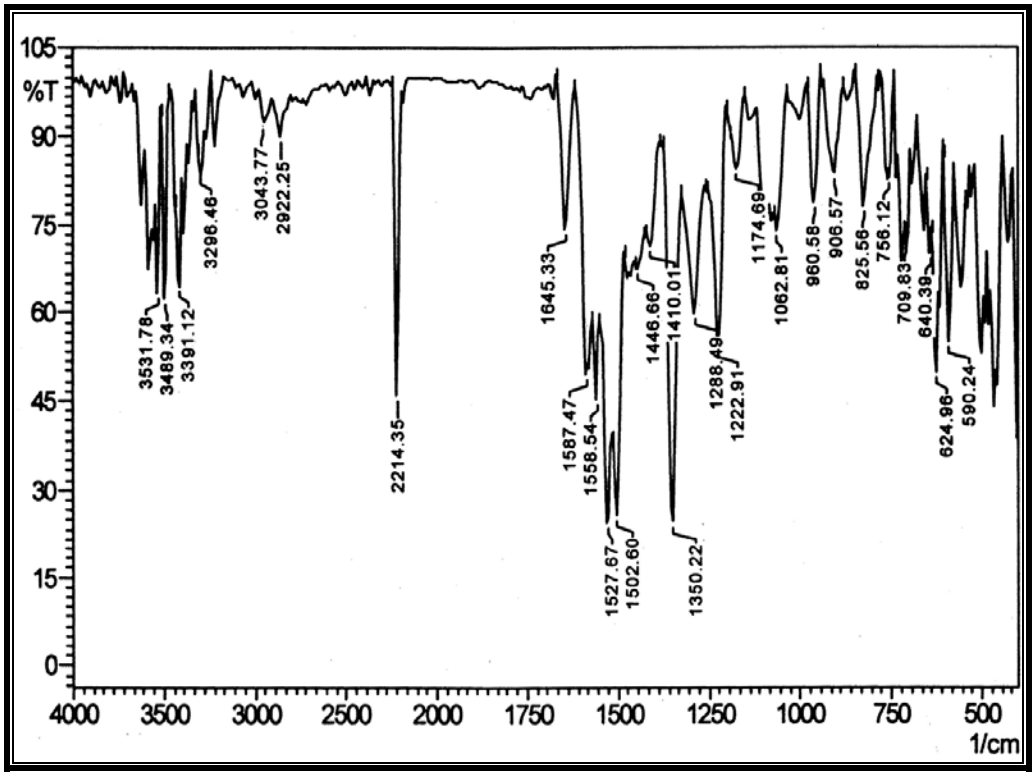
Expanded ^{1}H NMR spectrum of DDK-C-09



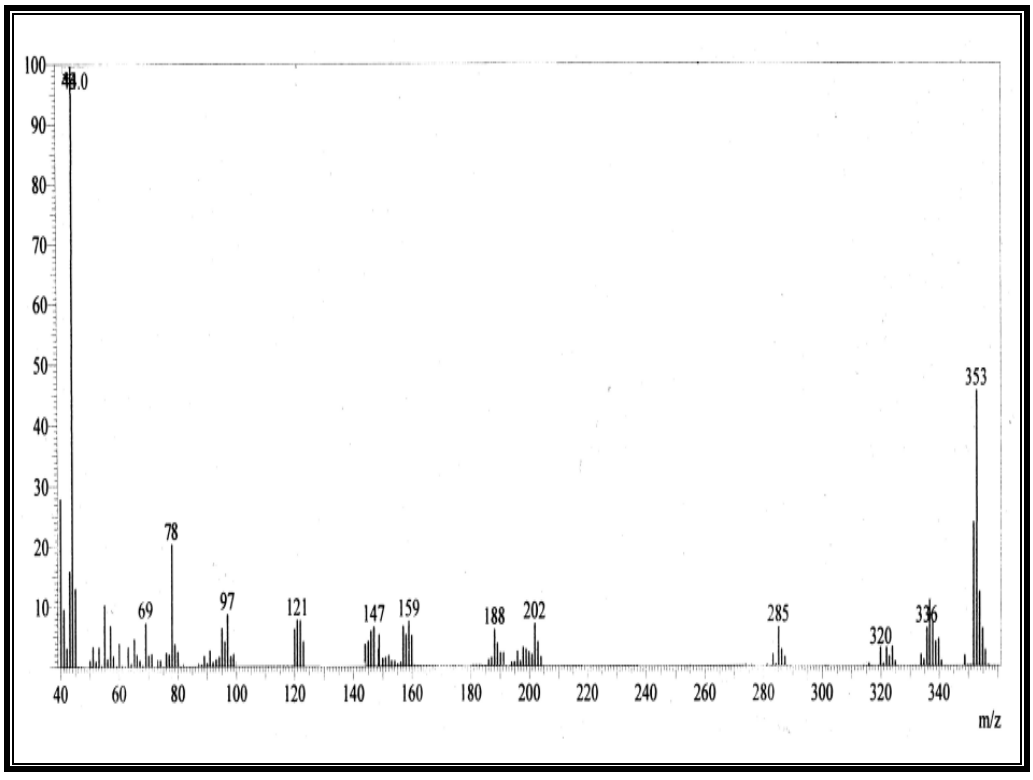
Expanded ^{1}H NMR spectrum of DDK-C-09



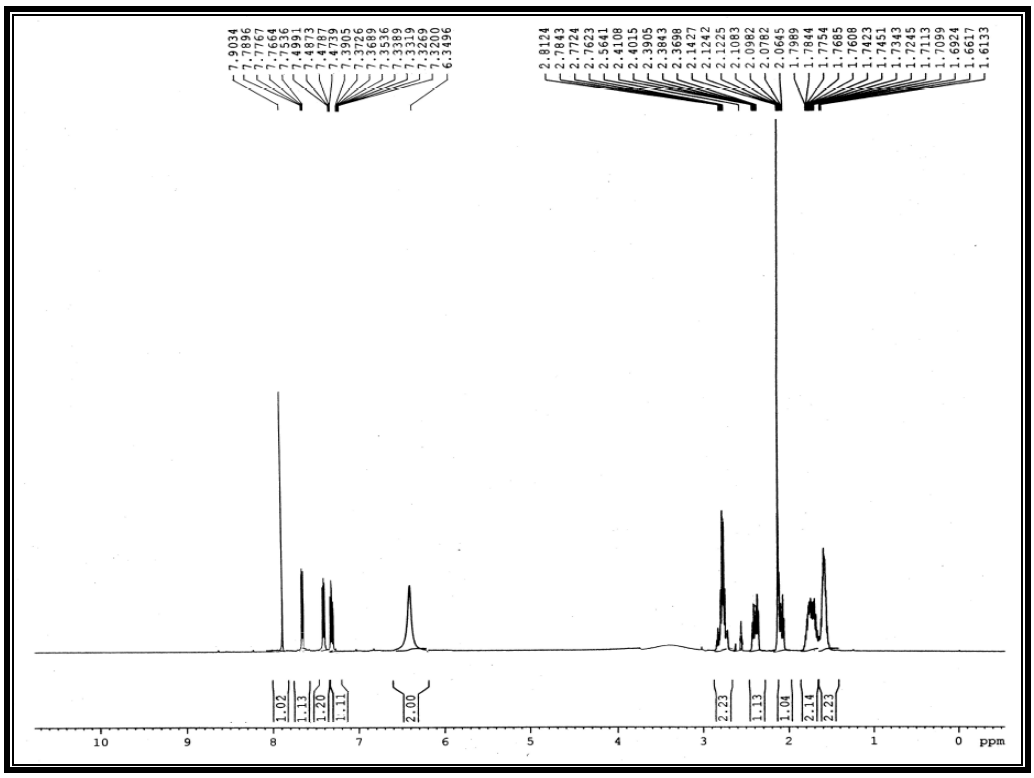
IR spectrum of DDK-C-11



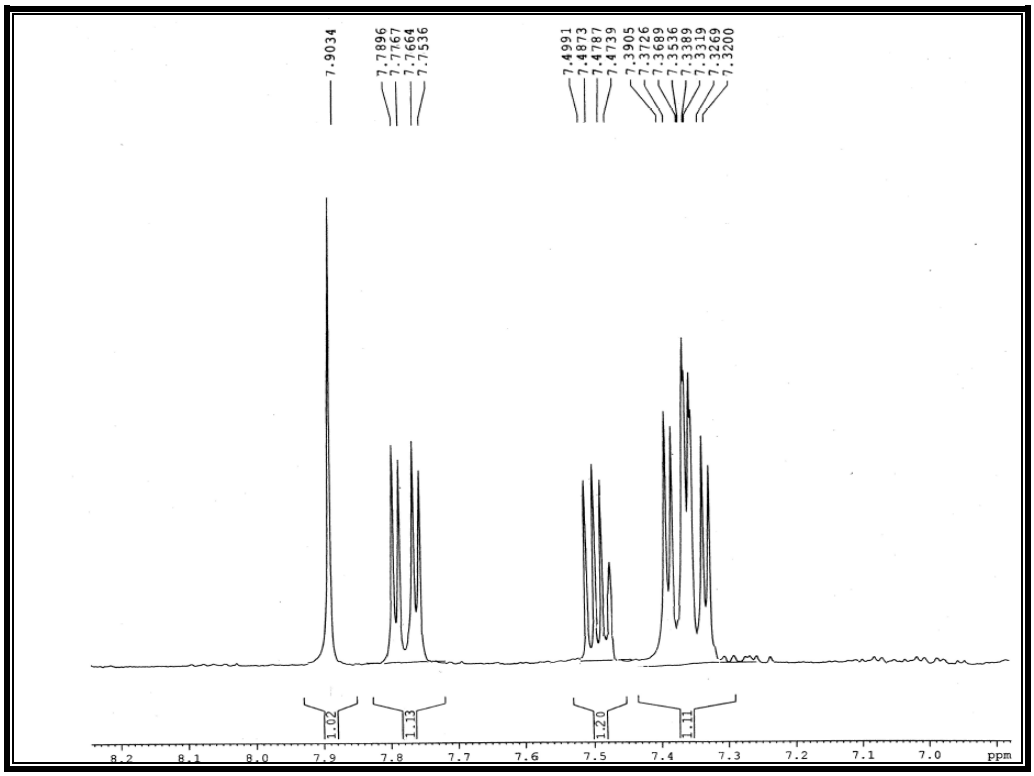
Mass spectrum of DDK-C-11



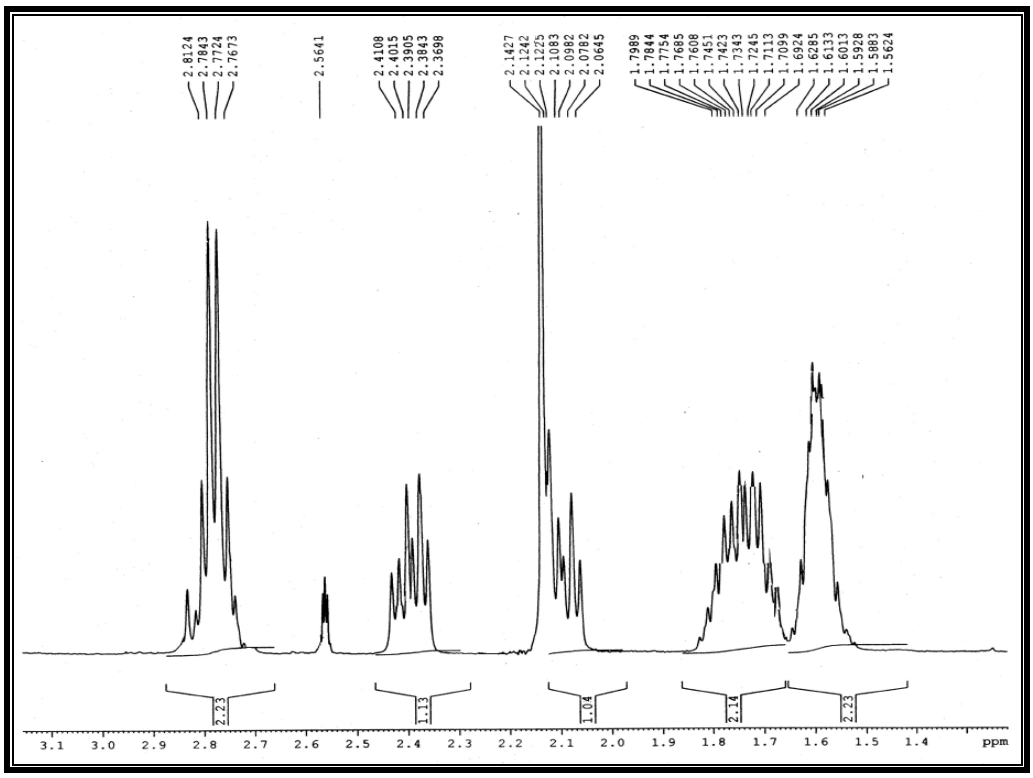
^{1}H NMR spectrum of DDK-C-11



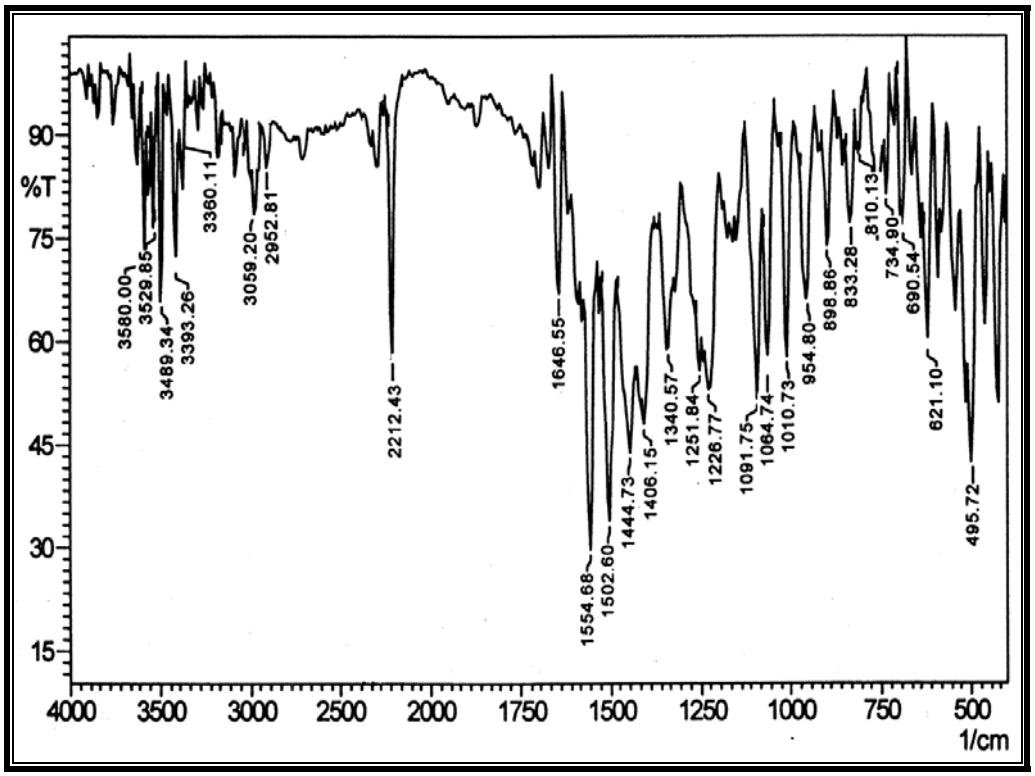
Expanded ^{1}H NMR spectrum of DDK-C-11



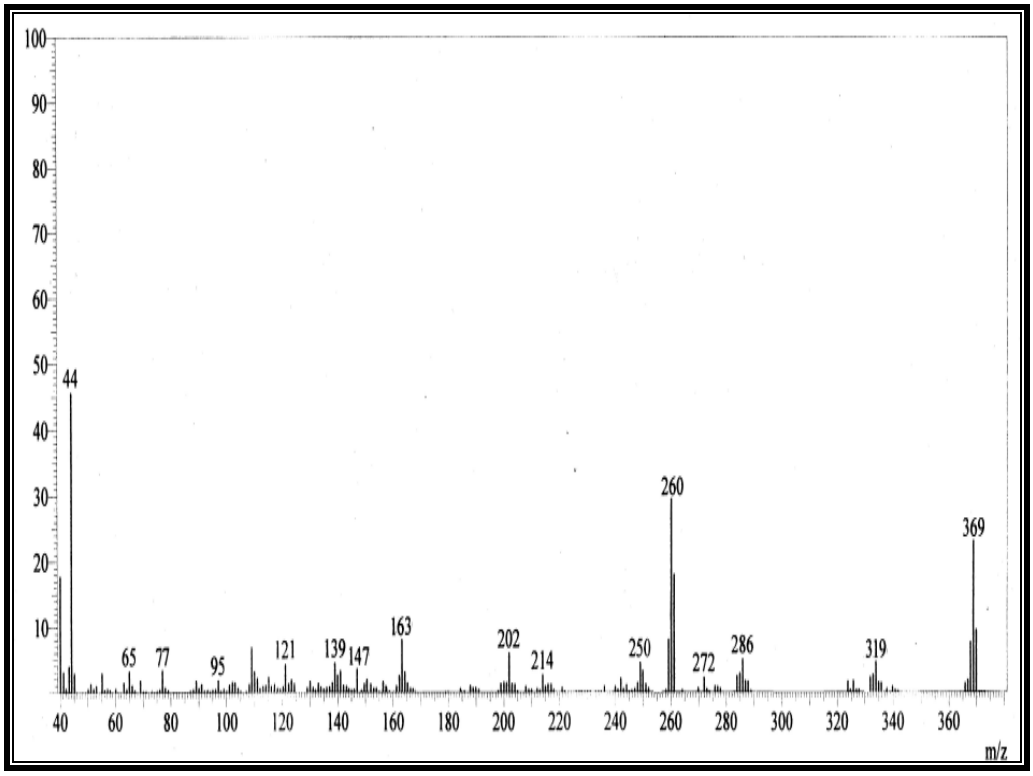
Expanded ^{1}H NMR spectrum of DDK-C-11



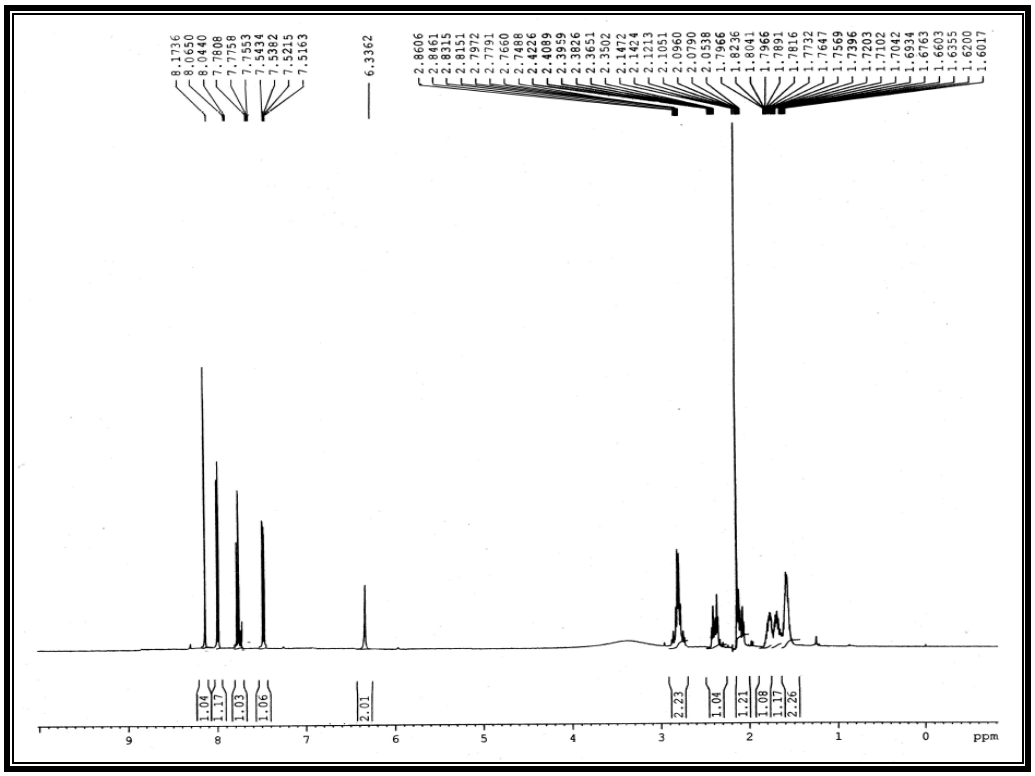
IR spectrum of DDK-C-12



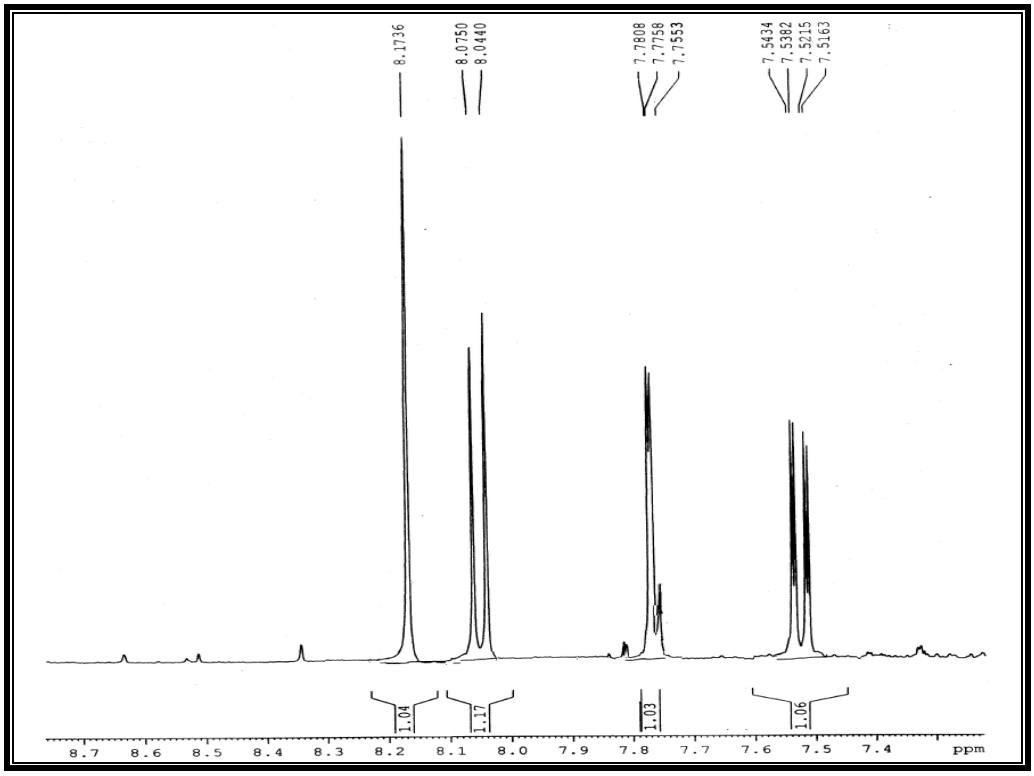
Mass spectrum of DDK-C-12



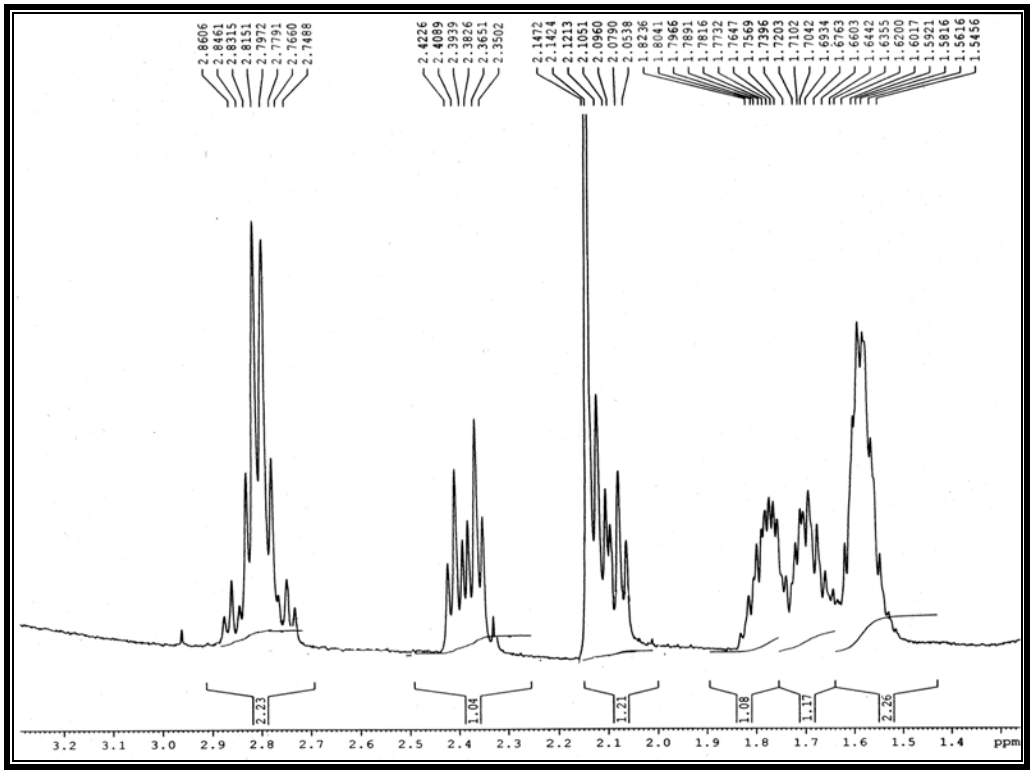
^{1}H NMR spectrum of DDK-C-12



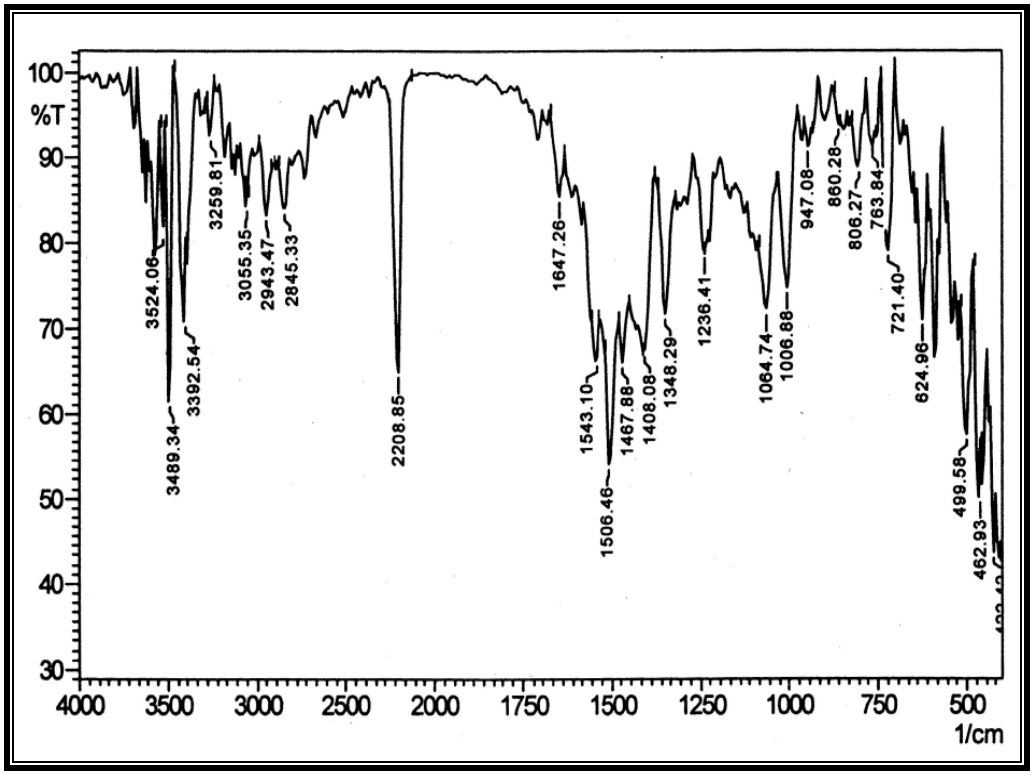
Expanded ^{1}H NMR spectrum of DDK-C-12



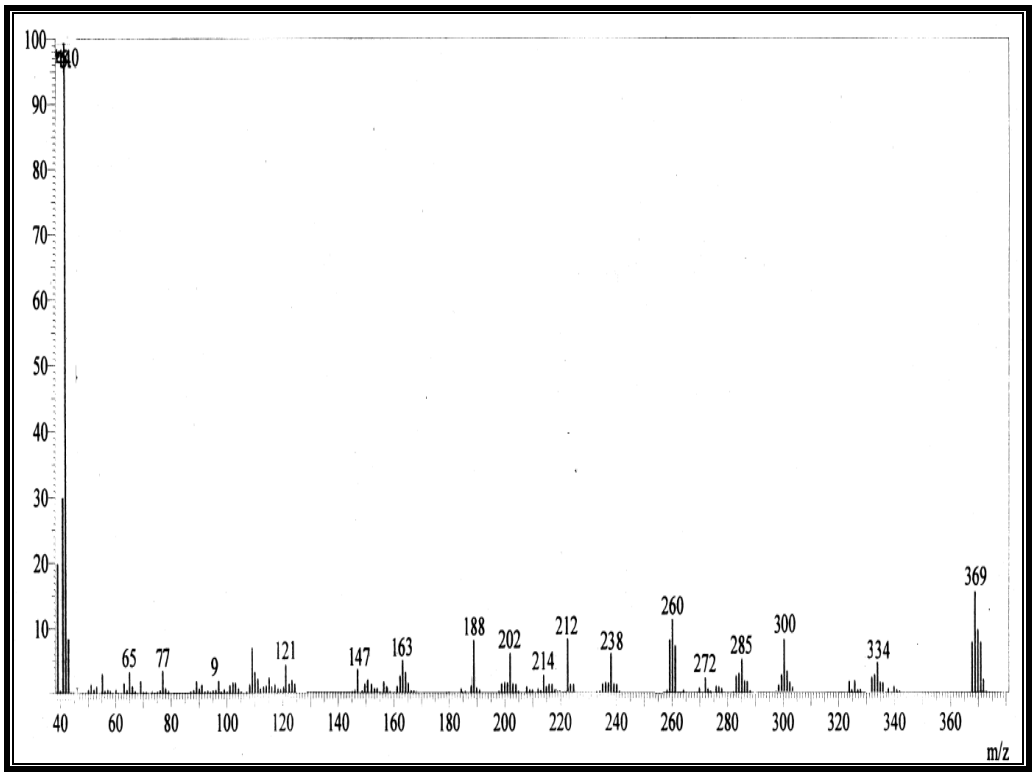
Expanded ^{1}H NMR spectrum of DDK-C-12



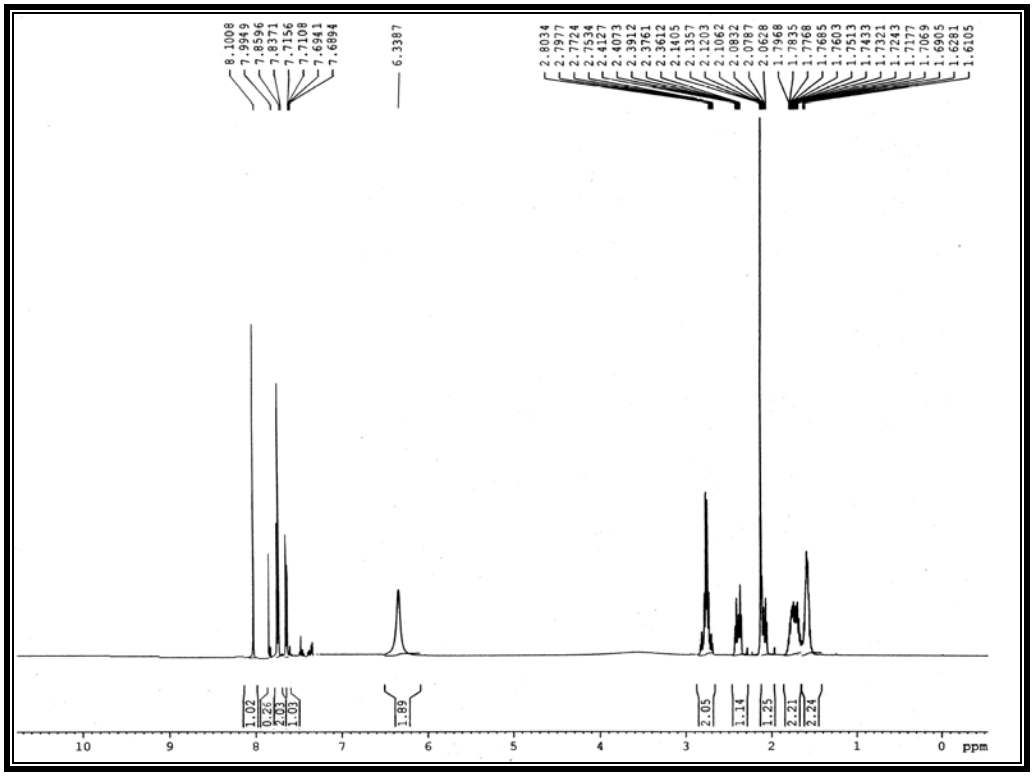
IR spectrum of DDK-C-13



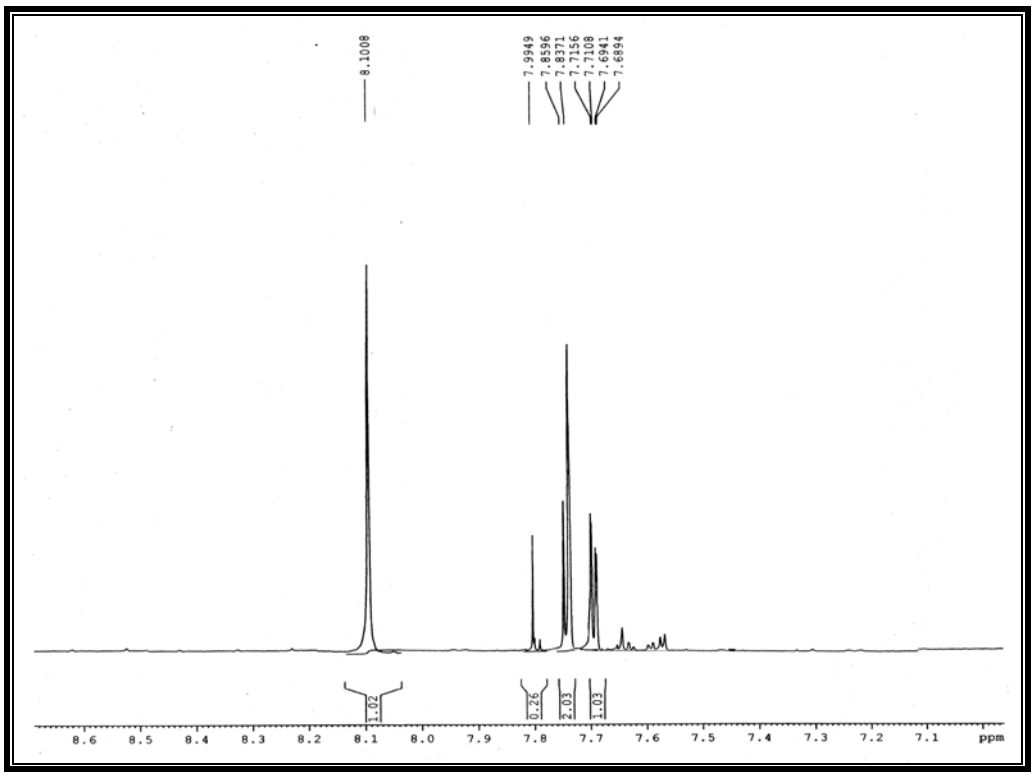
Mass spectrum of DDK-C-13



^{1}H NMR spectrum of DDK-C-13



Expanded ^{1}H NMR spectrum of DDK-C-13



5.1.8 Biological evaluation

5.1.8.1 Antimicrobial evaluation

All of the synthesized compounds (**DDK-C-01 to DDK-C-20**) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [57, 58] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin, and Griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using microdilution broth method according to NCCLS standards [57]. Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). The active synthesized drugs found in this primary screening were further tested in a second set of dilution at 125 μg mL^{-1}, 62.5 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1}, and 6.250 μg mL^{-1} concentration against all microorganisms. The tubes were inoculated with 10^8 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table 1.

Table 1. Antibacterial and antifungal activity of synthesized compounds DDK-C-01to DDK-C-20

Code	Minimum inhibition concentration (μg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	***S. p.***	***E.c.***	***P.a.***	***C. a.***	***A. n.***	***A.c.***
DDK-C-01	50	25	125	500	1000	1000	500
DDK-C-02	250	250	100	500	100	500	500
DDK-C-03	100	500	125	1000	500	1000	1000
DDK-C-04	250	1000	250	500	500	250	250
DDK-C-05	50	125	250	62.5	1000	1000	500
DDK-C-06	500	500	500	>1000	100	500	250
DDK-C-07	1000	500	1000	1000	500	500	100
DDK-C-08	500	1000	1000	500	500	100	500
DDK-C-09	62.5	100	250	125	1000	>1000	1000
DDK-C-10	1000	500	500	1000	500	100	500
DDK-C-11	1000	100	100	500	250	100	250
DDK-C-12	125	100	100	500	500	250	1000
DDK-C-13	500	500	1000	>1000	1000	500	125
DDK-C-14	125	100	50	250	500	1000	500
DDK-C-15	100	1000	250	1000	1000	500	1000
DDK-C-16	500	1000	500	100	1000	500	500
DDK-C-17	1000	500	1000	1000	500	500	1000
DDK-C-18	500	500	250	500	>1000	1000	500
DDK-C-19	25	62.5	10	25	100	500	250
DDK-C-20	125	100	1000	500	100	1000	500
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

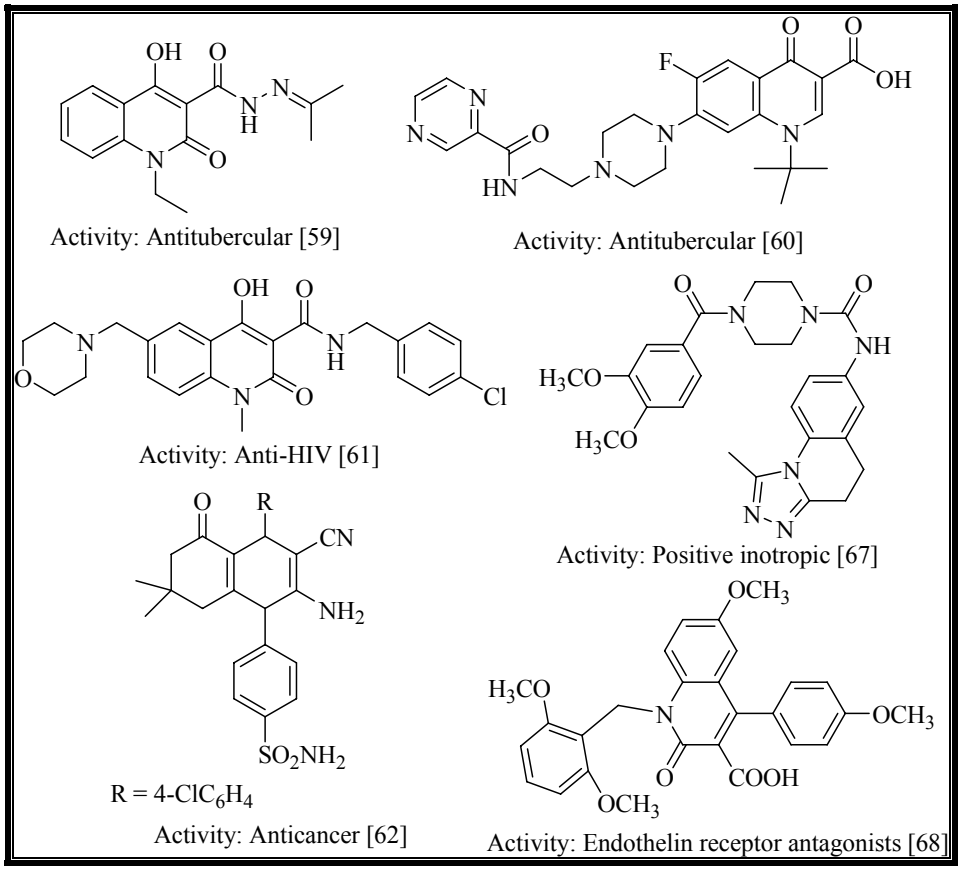
5.1.8.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds (**DDK-C-01** to **DDK-C-20**) is currently under investigation and results are awaited.

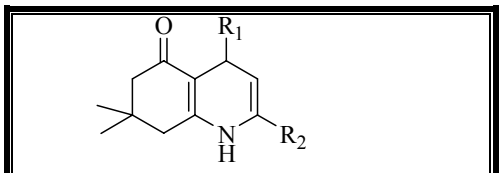
5.2: Synthesis and biological evaluation of 7,8-dihydro-7,7-dimethyl-2,4-di substituted-quinolin-5(*1H,4H,6H*)-ones

5.2.1 Introduction

Among quinoline derivatives, dihydroquinolines are an important structural subunit of bioactive molecules and many dihydroquinolines derivatives exhibit interesting biological and pharmaceutical activities including anti-tubercular [59, 60], anti-HIV [61], anti-cancer [62], apical sodium-dependent bile acid transporter (ASBT) inhibitors [63], anti-secretory [64], anti-inflammatory [65, 66] etc. Among various dihydroquinolines, chemistry and biological profile of 1,2-dihydroquinolines and 1,4-dihydroquinolines is extensively studied. Some examples of published derivatives of diydroquinolines with their biological activities are shown in the following figure.



The chemistry and biology of 7,8-dihydroquinoline is not widely studied in literature. However, the present 7,8-dihydroquinoline derivatives of type (1) are polyfunctionalized 1,4-dihydropyridine type derivatives. 1,4-Dihydropyridines (1,4-DHPs) are well-known compounds because of their biological activities [69-71]. The chemical modifications on the DHP ring, such as different substituents [69] or heteroatoms [71], have allowed the study of the extended structure and activity relationship and also provided some insight into the molecular interactions at the receptor level. In this context, It was thought worthwhile to study the biological activity profile of 7,8-dihydroquinolines of type (1), which may prove useful modification as 1,4-DHP type derivatives.

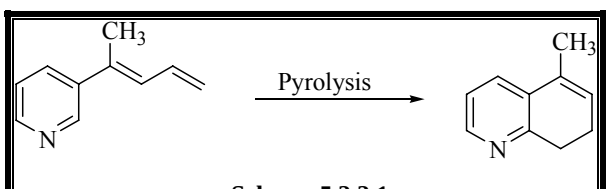


R_1 = aryl, R_2 = 2-thienyl, 4-fluorophenyl

(1) 7,8-dihydro-7,7-dimethyl-2,4-disubstituted-quinolin-5(1H,4H,6H)-ones

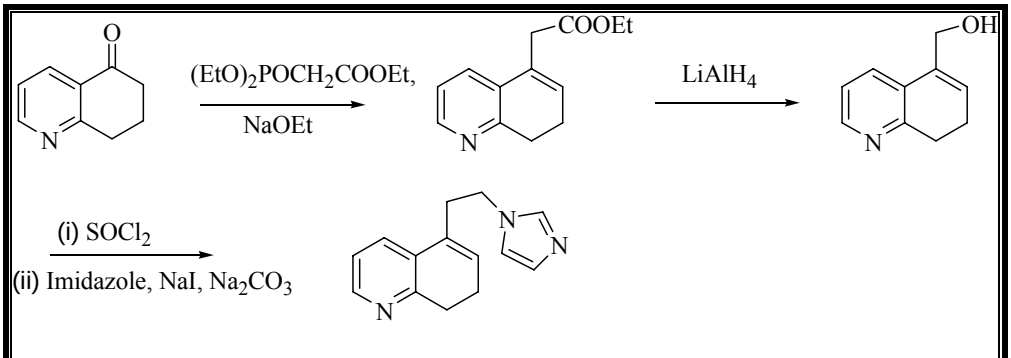
4.2 Reported synthetic strategies

Literature survey revealed a very few strategies describing the synthesis of 7,8-dihydroquinolines. Rosen et al. have reported the synthesis of 7,8-dihydroquinolines based on joining onto a pyridine ring a specific partially reduced aromatic ring. The critical ring closure reaction onto the pyridine nucleus was an electrocyclic reaction [72] (Scheme 5.2.2.1).



Scheme 5.2.2.1

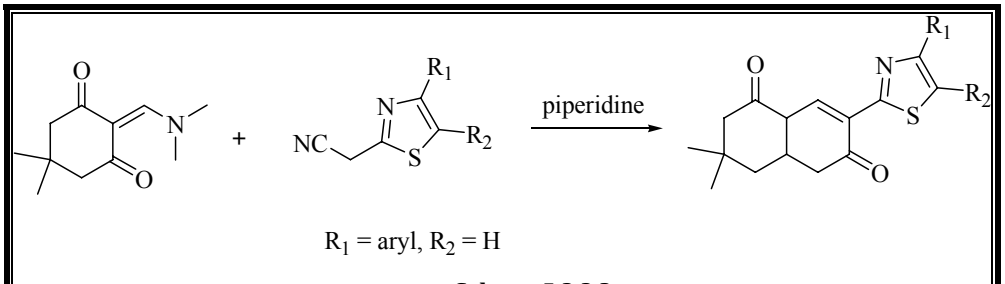
Jacobs et al. have prepared 6,7-dihydroquinolines starting from and tested them as dual inhibitors of Thromboxane A_2 Synthase and aromatase [73] (Scheme 5.2.2.1).



Scheme 5.2.2.2

Huang et al. have reported the synthesis of 7,8-dihydro-quinoline-5(6*H*)-one from1,3-diketones, ammonium acetate and 1,1,3,3-tetraethoxylpropane [74].

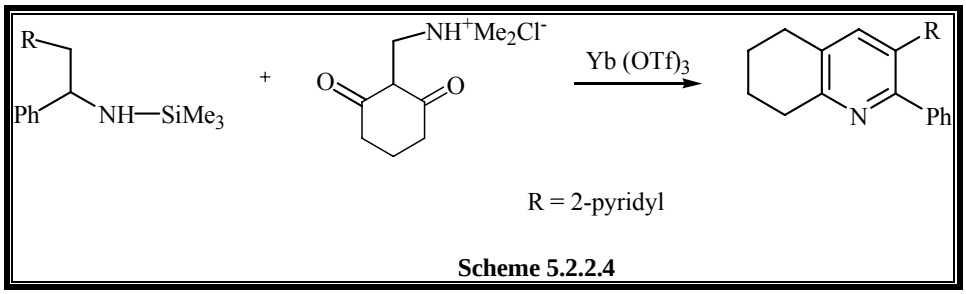
An efficient method for the synthesis of novel 3-(1,3-thiazolyl)-7,8-dihydroquinoline-2,5-(1*H*, 6*H*)-diones from various 2-dimethylaminomethylidene cyclohexanone-1,3-diones, (1,3-thiazol-2-yl)acetonitriles and dimethylfromamide dimethyl acetal was reported by Dzhavakhishvili et al. [75] (Scheme 5.2.2.3).



Scheme 5.2.2.3

Recently, number of approaches have been reported for the synthesis of 7,8-dihydro-7,7-dimethyl-2,4-disubstituted-quinolin-5(*1H,4H,6H*)-ones by the reaction of 1,3-diaryl-prop-2-en-1-ones with 5,5-dimethylcyclohexane-1,3-dione and ammonium acetate. The reaction is genrally carried out using DMF as solvent [76] and under solvent-free conditions using conventional heating [77].

Sakai et al. have reported $Yb(OTf)_3$-catalyzed cyclization of an N-silylenamine with 2-methylene-1,3-cyclohexanedione to afford a 7,8-dihydroquinolin-5(6H)-one derivative and its application to the one-pot conversion to a 2,3,5-trisubstituted quinoline derivative [78] (Scheme 5.2.2.4).



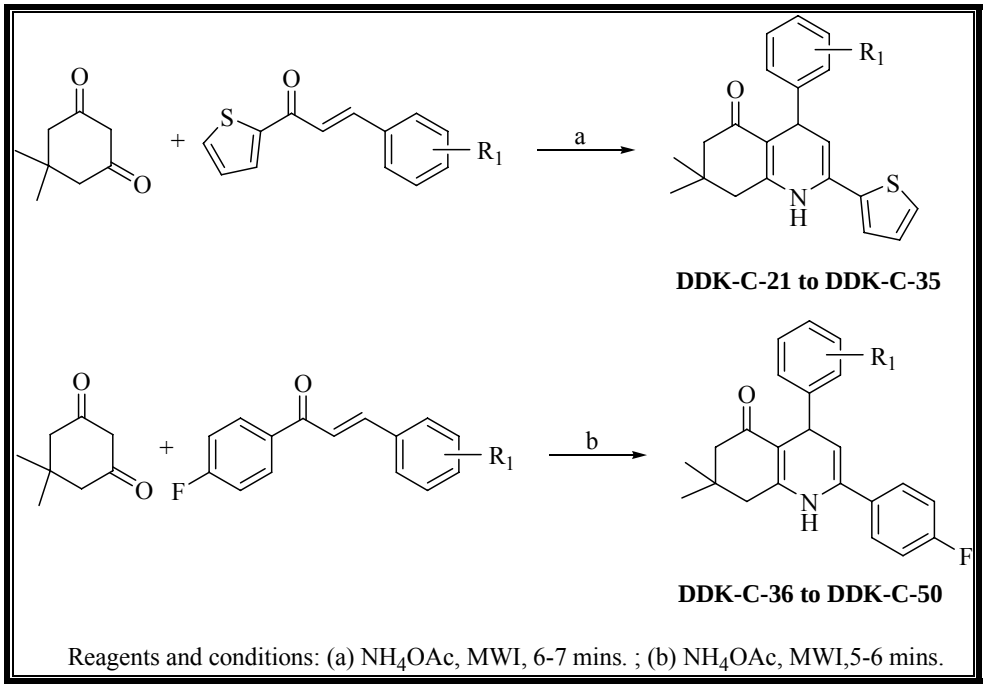
Scheme 5.2.2.4

5.2.3 Current work

1,4-Dihydropyridines (1,4-DHPs) are well-known compounds because of their biological activities [69-71]. The chemical modifications on the DHP ring, such as different substituents [69] or heteroatoms [71], have allowed the study of the extended structure and activity relationship and also provided some insight into the molecular interactions at the receptor level. In this context, It was thought worthwhile to study the biological activity profile of the present 7,8-dihydroquinolines, which may prove useful modification as 1,4-DHP type derivatives.

7,8-dihydro-7,7-dimethyl-2,4-disubstituted-quinolin-5(*1H,4H,6H*)-ones (**DDK-C-21 to DDK-C-35**) and (**DDK-C-21 to DDK-C-35**) were synthesized by the one pot solvent-free microwave-assisted reaction of 5,5-dimethylcyclohexane-1,3-dione and ammonium acetate with 3-(aryl)-1-(thiophen-2-yl)prop-2-en-1-ones and 3-(aryl)-1-(4-fluorophenyl)prop-2-en-1-ones respectively. The products were characterized by FT-IR, mass, ^{1}H NMR spectroscopy and elemental analyses. The newly synthesized compounds were subjected to antimicrobial activity.

5.2.4 Reaction scheme

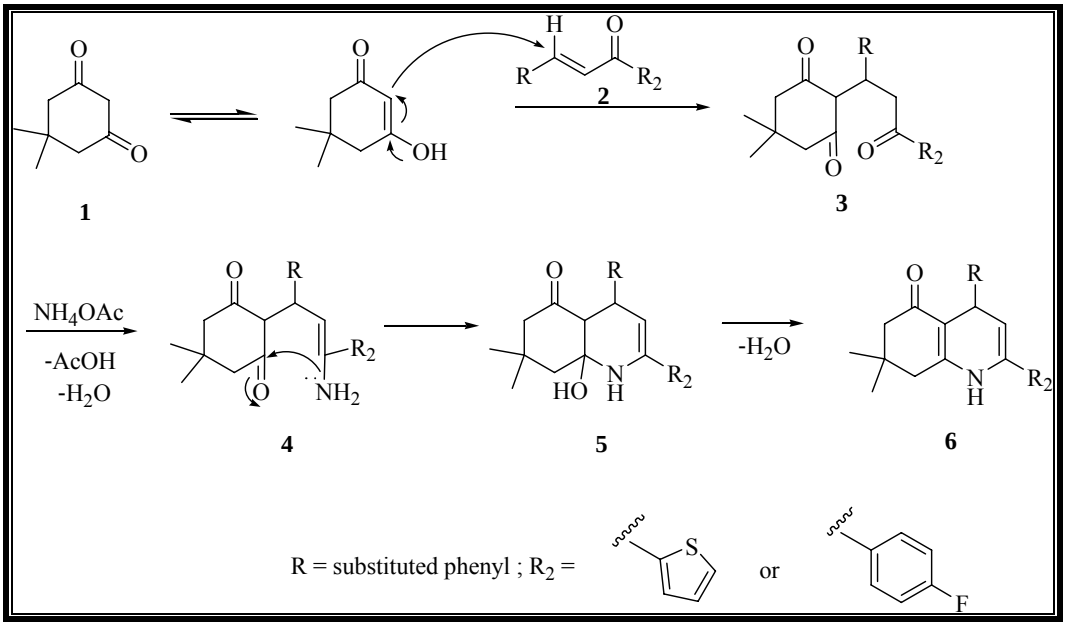


Reagents and conditions: (a) NH_4OAc, MWI, 6-7 mins. ; (b) NH_4OAc, MWI,5-6 mins.

Code	R_1	M.F.	M.W.	M.P. °C	Yield %	R_{f1}	R_{f2}
DDK-C-21	H	$C_{21}H_{21}NOS$	335	206-208	80	0.47	0.61
DDK-C-22	2-Cl	$C_{21}H_{20}ClNOS$	369	197-199	72	0.48	0.68
DDK-C-23	3-Cl	$C_{21}H_{20}ClNOS$	369	217-219	78	0.51	0.69
DDK-C-24	4-Cl	$C_{21}H_{20}ClNOS$	369	209-211	84	0.50	0.65
DDK-C-25	2-NO_2	$C_{21}H_{20}N_2O_3S$	380	225-227	66	0.48	0.65
DDK-C-26	3-NO_2	$C_{21}H_{20}N_2O_3S$	380	233-235	76	0.53	0.68
DDK-C-27	4-NO_2	$C_{21}H_{20}N_2O_3S$	380	241-243	78	0.53	0.72
DDK-C-28	4-OCH_3	$C_{22}H_{23}NO_2S$	365	199-201	83	0.51	0.67
DDK-C-29	4- CH_3	$C_{22}H_{23}NOS$	349	213-215	81	0.54	0.67
DDK-C-30	4- F	$C_{32}H_{32}BrN_3O_2$	353	244-246	79	0.52	0.69
DDK-C-31	2-OH	$C_{26}H_{26}ClFN_2O_2$	351	198-200	62	0.51	0.63
DDK-C-32	3- OH	$C_{26}H_{26}Cl_2N_2O_2$	351	203-205	63	0.54	0.65
DDK-C-33	4- OH	$C_{26}H_{26}Cl_2N_2O_2$	351	212-214	67	0.51	0.60
DDK-C-34	2-C_5H_4N	$C_{27}H_{29}ClN_2O_3$	452	237-239	70	0.53	0.60
DDK-C-35	2,3,4-$(OMe)_3$	$C_{27}H_{29}ClN_2O_3$	425	249-251	76	0.59	0.65
DDK-C-36	H	$C_{23}H_{23}FNO$	347	238-240	73	0.53	0.59
DDK-C-37	2-Cl	$C_{23}H_{22}ClFNO$	381	198-200	69	0.50	0.62
DDK-C-38	3-Cl	$C_{23}H_{22}ClFNO$	381	197-199	72	0.53	0.62
DDK-C-39	4-Cl	$C_{23}H_{22}ClFNO$	381	218-220	79	0.51	0.58
DDK-C-40	2-NO_2	$C_{23}H_{21}FN_2O_3$	392	229-231	60	0.58	0.65
DDK-C-41	3-NO_2	$C_{23}H_{21}FN_2O_3$	392	217-219	69	0.55	0.60
DDK-C-42	4-NO_2	$C_{23}H_{21}FN_2O_3$	392	206-208	74	0.52	0.61
DDK-C-43	4-OCH_3	$C_{24}H_{24}FNO_2$	377	237-239	83	0.53	0.65
DDK-C-44	4- CH_3	$C_{24}H_{24}FNO$	361	213-215	81	0.53	0.59
DDK-C-45	4- F	$C_{23}H_{21}F_2NO$	365	194-196	82	0.51	0.62
DDK-C-46	2-OH	$C_{23}H_{22}FNO_2$	448	201-203	60	0.53	0.65
DDK-C-47	3- OH	$C_{23}H_{22}FNO_2$	464	215-217	62	0.53	0.59
DDK-C-48	4- OH	$C_{23}H_{22}FNO_2$	363	194-196	65	0.51	0.62
DDK-C-49	2-C_5H_4N	$C_{22}H_{21}F_2NO$	348	239-241	72	0.53	0.63
DDK-C-50	2,3,4-$(OMe)_3$	$C_{26}H_{28}FNO_4$	437	222-224	75	0.52	0.58

TLC Solvent system R_{f1}: Hexane: Ethyl acetate – 6:4,
TLC Solvent system R_{f2}: Chloroform:Methanol – 9.5:0.5.

5.2.5 Mechanism



The proposed mechanism involves the Michael addition reaction of of 5,5-dimethyl-1,3-cyclo-hexanedione **1** reacted with 1,3-diaryl-2- propen-1-one **2** to give addition product **3**. Then the intermediate **3** upon condensation with ammonium acetate, loses the acetic acid and water to afford the intermediate **4**. Cyclization takes place by the nucleophilic attack of amine (NH_2) group on the carbonyl (C=O) group to give the intermediate **5**, which loses a water to give the 7,8-dihydroquinolines **6** [77].

5.1.6 Experimental

5.1.6.1 Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. Microwave assisted reactions were carried out in QPro-M microwave synthesizer. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

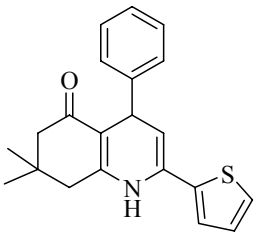
5.1.6.2 Synthesis of 3-(aryl)-1-(thiophen-2-yl)prop-2-en-1-ones

Synthesis of 3-(aryl)-1-(thiophen-2-yl)prop-2-en-1-ones was achieved using previously published method [79].

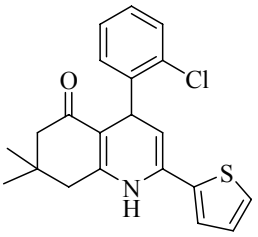
5.1.6.3 General procedure for the synthesis of 2-Amino-4-[3-(4-chloro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6,7,8-tetrahydro-quinoline-3-carbonitrile (DDK-C-21 to DDK-C-35)

A mixture of the 5,5-dimethyl-1,3-cyclohexanone (0.01 mol), 3-(aryl)-1-(thiophen-2-yl)prop-2-en-1-one (0.01 mol) and ammonium aceate (0.08 mol) was irradiated with microwave irradiation at 120 °C for 5-7 min. The microwave irradiation was operated in 30-second cycles. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the reaction mass was poured into ice-cold water, the product was filtered, washed with water, dried and crystallized from ethanol-DMF (9:1) mixture.

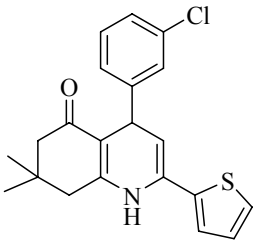
5.2.6.3.1 7,8-dihydro-7,7-dimethyl-4-phenyl-2-(thiophen-2-yl)quinolin-5(1H,4H, 6H)-one (DDK-C-21) Yield: 80%; m.p. 206-208 ºC; MS: *m/z* 335; Anal. Calcd. for $C_{21}H_{21}NOS$: C, 75.19; H, 6.31; N, 4.18. Found: C, 75.12; H, 6.23; N, 4.11%



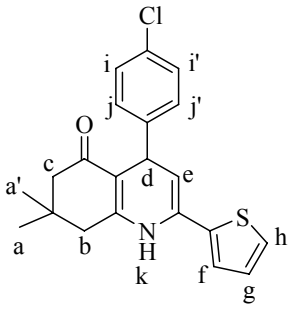
5.2.6.3.2 4-(2-chlorophenyl)-7,8-dihydro-7,7-dimethyl-2-(thiophen-2-yl)quinolin-5(1H,4H,6H)-one (DDK-C-22) Yield: 72%; m.p. 197-199 ºC; MS: *m/z* 369; Anal. Calcd. for $C_{21}H_{20}ClNOS$: C, 68.19; H, 5.45; N, 3.79. Found: C, 68.10; H, 5.37; N, 3.72%.



5.2.6.3.3 4-(3-chlorophenyl)-7,8-dihydro-7,7-dimethyl-2-(thiophen-2-yl)quinolin-5(1H,4H,6H)-one (DDK-C-23) Yield: 78%; m.p. 217-219 ºC; MS: *m/z* 369; Anal. Calcd. for $C_{21}H_{20}ClNOS$: C, 68.19; H, 5.45; N, 3.79. Found: C, 68.11; H, 5.38; N, 3.73%.

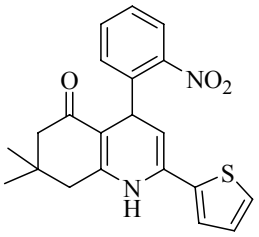


5.2.6.3.4 4-(4-chlorophenyl)-7,8-dihydro-7,7-dimethyl-2-(thiophen-2-yl)quinolin-5(1H,4H,6H)-one (DDK-C-24) Yield: 84%; m.p. 209-211 ºC; IR (cm^{-1}): 3277 (N-H stretching of secondary amine), 3028 (C-H stretching of aromatic ring), 2924 (C-H asymmetrical stretching of CH_2 group), 2874 (C-H symmetrical stretching of CH_3 group), 1681 (C=O stretching of carbonyl group), 1647 (N-H deformation of secondary amine), 1581, 1487 and 1444 (C=C stretching of aromatic ring), 1406 (C-H asymmetrical deformation of CH_3 group), 1300 (C-H symmetrical deformation of CH_3 group), 1006 (C-H in plane bending for aromatic ring), 823 (C-H out of plane bending for 1,4-disubstituted aromatic ring), 700 (C-Cl stretching), 628 (C-S-C

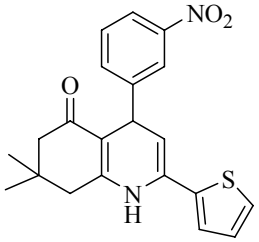


stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 1.08 (s, 3H, $H_{a,}$), 1.10 (s, 3H, $H_{a'}$), 2.05-2.21 (m, 2H, H_b), 2.39 (s, 2H, H_c), 4.59-4.61 (d, 1H, H_d), 5.21-5.23 (d, 1H, H_e), 6.92-6.94 (d, 2H, $H_{j, j'}$, J = 8.6 Hz), 7.23-7.25 (d, 2H, $H_{i, i'}$, J = 8. 08 Hz), 7.00-7.02 (m, 1H, H_f), 7.13-7.17 (m, 1H, H_g), 7.33-7.34 (m, 1H, H_h), 8.40 (s , 1H, H_k); MS: *m/z* 369; Anal. Calcd. for $C_{21}H_{20}ClNOS$: C, 68.19; H, 5.45; N, 3.79. Found: C, 68.10; H, 5.37; N, 3.71.%.

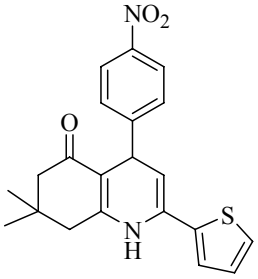
5.2.6.3.5 7,8-dihydro-7,7-dimethyl-4-(2-nitrophenyl)-2-(thiophen-2-yl)quinolin-5(1H,4H,6H)-one (DDK-C-25) Yield: 66%; m.p. 225-227 ºC; MS: *m/z* 380; Anal. Calcd. for $C_{21}H_{20}N_2O_3S$: C, 66.29; H, 5.30; N, 7.36. Found: C, 66.23; H, 5.22; N, 7.29%.



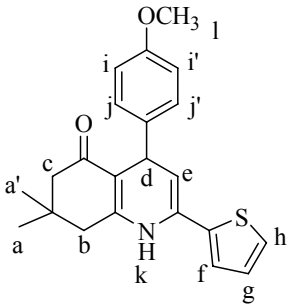
5.2.6.3.6 7,8-dihydro-7,7-dimethyl-4-(3-nitrophenyl)-2-(thiophen-2-yl)quinolin-5(1H,4H,6H)-one (DDK-C-26) Yield: 76%; m.p. 233-235 ºC; MS: *m/z* 380; Anal. Calcd. for $C_{21}H_{20}N_2O_3S$: C, 66.29; H, 5.30; N, 7.36. Found: C, 66.22; H, 5.22; N, 7.28%.



5.2.6.3.7 7,8-dihydro-7,7-dimethyl-4-(3-nitrophenyl)-2-(thiophen-2-yl)quinolin-5(1H,4H,6H)-one (DDK-C-27) Yield: 78%; m.p. 241-243 ºC; MS: *m/z* 380; Anal. Calcd. for $C_{21}H_{20}N_2O_3S$: C, 66.29; H, 5.30; N, 7.36. Found: C, 66.22; H, 5.21; N, 7.28%.

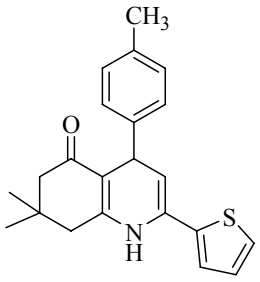


5.2.6.3.8 7,8-dihydro-4-(4-methoxyphenyl)-7,7-dimethyl-2-(thiophen-2-yl)



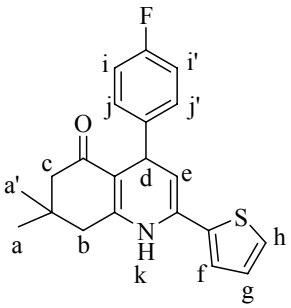
quinolin-5(1H,4H,6H)-one (DDK-C-28) Yield: 83%; m.p. 199-201 ºC; IR (cm^{-1}): 3255 (N-H stretching of secondary amine), 3061 (C-H stretching of aromatic ring), 2955 (C-H asymmetrical stretching of CH_2 group), 2866 (C-H symmetrical stretching of CH_3 group), 1653 (C=O stretching of carbonyl group), 1577 and 1498 (C=C stretching of aromatic ring), 1477 (C-H asymmetrical deformation of CH_3 group), 1388 (C-H symmetrical deformation of CH_3 group), 1251 (C-O-C asymmetrical stretching of OCH_3 group), 1165 (C-O-C symmetrical stretching OCH_3 group), 1035 (C-H in plane bending for aromatic ring), 825 (C-H out of plane bending for 1,4-disubstituted aromatic ring), 692 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 1.00 (s, 3H, $H_{a,}$), 1.08 (s, 3H, $H_{a'}$), 2.04-2.21 (m, 2H, H_b), 2.47 (s, 2H, H_c), 4.52-4.53 (d, 1H, H_d), 5.21-5.23 (d, 1H, H_e), 6.76-6.78 (d, 2H, $H_{j, j'}$, J = 7.92 Hz), 7.23-7.25 (d, 2H, $H_{i, i'}$, J = 8.56 Hz), 7.00-7.02 (m, 1H, H_f), 7.15-7.17 (m, 1H, H_g), 7.33-7.34 (t, 1H, H_h), 8.42 (s , 1H, H_k), 3.75 (s, 1H, H_l); MS: *m/z* 365; Anal. Calcd. for $C_{22}H_{23}NO_2S$: C, 72.30; H, 6.34; N, 3.83. Found: C, 72.23; H, 6.26; N, 3.76%.

5.2.6.3.9 7,8-dihydro-4-(4-methoxyphenyl)-7,7-dimethyl-2-(thiophen-2- yl)



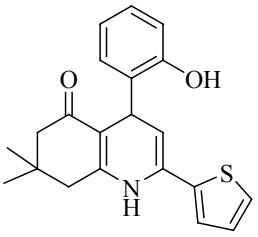
quinolin-5(1H,4H,6H)-one (DDK-C-29) Yield: 81%; m.p. 213-215 ºC; MS: *m/z* 349; Anal. Calcd. for $C_{23}H_{22}NOS$: C, 75.61; H, 6.63; N, 4.01. Found: C, 75.55; H, 6.55; N, 3.92%

5.2.6.3.10 4-(4-fluorophenyl)-7,8-dihydro-7,7-dimethyl-2-(thiophen-2-yl)quinolin-



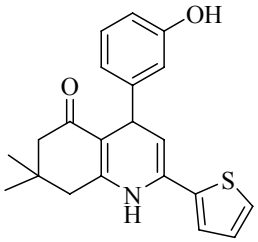
5(1H,4H,6H)-one (DDK-C-30) Yield: 79%; m.p. 244-246 ºC; IR (cm^{-1}): 3287 (N-H stretching of secondary amine), 3032 (C-H stretching of aromatic ring), 2929 (C-H asymmetrical stretching of CH_2 group), 2837 (C-H symmetrical stretching of CH_3 group), 1701 (C=O stretching of carbonyl group), 1581, 1552 and 1496 (C=C stretching of aromatic ring), 1440 (C-H asymmetrical deformation of CH_3 group), 1350 (C-H symmetrical deformation of CH_3 group), 1093 (C-H in plane bending for aromatic ring), 1006 (C-F stretching), 823 (C-H out of plane bending for 1,4-disubstituted aromatic ring), 709 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 1.03 (s, 3H, $H_{a,}$), 1.08 (s, 3H, $H_{a'}$), 2.02-2.19 (m, 2H, H_b), 2.50 (s, 2H, H_c), 4.57-4.58 (d, 1H, H_d), 5.19-5.21 (d, 1H, H_e), 6.92-6.97 (t, 2H, $H_{j, j'}$), 7.22-7.29 (m, 2H, $H_{i, i'}$), 7.01-7.03 (m, 1H, H_f), 7.12-7.16 (m, 1H, H_g), 7.35-7.36 (m, 1H, H_h), 8.49 (s , 1H, H_k); MS: *m/z* 353; Anal. Calcd. for $C_{21}H_{21}FNOS$: C, 71.36; H, 5.70; N, 3.96. Found: C, 71.27; H, 5.62; N, 3.90%.

5.2.6.3.11 7,8-dihydro-4-(2-hydroxyphenyl)-7,7-dimethyl-2-(thiophen-2-yl)



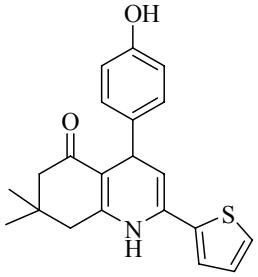
quinolin-5(1H,4H,6H)-one (DDK-C-31) Yield: 62%; m.p. 198-200 ºC; MS: *m/z* 351; Anal. Calcd. for $C_{21}H_{21}NO_2S$: C, 71.76; H, 6.02; N, 3.99. Found: C, 71.68; H, 5.95; N, 3.93%.

5.2.6.3.12 7,8-dihydro-4-(3-hydroxyphenyl)-7,7-dimethyl-2-(thiophen-2-yl)

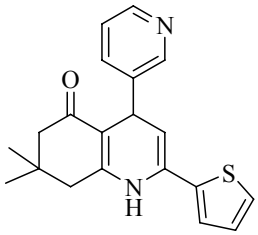


quinolin-5(1H,4H,6H)-one (DDK-C-32) Yield: 63%; m.p. 203-205 ºC; MS: *m/z* 351; Anal. Calcd. for $C_{21}H_{21}NO_2S$: C, 71.76; H, 6.02; N, 3.99. Found: C, 71.69; H, 5.94; N, 3.92%.

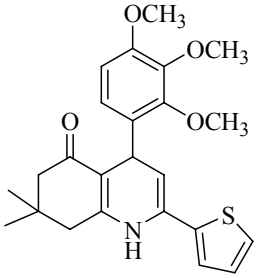
5.2.6.3.13 7,8-dihydro-4-(4-hydroxyphenyl)-7,7-dimethyl-2-(thiophen-2-yl) quinolin-5(1H,4H,6H)-one (DDK-C-33) Yield: 67%; m.p. 212-214 ºC; MS: *m/z* 351; Anal. Calcd. for $C_{21}H_{21}NO_2S$: C, 71.76; H, 6.02; N, 3.99. Found: C, 71.69; H, 5.95; N, 3.91%.



5.2.6.3.14 7,8-dihydro-7,7-dimethyl-4-(pyridin-3-yl)-2-(thiophen-2-yl)quinolin-5(1H,4H,6H)-one (DDK-C-34) Yield: 70%; m.p. 237-239 ºC; MS: *m/z* 352; Anal. Calcd. for $C_{21}H_{24}N_2OS$: C, 71.55; H, 6.86; N, 7.95. Found: C, 71.47; H, 6.79; N, 7.87%.



5.2.6.3.15 7,8-dihydro-4-(2,3,4-trimethoxyphenyl)-7,7-dimethyl-2-(thiophen-2-yl) quinolin-5(1H,4H,6H)-one (DDK-C-35) Yield: 76%; m.p. 249-251 ºC; MS: *m/z* 425; Anal. Calcd. for $C_{24}H_{27}NO_4S$: C, 67.74; H, 6.40; N, 3.29. Found: C, 67.65; H, 6.33; N, 3.23%



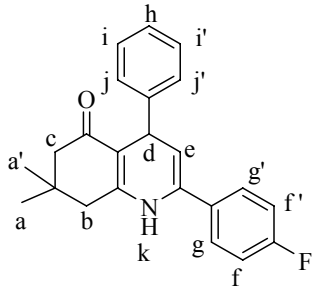
5.1.6.4 Synthesis of 3-(aryl)-1-(4-fluorophenyl)prop-2-en-1-ones

Synthesis of 3-(aryl)-1-(4-fluorophenyl)prop-2-en-1-ones was achieved using previously published method [80].

5.2.6.5 General procedure for the synthesis of 2-Amino-5,6,7,8-tetrahydro-4-quinoline-3-carbonitriles (DDK-C-36 to DDK-C-50)

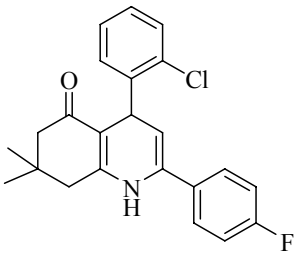
A mixture of the 5,5-dimethyl-1,3-cyclohexanone (0.01 mol), 3-(aryl)-1-(4-fluorophenyl)prop-2-en-1-ones (0.01 mol) and ammonium aceate (0.08 mol) was irradiated with microwave irradiation at 120 °C for 5-7 min. The microwave irradiation was operated in 30-second cycles. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the reaction mass was poured into ice-cold water, the product was filtered, washed with water, dried and crystallized from ethanol-DMF (9:1) mixture.

5.2.6.5.1 2-(4-fluorophenyl)-7,8-dihydro-7,7-dimethyl-4-phenylquinolin-5(1H,4H,6H)-one (DDK-C-36)

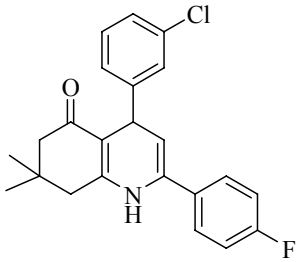


Yield: 73%; m.p. 198-200 ºC; IR (cm^{-1}): 3377 (N-H stretching of secondary amine), 3084 (C-H stretching of aromatic ring), 2956 (C-H asymmetrical stretching of CH_2 group), 2860 (C-H symmetrical stretching of CH_3 group), 1683 (C=O stretching of carbonyl group), 1651 (N-H deformation of secondary amine), 1581 and 1494 (C=C stretching of aromatic ring), 1423 (C-H asymmetrical deformation of CH_3 group), 1332 (C-H symmetrical deformation of CH_3 group), 1035 (C-F stretching), 956 (C-H in plane bending for aromatic ring), 833 (C-H out of plane bending for 1,4-disubstituted aromatic ring), 719 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 1.01 (s, 3H, $H_{a,}$), 1.08 (s, 3H, $H_{a'}$), 1.99-2.20 (m, 2H, H_b), 2.46 (s, 2H, H_c), 4.60-4.61 (d, 1H, H_d), 5.17-5.18 (d, 1H, H_e), 7.21=7.26 (m, 1H, H_h), 7.27-7.35 (m, 4H, $H_{i, i, j, j'}$), 7.07-7.11 (m, 1H, $H_{f,f'}$), 7.43-7.47 (t, 2H, $H_{g, g'}$), 8.52 (s , 1H, H_k); MS: *m/z* 347; Anal. Calcd. for $C_{23}H_{22}FNO$: C, 79.51; H, 6.38; N, 4.03. Found: C, 79.43; H, 6.31; N, 3.97%.

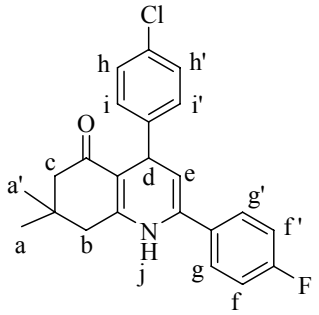
5.2.6.5.2 4-(2-chlorophenyl)-2-(4-fluorophenyl)-7,8-dihydro-7,7-dimethyl quinolin-5(1H,4H,6H)-one (DDK-C-37) Yield: 69%; m.p. 197-199 ºC; MS: *m/z* 381; Anal. Calcd. for $C_{23}H_{22}Cl$ FNO: C, 72.34; H, 5.54; N, 3.67. Found: C, 72.27; H, 5.46; N, 3.59%.



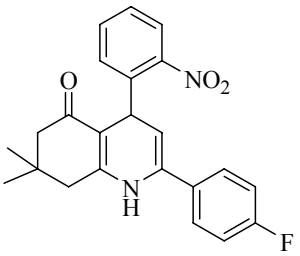
5.2.6.5.3 4-(3-chlorophenyl)-2-(4-fluorophenyl)-7,8-dihydro-7,7-dimethyl quinolin-5(1H,4H,6H)-one (DDK-C-38) Yield: 72%; m.p. 203-205 ºC; MS: *m/z* 381; Anal. Calcd. for $C_{23}H_{22}ClFNO$: C, 72.34; H, 5.54; N, 3.67. Found: C, 72.26; H, 5.47; N, 3.60%.



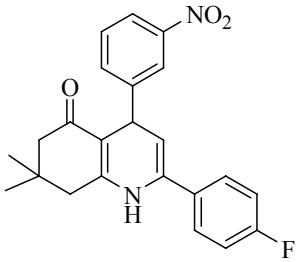
5.2.6.5.4 4-(3-chlorophenyl)-2-(4-fluorophenyl)-7,8-dihydro-7,7-dimethyl quinolin-5(1H,4H,6H)-one (DDK-C-39) Yield: 79%; m.p. 218-220 ºC; IR (cm^{-1}): 3257 (N-H stretching of secondary amine), 3032 (C-H stretching of aromatic ring), 2947 (C-H asymmetrical stretching of CH_2 group), 2850 (C-H symmetrical stretching of CH_3 group), 1680 (C=O stretching of carbonyl group), 1584 1558 and 1485 (C=C stretching of aromatic ring), 1452 (C-H asymmetrical deformation of CH_3 group), 1334 (C-H symmetrical deformation of CH_3 group), 1085 (C-F stretching), 1001 (C-H in plane bending for aromatic ring), 813 (C-H out of plane bending for 1,4-disubstituted aromatic ring), 723 (C-Cl stretching), 692 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 0.98 (s, 3H, $H_{a,}$), 1.03 (s, 3H, $H_{a'}$), 2.03-2.07 (d, 1H, H_b), 2.17-2.20 (d, 1H, H_b), 2.45 (s, 2H, H_c), 4.59-4.61 (d, 1H, H_d), 5.21-5.23 (d, 1H, H_e), 7.00-7.02 (d, 2H, $H_{h, h'}$, J = 8.76 Hz), 7.23-7.25 (d, 2H, $H_{i, i}$, J = 8.2 Hz), 6.90-6.95 (t, 2H, $H_{f, f'}$), 7.13-7.17 (t, 2H, $H_{g, g'}$), 8.52 (s, 1H, H_j); MS: *m/z* 381; Anal. Calcd. for $C_{23}H_{22}ClFNO$: C, 72.34; H, 5.54; N, 3.67. Found: C, 72.27; H, 5.46; N, 3.58%.



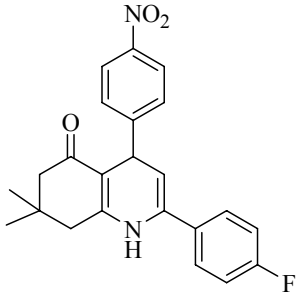
5.2.6.5.5 2-(4-fluorophenyl)-7,8-dihydro-7,7-dimethyl-4-(2-nitrophenyl)quinolin-5(1H,4H,6H)-one (DDK-C-40) Yield: 60%; m.p. 229-231 ºC; MS: *m/z* 392; Anal. Calcd. for $C_{23}H_{21}FN_2O_3$: C, 70.40; H, 5.39; N, 7.14. Found: C, 70.34; H, 5.32; N, 7.07%.



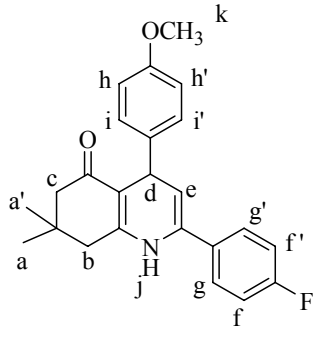
5.2.6.5.6 2-(4-fluorophenyl)-7,8-dihydro-7,7-dimethyl-4-(3-nitrophenyl)quinolin-5(1H,4H,6H)-one (DDK-C-40) Yield: 69%; m.p. 217-219 ºC; MS: *m/z* 392; Anal. Calcd. for $C_{23}H_{21}FN_2O_3$: C, 70.40; H, 5.39; N, 7.14. Found: C, 70.34; H, 5.32; N, 7.07%.



5.2.6.4.7 2-(4-fluorophenyl)-7,8-dihydro-7,7-dimethyl-4-(4-nitrophenyl)quinolin-5(1H,4H,6H)-one (DDK-C-42) Yield: 74%; m.p. 206-208 ºC; MS: *m/z* 392; Anal. Calcd. for $C_{23}H_{21}FN_2O_3$: C, 70.40; H, 5.39; N, 7.14. Found: C, 70.34; H, 5.32; N, 7.07%.

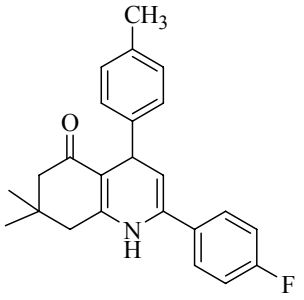


5.2.6.4.8 2-(4-fluorophenyl)-7,8-dihydro-4-(4-methoxyphenyl)-7,7-dimethyl quinolin-5(1H,4H,6H)-one (DDK-C-43) Yield: 83%; m.p. 237-239 ºC; IR (cm^{-1}): 3296 (N-H stretching of secondary amine), 3043 (C-H stretching of aromatic ring), 2945 (C-H asymmetrical stretching of CH_2 group), 2847 (C-H symmetrical stretching of CH_3 group), 1645 (C=O stretching of carbonyl group), 1587 1558 and 1502 (C=C stretching of aromatic ring), 1446 (C-H asymmetrical deformation of CH_3 group), 1350 (C-H symmetrical deformation of CH_3 group), 1222



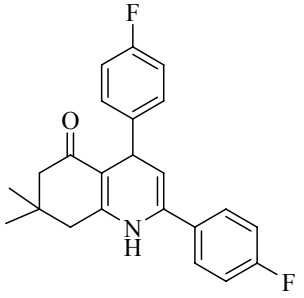
(C-O-C asymmetrical stretching of OCH_3 group), 1174 (C-O-C symmetrical stretching OCH_3 group), 1022 (C-F stretching), 960 (C-H in plane bending for aromatic ring), 825 (C-H out of plane bending for 1,4-disubstituted aromatic ring), 709 (C-S-C stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 0.99 (s, 3H, $H_{a,}$), 1.07 (s, 3H, $H_{a'}$), 2.02-2.06 (d, 1H, H_b), 2.16-2.20 (d, 1H, H_b), 2.45 (s, 2H, H_c), 4.54-4.55 (d, 1H, H_d), 5.10-5.12 (d, 1H, H_e), 6.75-6.77 (d, 2H, $H_{h, h'}$, J = 8.32 Hz), 7.16-7.18 (d, 2H, $H_{i, i}$, J = 7.84 Hz), 7.06-7.10 (t, 2H, $H_{f, f'}$), 7.46-7.50 (t, 2H, $H_{g, g'}$), 8.51 (s, 1H, H_j), 3.72 (s, 1H, H_k); MS: *m/z* 377; Anal. Calcd. for $C_{24}H_{24}FNO_2$: C, 76.37; H, 6.41; N, 3.71. Found: C, 76.30; H, 6.33; N, 3.65%.

5.2.6.4.9 2-(4-fluorophenyl)-7,8-dihydro-4-(4-methoxyphenyl)-7,7-dimethyl quinolin-5(1H,4H,6H)-one (DDK-C-44)



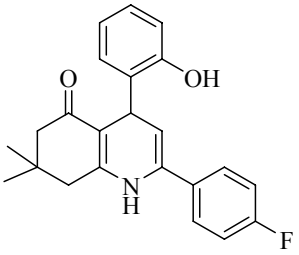
Yield: 81%; m.p. 213-215 ºC; MS: *m/z* 361; Anal. Calcd. for $C_{24}H_{24}FNO$: C, 79.75; H, 6.69; N, 3.88. Found: 79.68; H, 6.61; N, 3.82%

5.2.6.4.10 2,4-bis(4-fluorophenyl)-7,8-dihydro-7,7-dimethylquinolin-5(1H,4H,6H)-one (DDK-C-45)



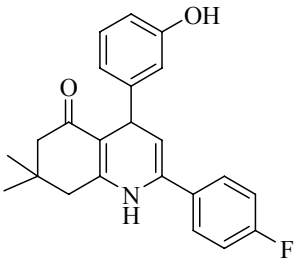
Yield: 82%; m.p. 194-196 ºC; MS: *m/z* 365; Anal. Calcd. for $C_{23}H_{21}F_2NO$: C, 75.60; H, 5.79; N, 3.83. Found: C, 75.54; H, 5.72; N, 3.76%.

5.2.6.4.11 2-(4-fluorophenyl)-7,8-dihydro-4-(2-hydroxyphenyl)-7,7-dimethyl quinolin-5(1H,4H,6H)-one (DDK-C-46)

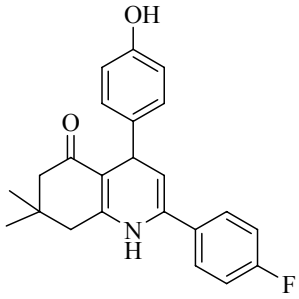


Yield: 60%; m.p. 201-203 ºC; MS: *m/z* 363; Anal. Calcd. for $C_{23}H_{22}FNO_2$: C, 76.01; H, 6.10; N, 3.85. Found: C, 75.94; H, 6.04; N, 3.78%.

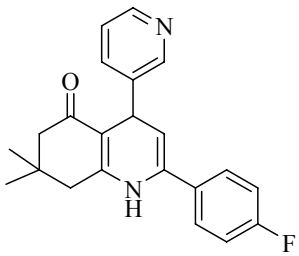
5.2.6.4.12 2-(4-fluorophenyl)-7,8-dihydro-4-(3-hydroxyphenyl)-7,7-dimethyl quinolin-5(1H,4H,6H)-one (DDK-C-47) Yield: 62%; m.p. 215-217 ºC; MS: *m/z* 363; Anal. Calcd. for $C_{23}H_{22}FNO_2$: C, 76.01; H, 6.10; N, 3.85. Found: C, 75.93; H, 6.02; N, 3.78%.



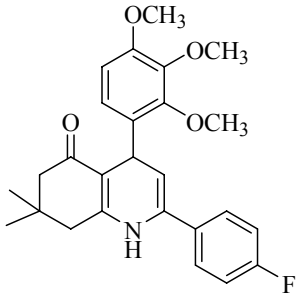
5.2.6.4.13 2-(4-fluorophenyl)-7,8-dihydro-4-(4-hydroxyphenyl)-7,7-dimethyl quinolin-5(1H,4H,6H)-one (DDK-C-48) Yield: 65%; m.p. 194-196 ºC; MS: *m/z* 363; Anal. Calcd. for $C_{23}H_{22}FNO_2$: C, 76.01; H, 6.10; N, 3.85. Found: C, 75.94; H, 6.03; N, 3.77%.



5.2.6.4.14 2-(4-fluorophenyl)-7,8-dihydro-7,7-dimethyl-4-(pyridin-3-yl)quinolin-5(1H,4H,6H)-one (DDK-C-49) Yield: 72%; m.p. 239-241 ºC; MS: *m/z* 348; Anal. Calcd. for $C_{22}H_{21}F_2NO$: C, 75.84; H, 6.08; N, 8.04. Found: C, 75.78; H, 6.00; N, 7.97%.



5.2.6.4.15 2-(4-fluorophenyl)-7,8-dihydro-4-(2,3,4-trimethoxyphenyl)-7,7-dimethyl quinolin-5(1H,4H,6H)-one (DDK-C-50) Yield: 75%; m.p. 222-224 ºC; MS: *m/z* 437; Anal. Calcd. for $C_{26}H_{28}$ FNO_4: C, 71.38; H, 6.45; N, 3.20. Found: C, 71.32; H, 6.38; N, 3.14%.

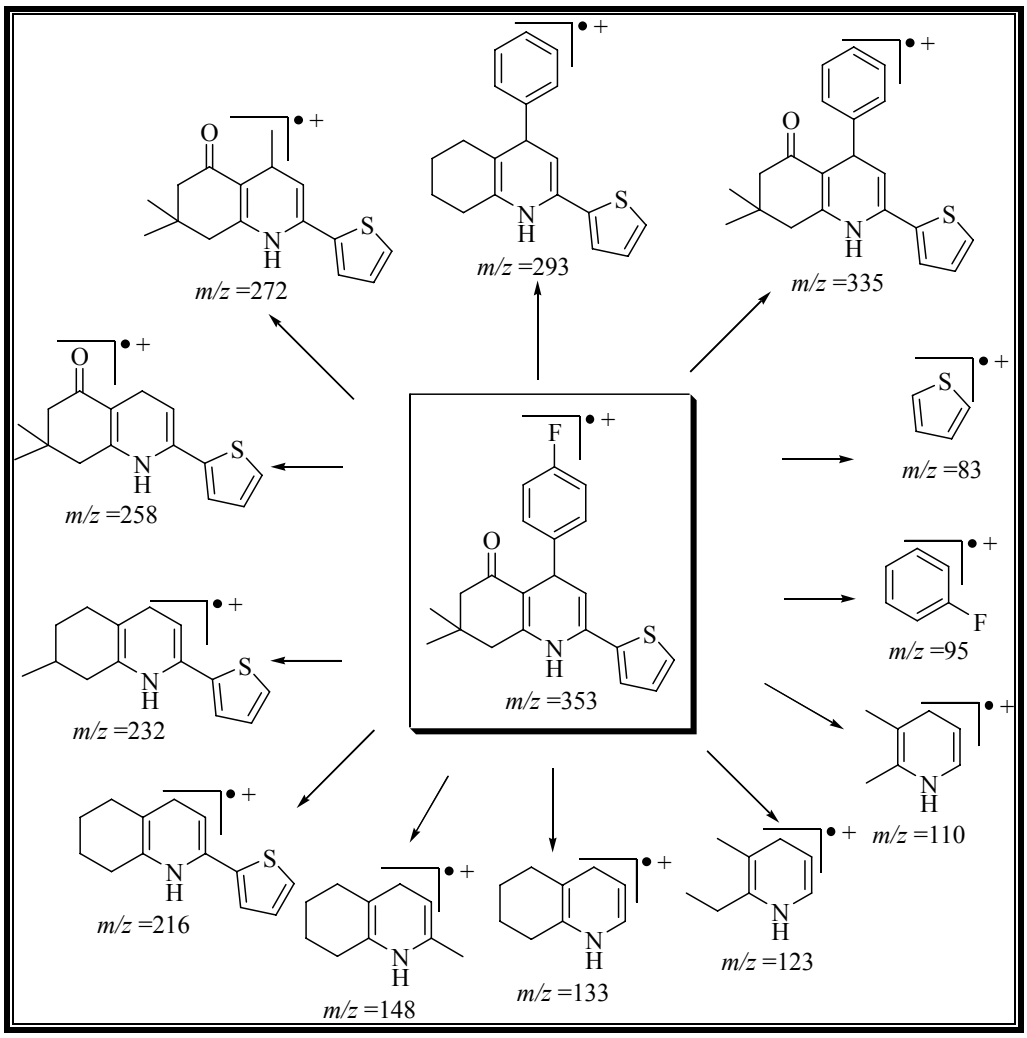


5.2.7 Spectral discussion

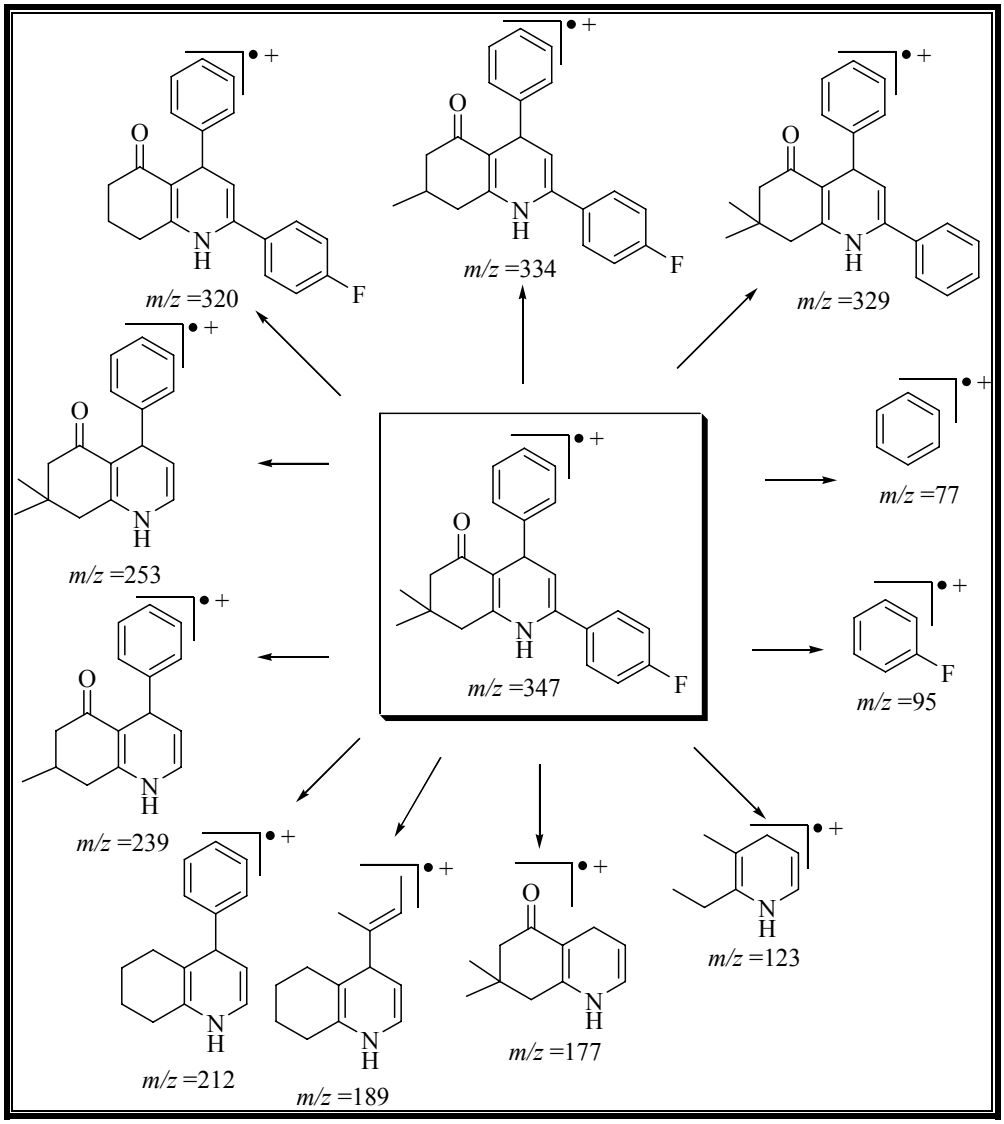
5.2.7.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of respective compound. Mass fragmentation pattern for a representative compound of each series is depicted below.

5.2.7.1.1 Mass fragmentation pattern for DDK-C-30



5.2.7.1.2 Mass fragmentation pattern for DDK-C-36



5.2.7.2 IR spectral study

IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For 7,8-dihydroquinolines (**DDK-C-21 to DDK-C-50**), a characteristic band of carbonyl group was observed in the range of 1645-1701 cm^{-1}. Another characteristic C-H asymmetrical and symmetrical stretching bands of methyl groups were observed at 2924-2956 cm^{-1} and 2847-2874 cm^{-1} respectively. Also, N-H stretching band of secondary amine was observed at 3255-3377 cm^{-1} suggesting formation of desired products (**DDK-C-21 to DDK-C-50**).

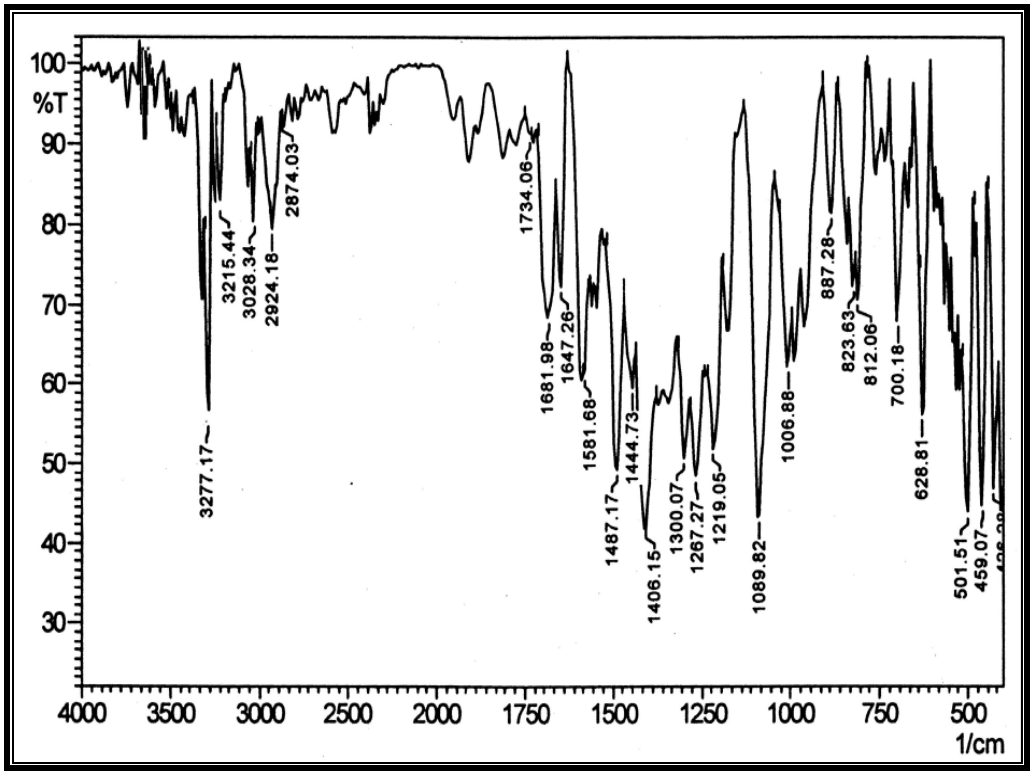
5.2.7.3 ^{1}H NMR spectral study

^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

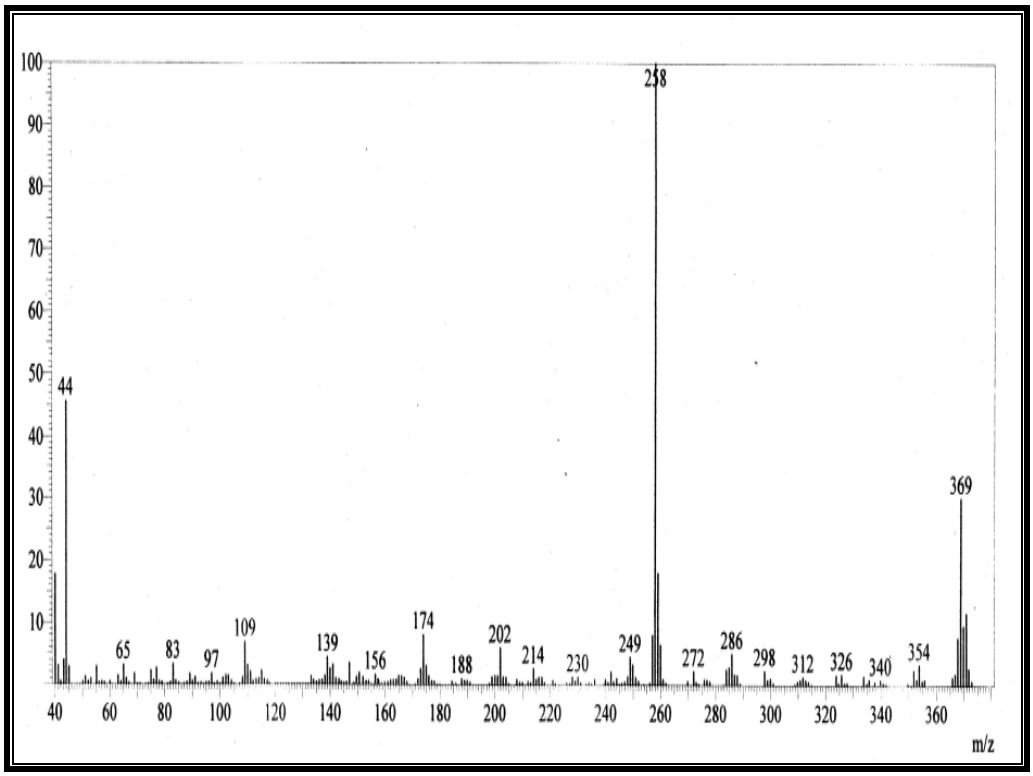
For 7,8-dihydroquinolines (**DDK-C-21 to DDK-C-35**), characteristic singlets were observed for methyl and methylene groups at 1.00-1.10 δ ppm and 2.02-2.50 δ ppm respectively. Confirmatory signal of two adjacent methine protons were observed at 4.52-4.61 δ ppm and 5.19-5.23 δ ppm. The aromatic ring protons were observed at 6.76-7.36 δ ppm and J value were found to be in accordance with substitution pattern on phenyl ring. The singlet for secondary amine (-NH) proton was observed at 8.40-8.49 δ ppm.

While, for 7,8-dihydroquinolines (**DDK-C-36 to DDK-C-50**), characteristic singlets were observed for methyl and methylene groups at 0.98-1.08 δ ppm and 1.99-2.46 δ ppm respectively. Confirmatory signal of two adjacent methine protons were observed at 4.54-4.61 δ ppm and 5.10-5.23 δ ppm. The aromatic ring protons were observed at 6.75-7.50 δ ppm and J value were found to be in accordance with substitution pattern on phenyl ring. The singlet for secondary amine (-NH) proton was observed at 8.51-8.52 δ ppm.

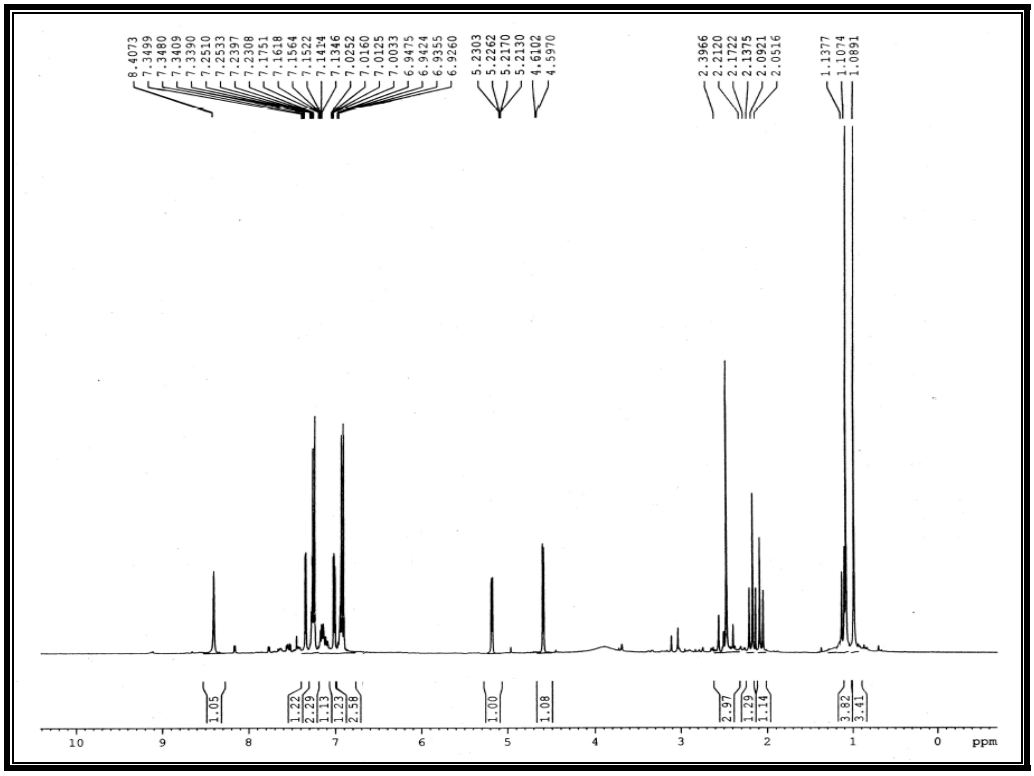
IR spectrum of DDK-C-24



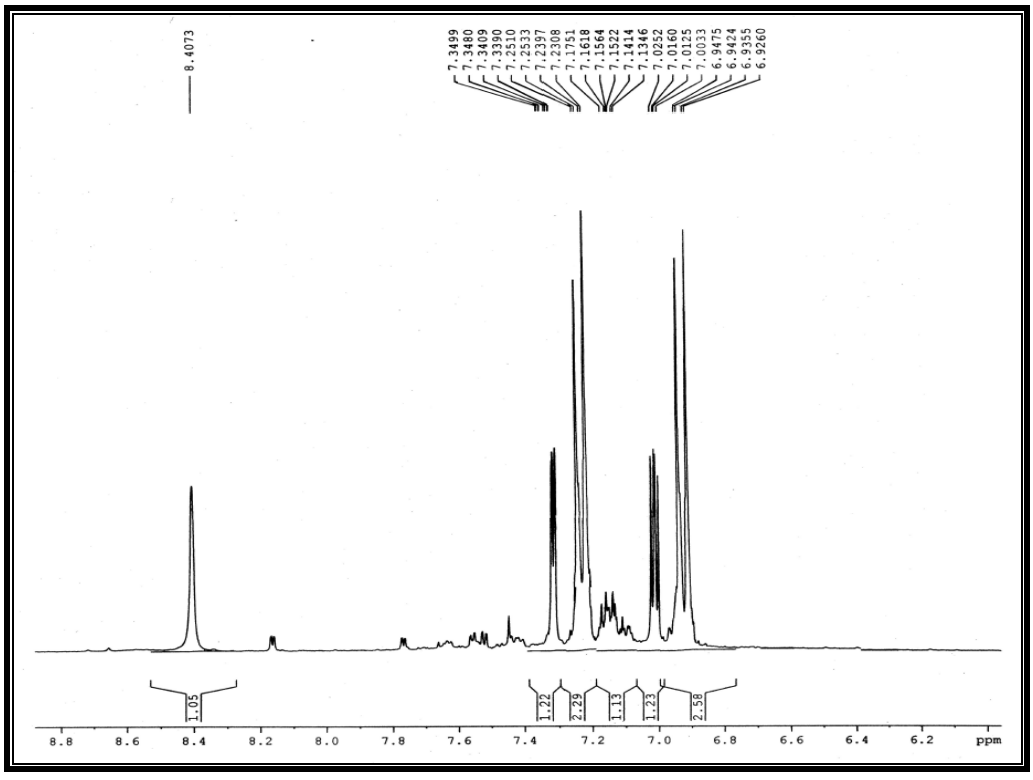
Mass spectrum of DDK-C-24



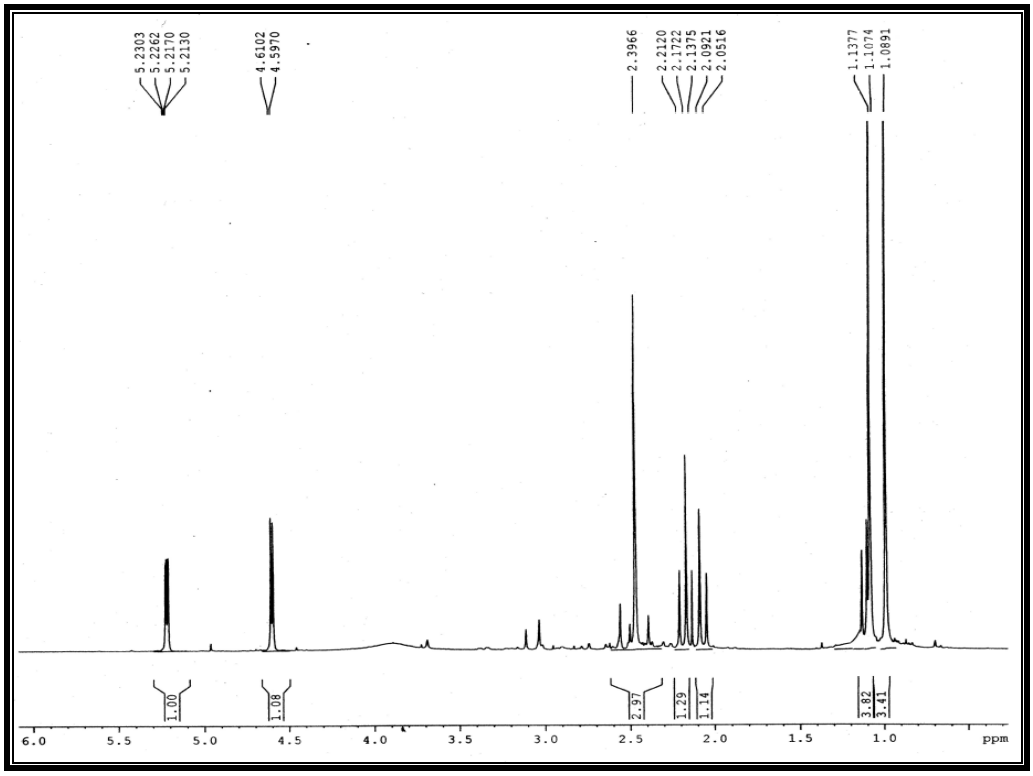
^{1}H NMR spectrum of DDK-C-24



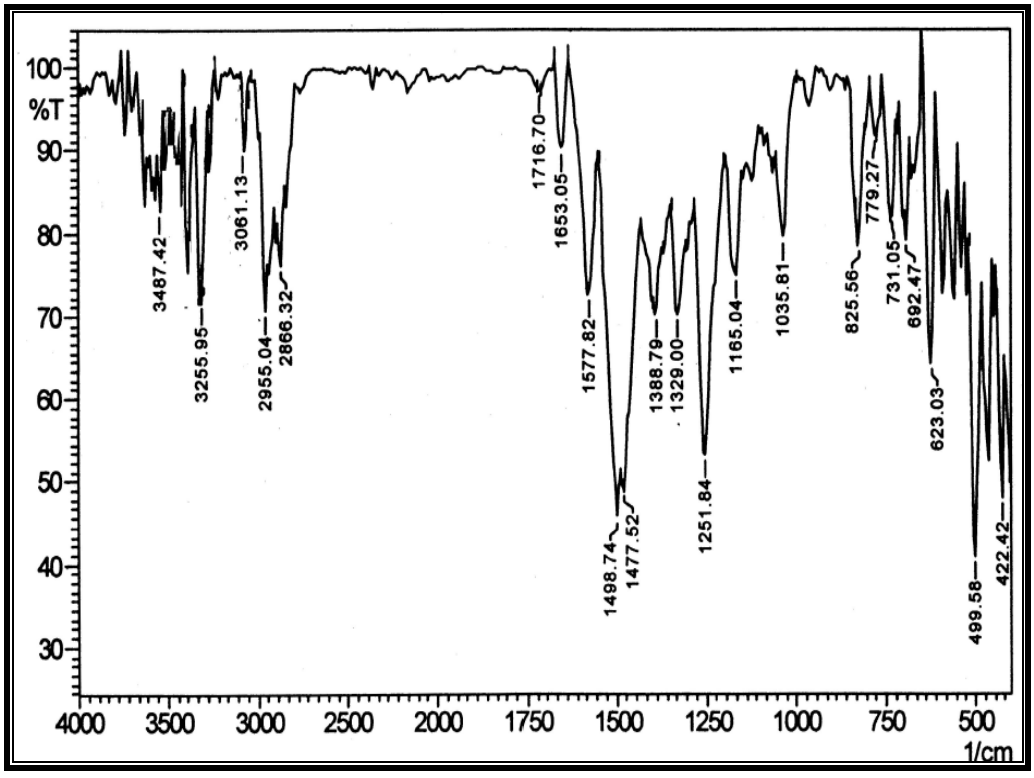
Expanded ^{1}H NMR spectrum of DDK-C-24



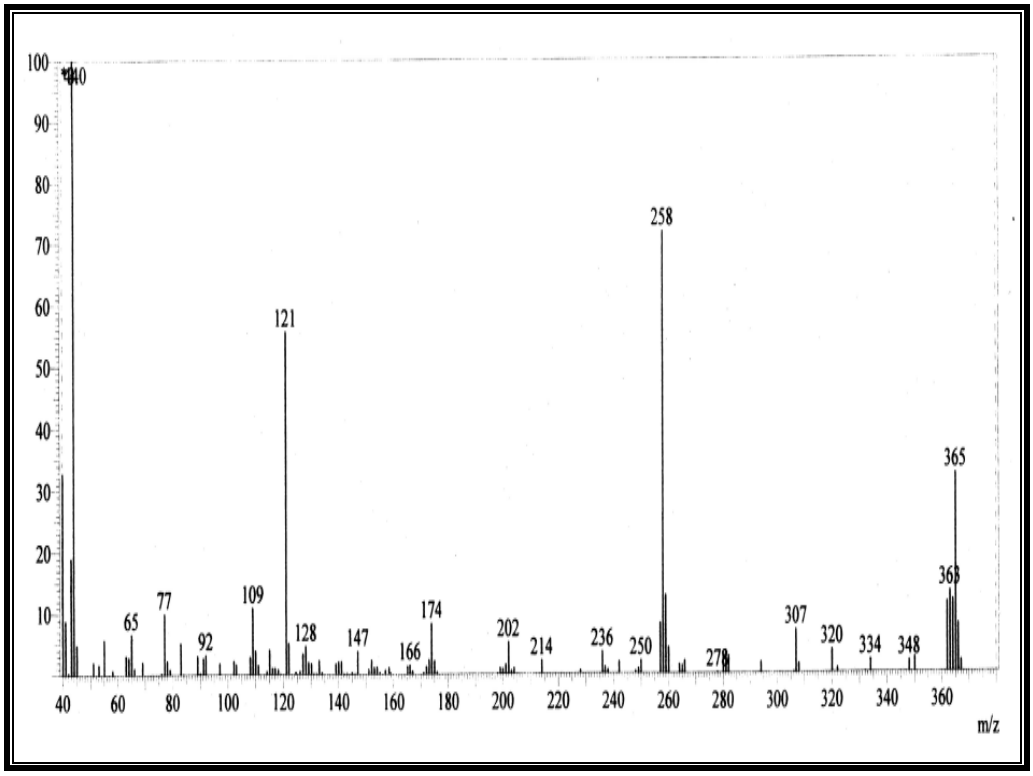
Expanded ^{1}H NMR spectrum of DDK-C-24



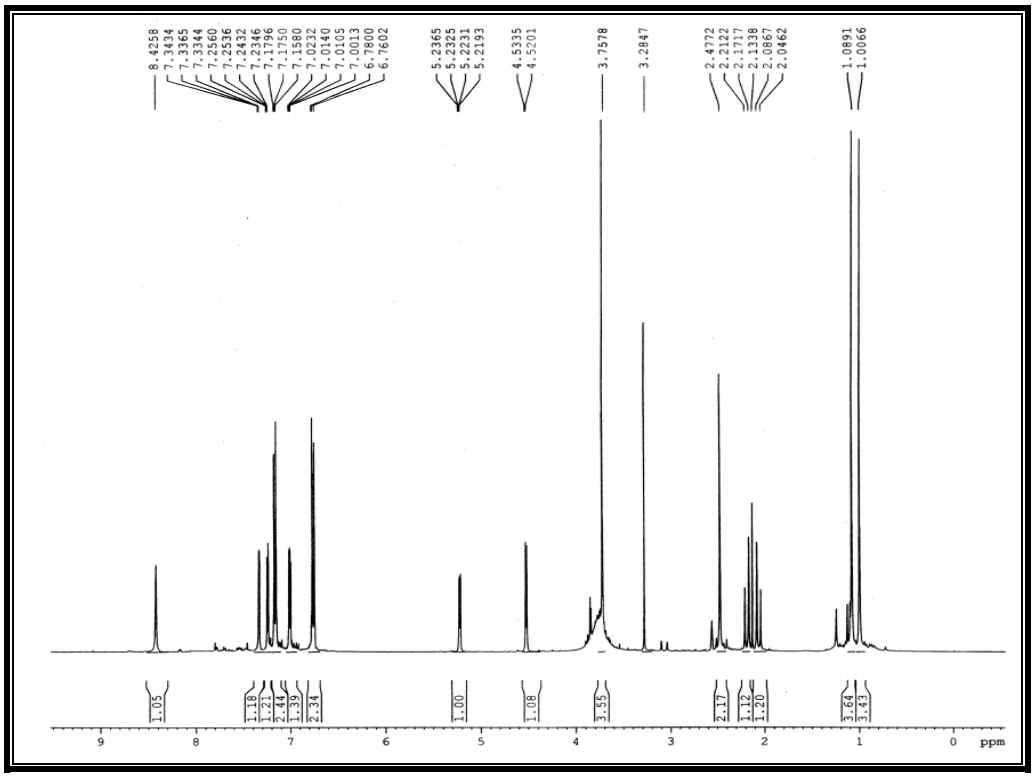
IR spectrum of DDK-C-28



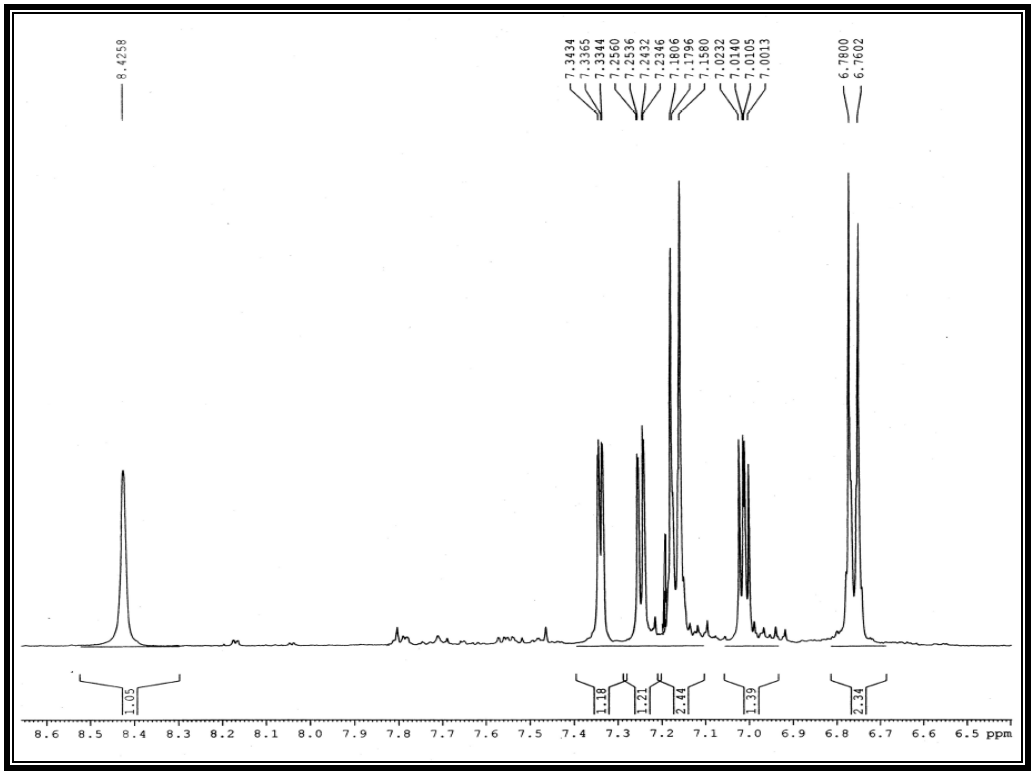
Mass spectrum of DDK-C-28



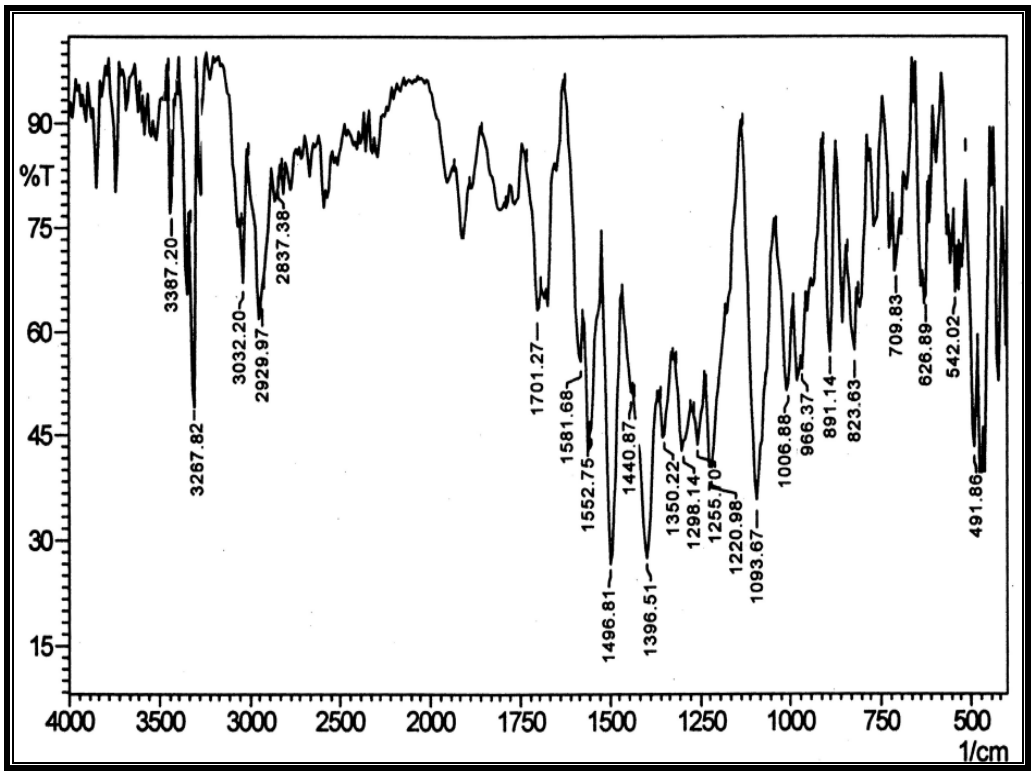
^{1}H NMR spectrum of DDK-C-28



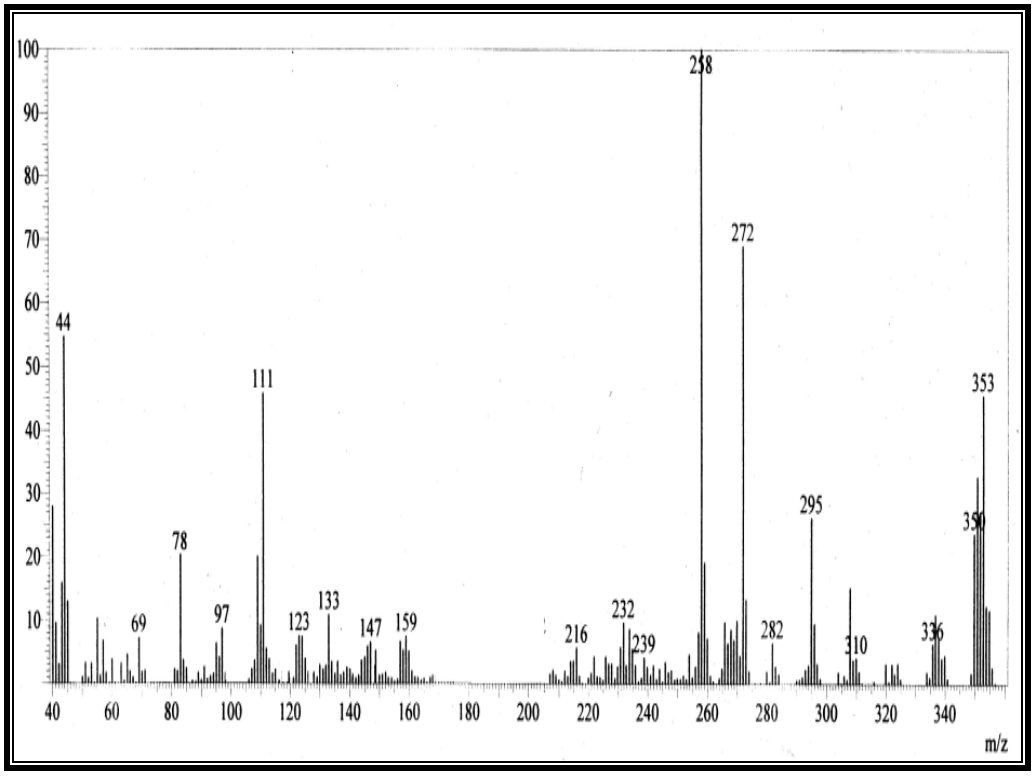
Expanded ^{1}H NMR spectrum of DDK-C-28



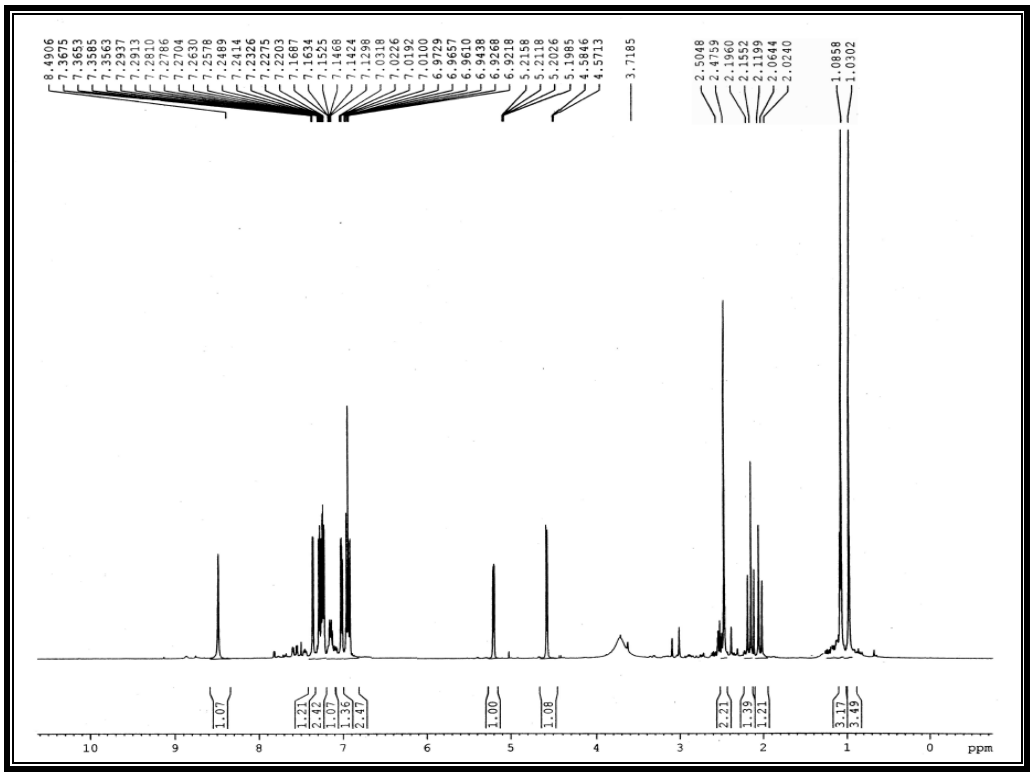
IR spectrum of DDK-C-30



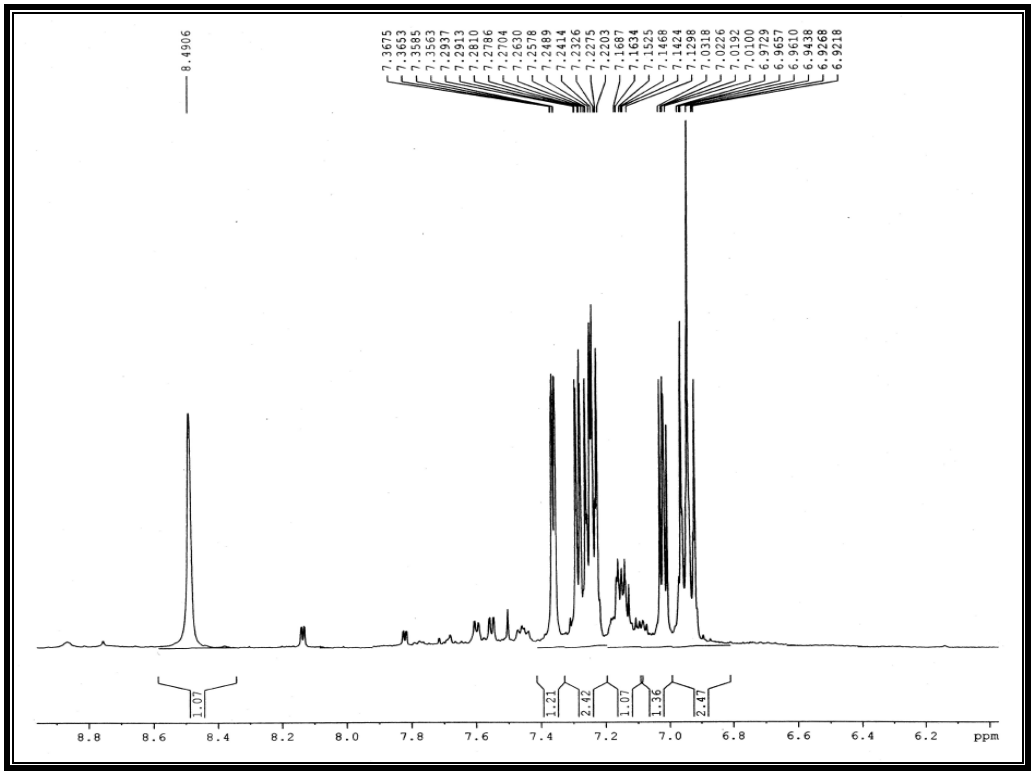
Mass spectrum of DDK-C-30



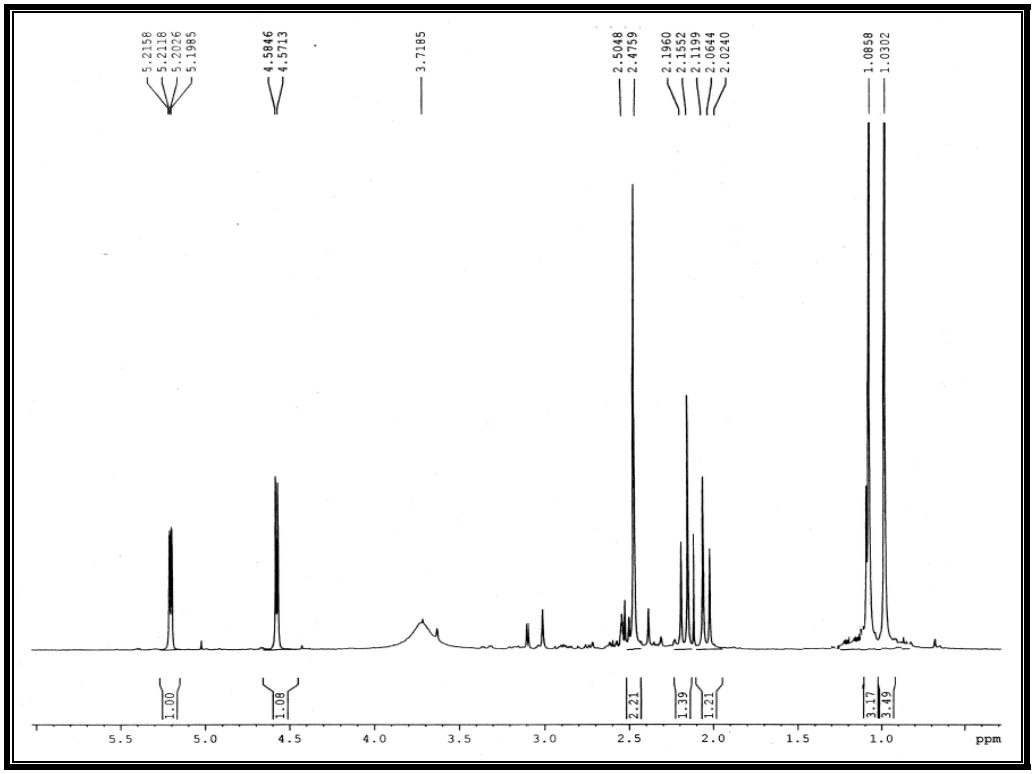
^{1}H NMR spectrum of DDK-C-30



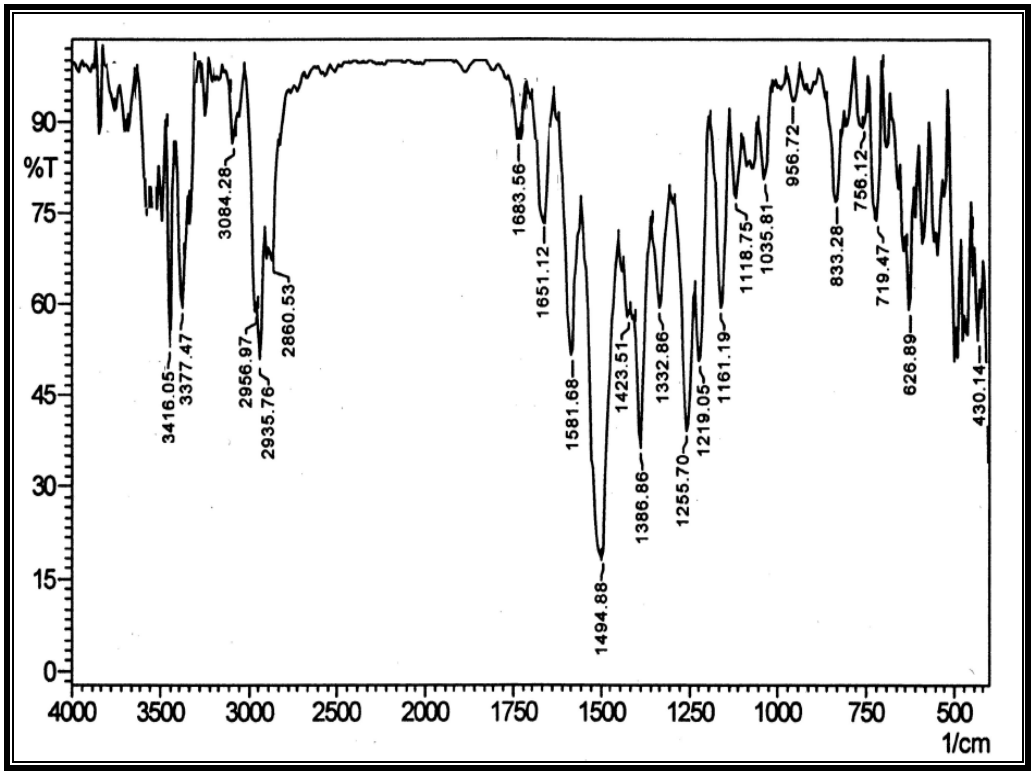
Expanded ^{1}H NMR spectrum of DDK-C-30



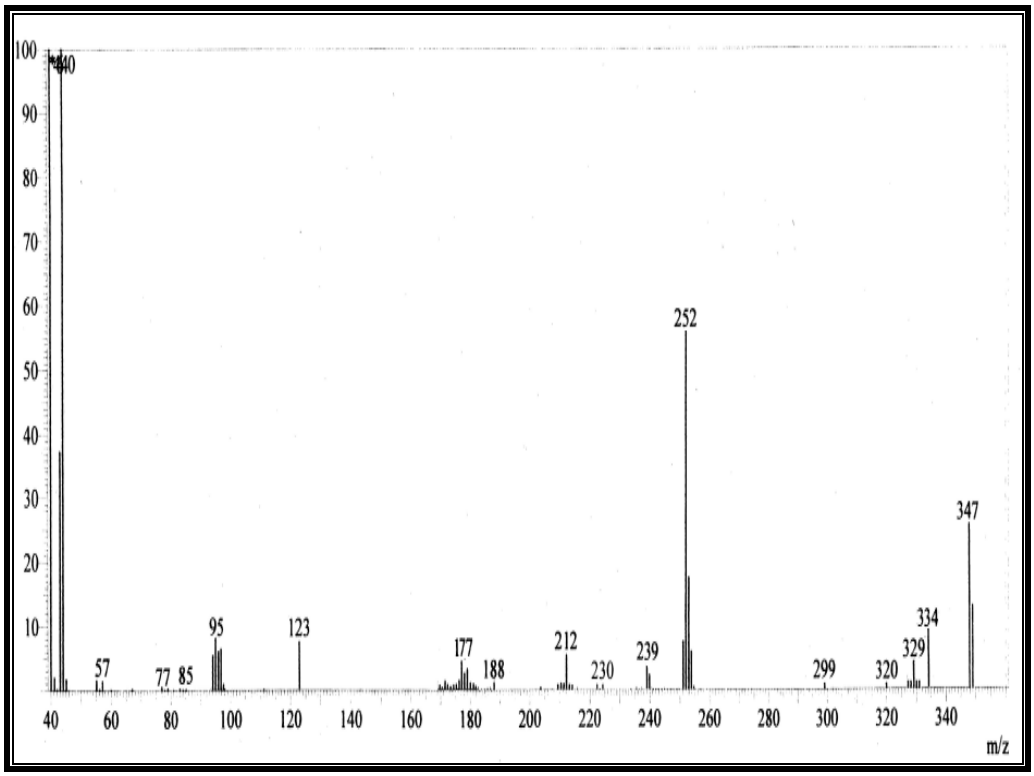
Expanded ^{1}H NMR spectrum of DDK-C-30



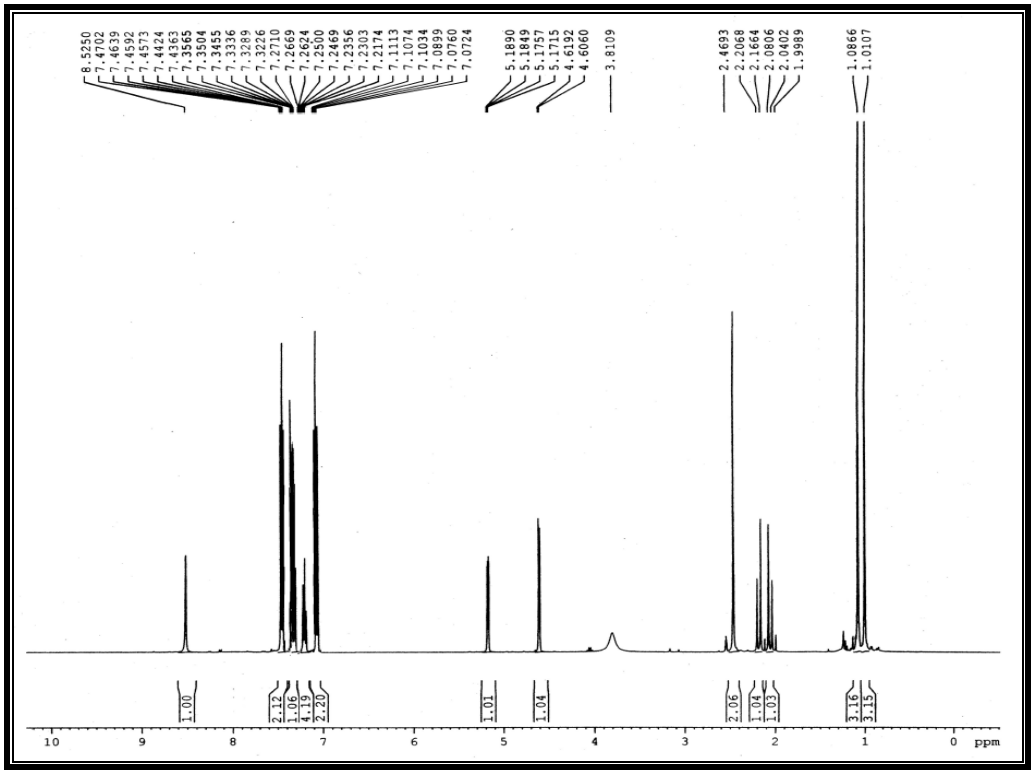
IR spectrum of DDK-C-36



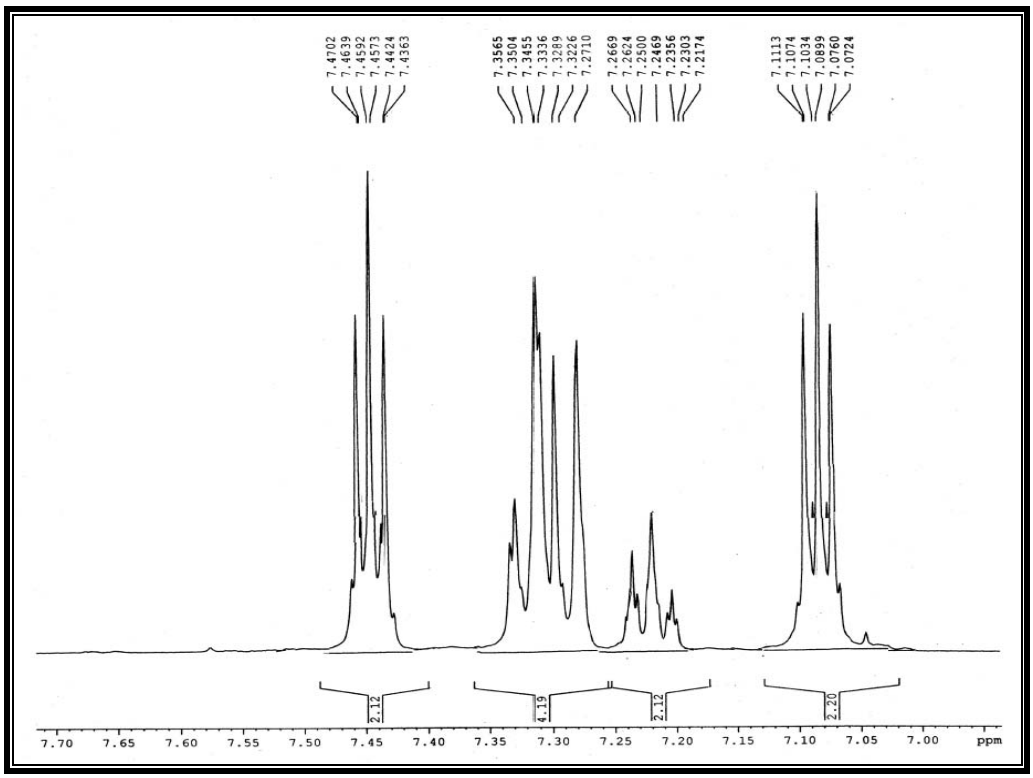
Mass spectrum of DDK-C-36



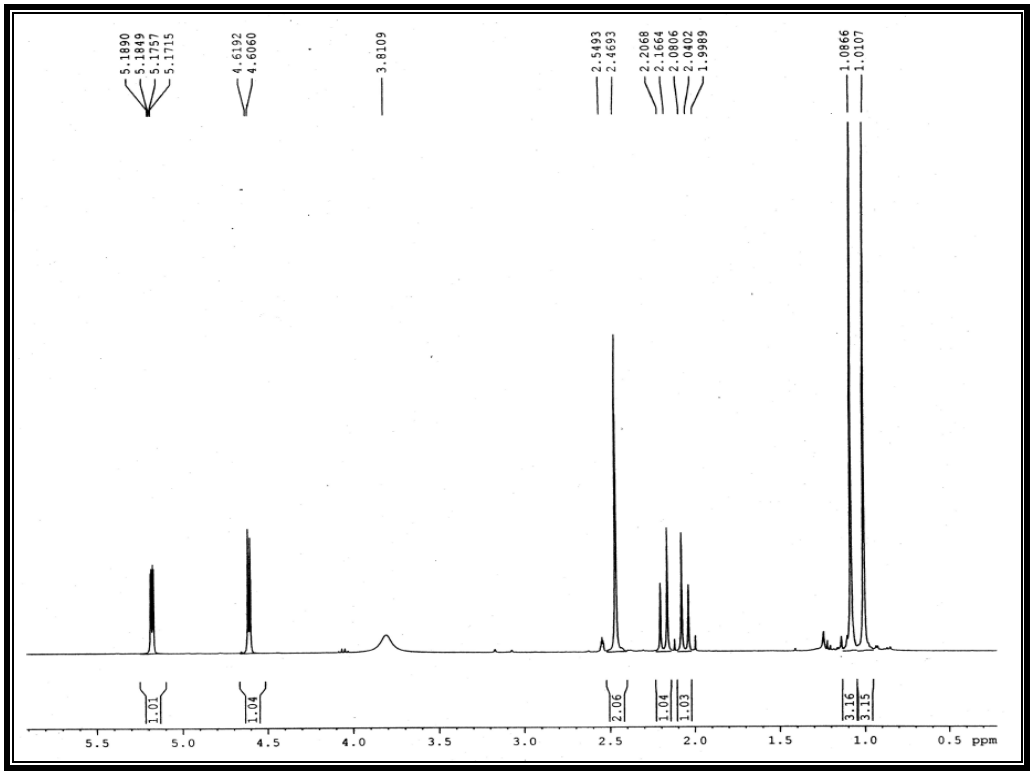
^{1}H NMR spectrum of DDK-C-36



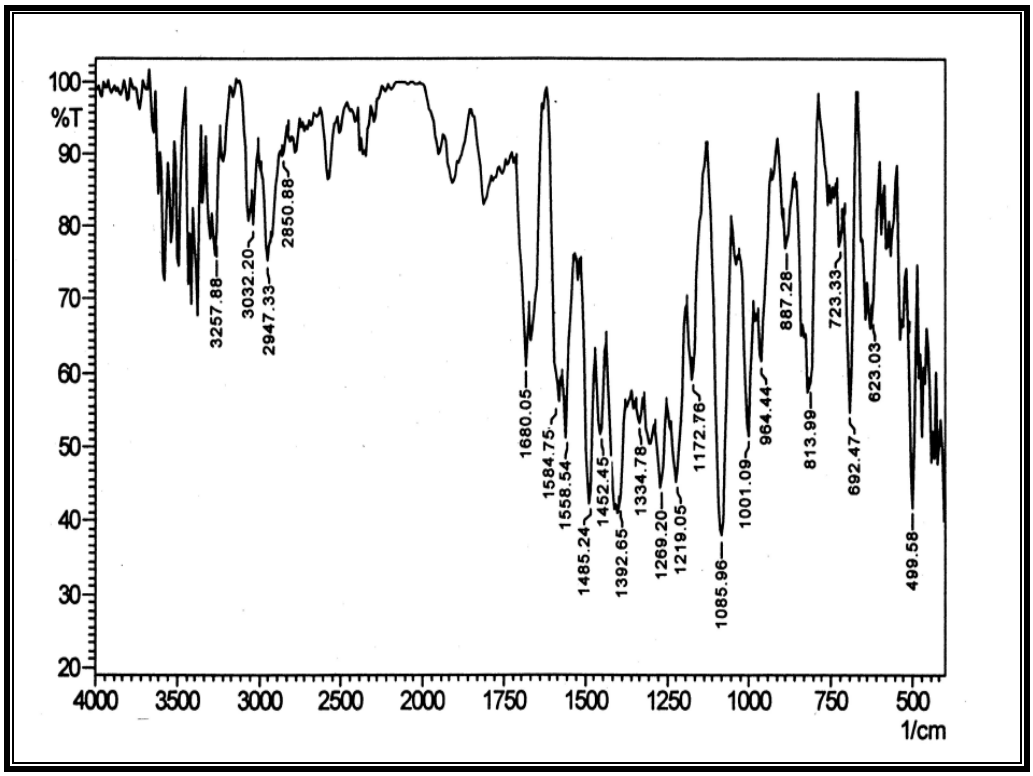
Expanded ^{1}H NMR spectrum of DDK-C-36



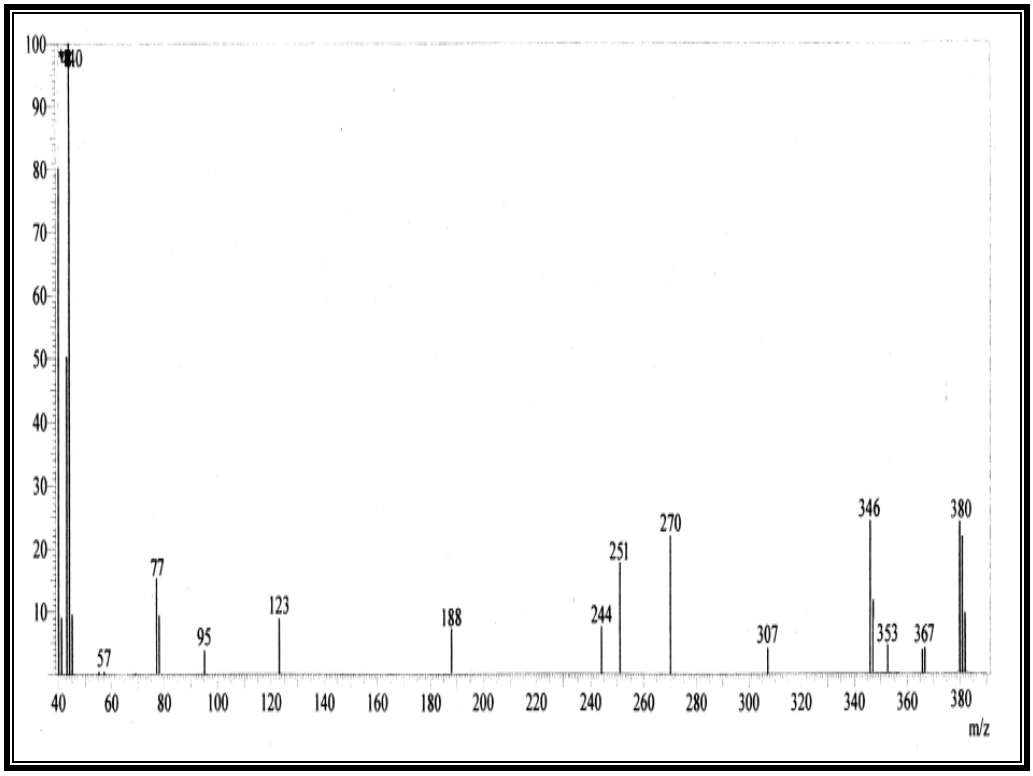
Expanded ^{1}H NMR spectrum of DDK-C-36



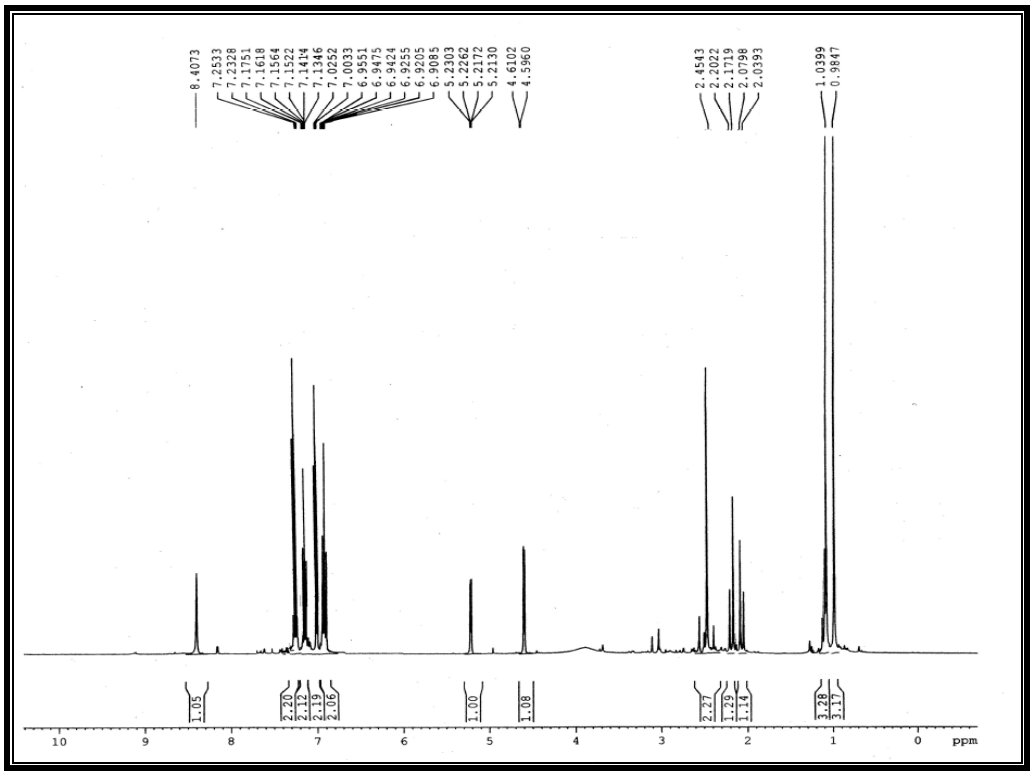
IR spectrum of DDK-C-39



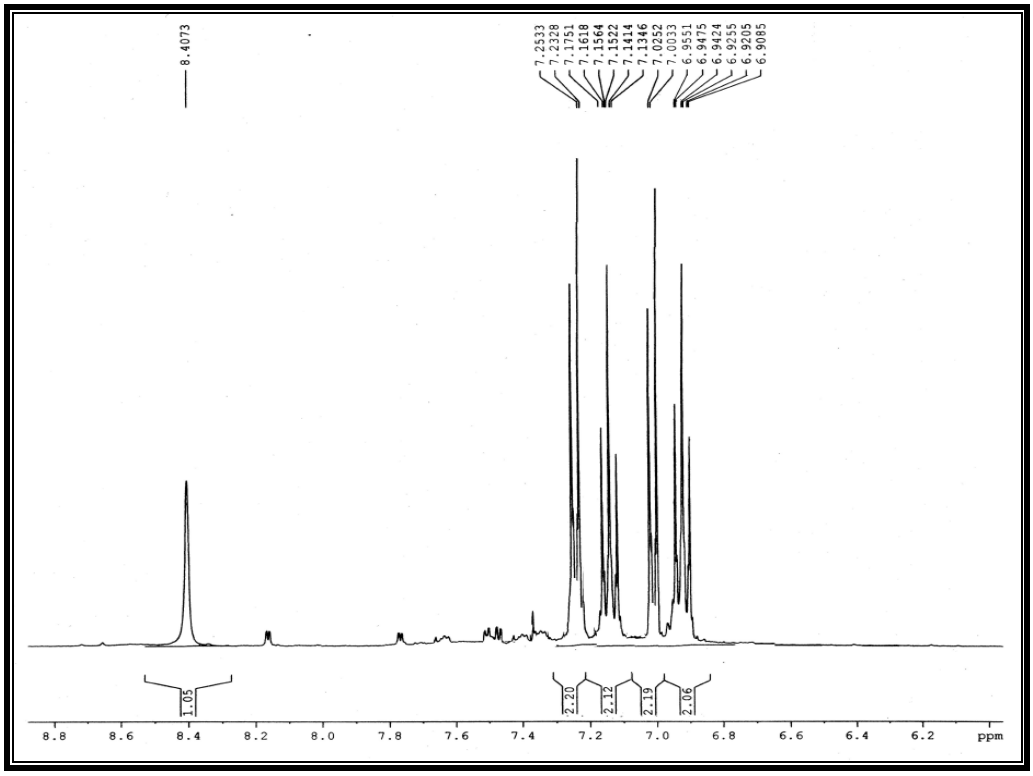
Mass spectrum of DDK-C-39



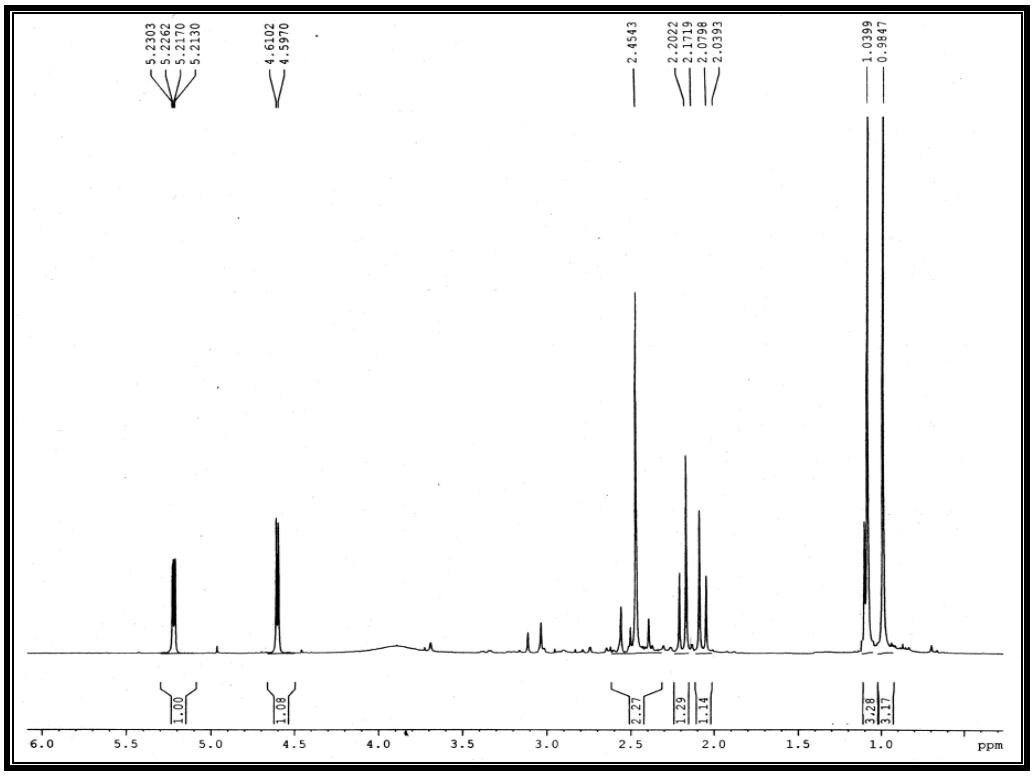
^{1}H NMR spectrum of DDK-C-39



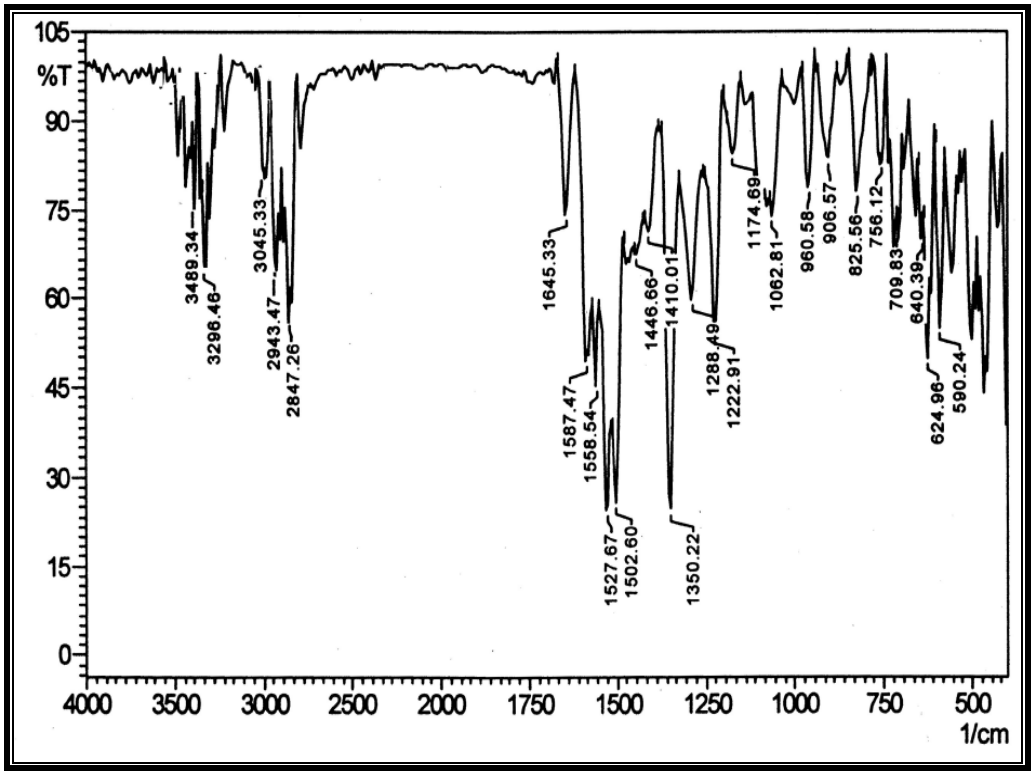
Expanded ^{1}H NMR spectrum of DDK-C-39



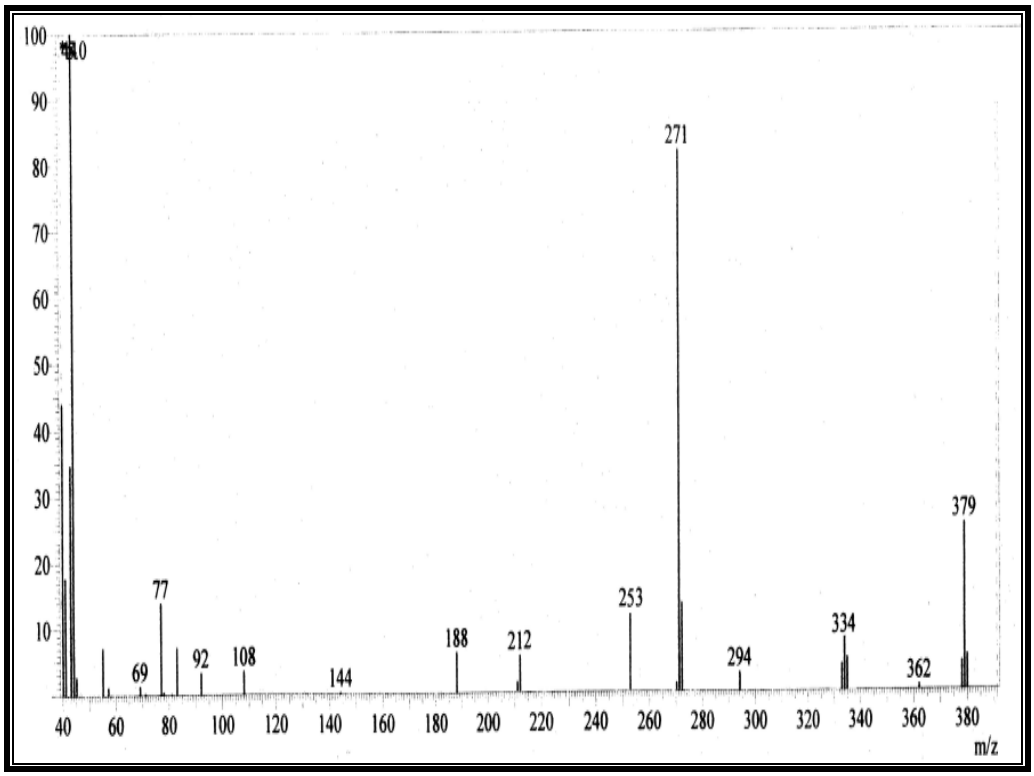
Expanded ^{1}H NMR spectrum of DDK--C-39



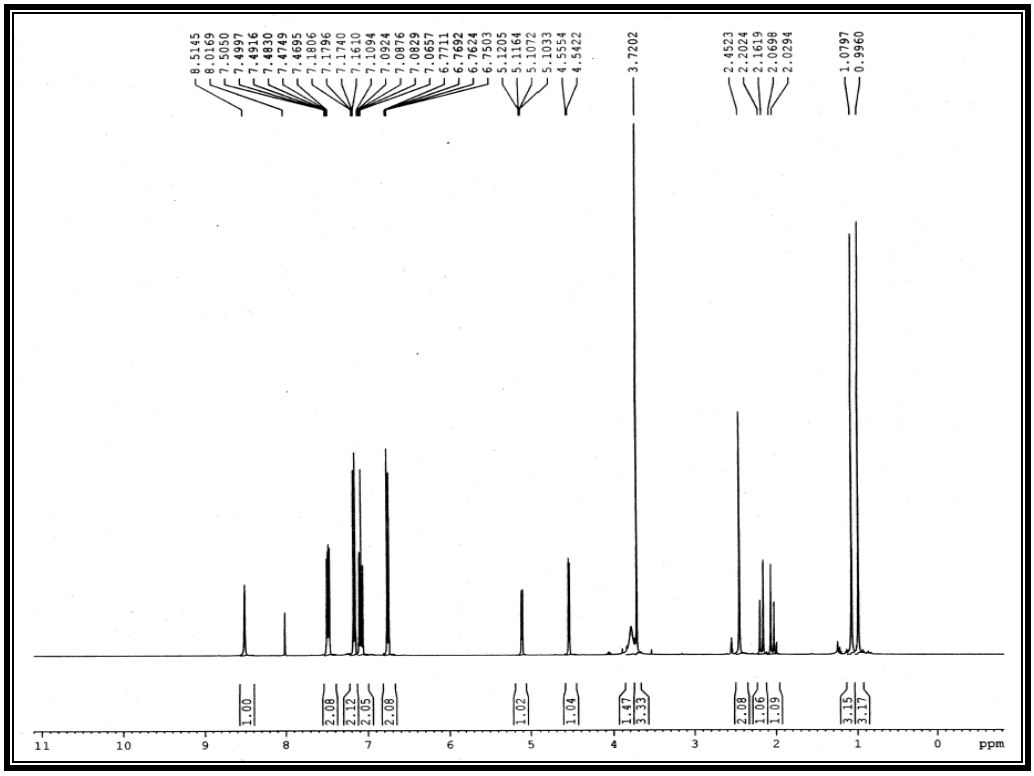
IR spectrum of DDK-C-43



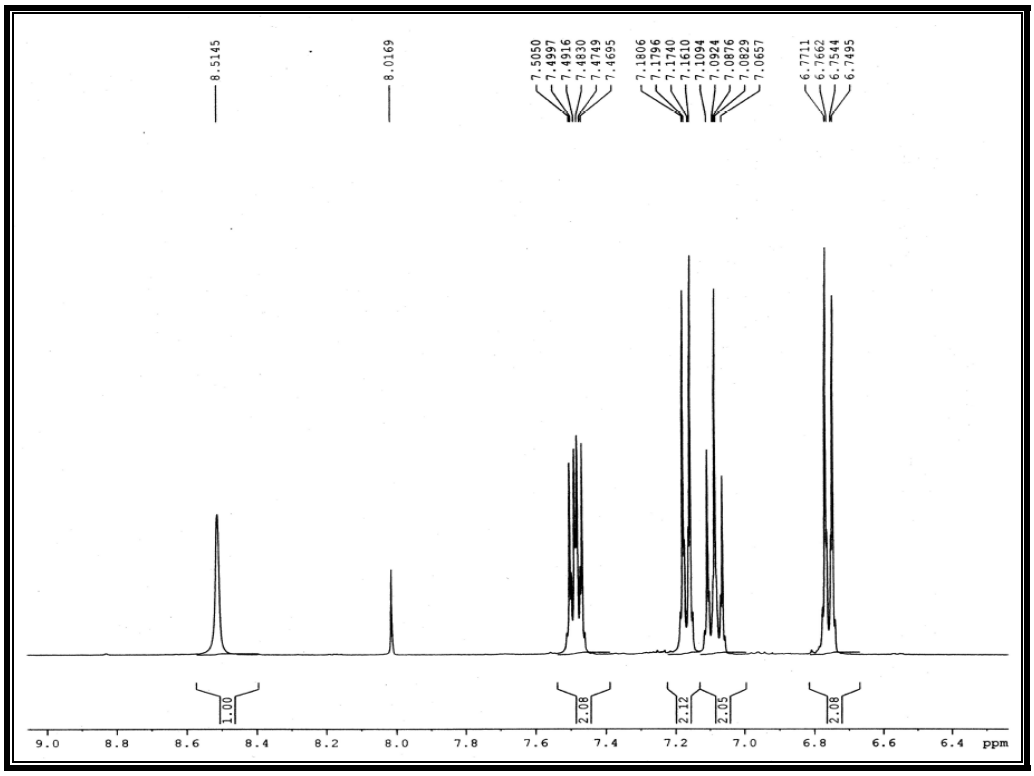
Mass spectrum of DDK-C-43



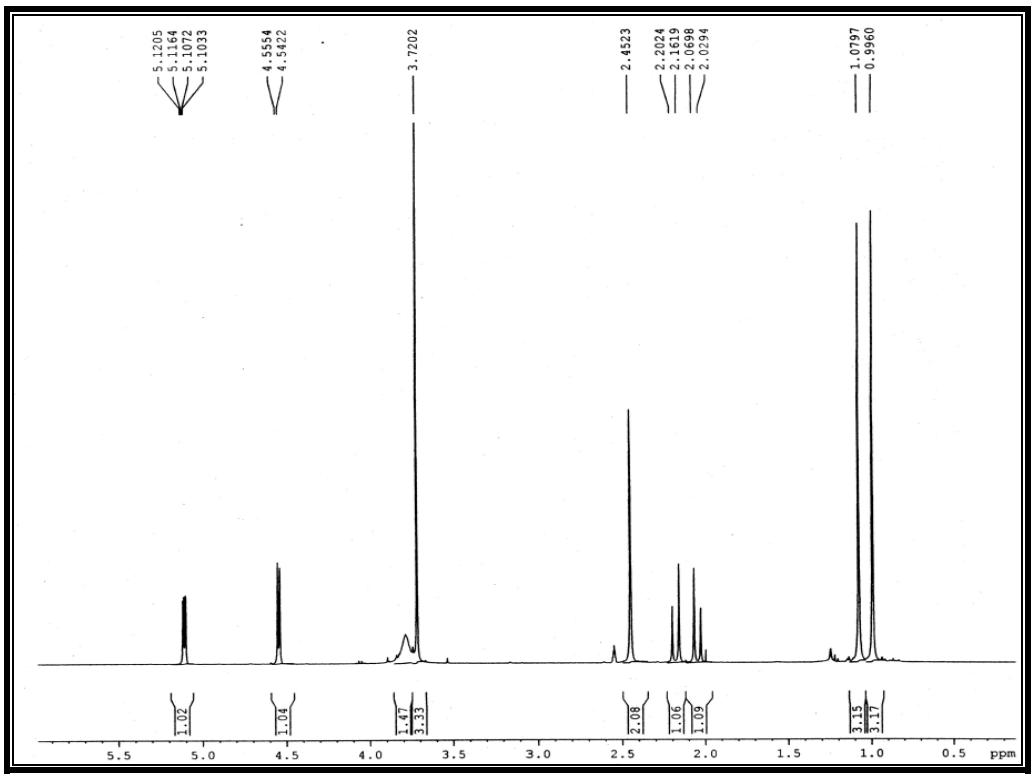
^{1}H NMR spectrum of DDK-C-43



Expanded ^{1}H NMR spectrum of DDK-C-43



Expanded ^{1}H NMR spectrum of DDK-C-43



5.2.8 Biological evaluation

5.2.8.1 Antimicrobial evaluation

All of the synthesized compounds (**DDK-C-21 to DDK-C-50**) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [57, 58] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin, and Griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using microdilution broth method

according to NCCLS standards [57]. Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations. In primary screening 1000 μg mL^{-1}, 500 μg mL^{-1} and 250 μg mL^{-1} concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution at 125 μg mL^{-1}, 62.5 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1}, and 6.250 μg mL^{-1} concentration against all microorganisms. The tubes were inoculated with 10^8 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table 1.

Table 1. Antibacterial and antifungal activity of synthesized compounds DDK-C-21 to DDK-C-50

Code	Minimum inhibition concentration (μg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	***S. p.***	***E.c.***	***P.a.***	***C. a.***	***A. n.***	***A.c.***
DDK-C-21	250	1000	1000	250	>1000	1000	500
DDK-C-22	125	500	>1000	1000	500	250	500
DDK-C-23	100	500	500	500	125	250	250
DDK-C-24	500	1000	250	1000	500	1000	500
DDK-C-25	250	100	500	>1000	1000	500	1000
DDK-C-26	500	1000	250	1000	500	500	>1000
DDK-C-27	1000	250	500	500	500	100	250
DDK-C-28	100	125	100	62.5	>1000	1000	1000
DDK-C-29	50	25	50	25	10	25	10
DDK-C-30	500	1000	500	100	1000	500	500
DDK-C-31	1000	500	1000	1000	500	500	1000
DDK-C-32	500	500	250	500	>1000	1000	500
DDK-C-33	250	62.5	125	250	1000	500	250
DDK-C-34	500	1000	500	100	1000	500	500
DDK-C-35	250	250	500	500	1000	100	1000
DDK-C-36	500	500	500	500	500	500	>1000
DDK-C-37	1000	1000	1000	1000	1000	500	500
DDK-C-38	>1000	500	250	500	1000	250	250
DDK-C-39	100	62.5	125	100	500	500	500
DDK-C-40	1000	1000	1000	1000	1000	>1000	500
DDK-C-41	25	100	200	100	1000	500	500

DDK-C-42	125	200	500	250	500	500	250
DDK-C-43	100	125	100	100	1000	500	500
DDK-C-44	50	100	25	100	>1000	500	1000
DDK-C-45	25	50	100	50	500	500	1000
DDK-C-46	500	500	500	500	500	500	>1000
DDK-C-47	50	125	100	200	>1000	500	>1000
DDK-C-48	125	100	250	125	500	500	>1000
DDK-C-49	125	250	125	50	500	1000	500
DDK-C-50	100	125	100	250	500	1000	>1000
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

5.3 References and notes

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Summary

The work presented in the Thesis entitled "**Studies on Heterocyclic Compounds of Medicinal Interest**" can be summarized as below.

Chapter 1 briefly introduces importance of bicyclic and tricyclic aromatic heterocycles in drug discovery as well as concept of "privileged structures". Chapter 1 further describes aims and objectives of the proposed research work.

Chapter 2 covers the synthesis of two types of cyanopyridines in two sections. The Section-1 of Chapter 2 includes the synthesis of twenty novel 2-alkoxy-pyridine-3-carbonitriles (2-alkoxy-3-cyanopyridines), which occupy a special position among various pyridine derivatives due to their wide spectrum biological activities along with their importance and utility as intermediates in preparing variety of heterocyclic compounds. The synthesis of these derivatives was achieved by Michael addition of α,β-unsaturated ketones (chalcones) to malononitrile in sodium methoxide/methanol and sodium ethoxide/ethanol systems resepectively. While Section-2 of Chapter 2 covers the synthesis of twenty novel fused cyanopyridines viz. pyrazolo[3,4-*b*]pyridine-5-carbonitriles. The pyrazolo[3,4-*b*]pyridines occupy a special position among fused pyridine systems containing an annelated five membered heteroaromatic ring due to their wide spectrum biological activities. The synthesis of 6-alkoxy-pyrazolo[3,4-*b*]pyridine-5-carbonitriles was achieved by the reaction of arylidene malononitrile and 3-methyl-1H-pyrazol-5*H*-one in sodium methoxide/methanol and sodium ethoxide/ethanol systems resepectively.

Chapter-3 briefly discusses the history of microwave assisted organic synthesis and their application in the synthesis of different heterocycles. Further it describes our aims and objectives to explore the microwave-assisted chemistry for the synthesis of two of our aimed heterocyclic scaffolds viz. acridines and polyhydroquinolines.

In Chapter 4, synthesis of twenty novel polyhydroacridine-1,8-diones is reported. Acridines posses a wide variety of pharmacological activities and many acridine derivatives are in clinical use as antimalarials and anticancer agents. Polyhydroacridine-1,8-diones are polyfunctionalized 1,4-dihydropyridine type derivatives. Synthesis of these derivatives may prove useful modification of 1,4-dihydropyridine nucleus in terms of biological activity. The synthesis of

polyhydroacridine-1,8-dione derivatives was achieved by microwave-assisted one pot reaction of 5,5-dimethylcyclohexane-1,3-dione (dimedone) with 3-(aryl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde and substituted 2-chloro-quinoline-3-carbaldehyde respectively in presence of excess of ammonium acetate.

Chapter 5 covers the synthesis of two types of polyhydroquinolines viz. 5,6,7,8-tetrahydroquinolines and 7,8-dihydro-2,4-disubstituted-quinolin-5(*1H,4H,6H*) -ones in two sections. The Section-1 of Chapter 5 includes the synthesis of twenty novel 2-amino-5,6,7,8-tetrahydro-4-heteroaryl-quinoline-3-carbonitriles. The synthesis was accomplished by the one pot microwave-assisted reaction of cyclohexanone with 2-((3-(aryl)-1-phenyl-1H-pyrazol-4-yl)methylene)malononitrile & 2-((2-chloro-substitutedquinolin-3-yl)methylene)malononitrile respectively in presence of excess of ammonium acetate. While Section-2 of Chapter-5 includes the synthesis of 7,8-dihydro-2,4-disubstituted-quinolin-5(*1H,4H,6H*) –ones by the one pot solvent-free microwave-assisted reaction of 5,5-dimethylcyclohexane-1,3-dione with 3-(aryl)-1-(thiophen-2-yl)prop-2-en-1-ones and 3-(aryl)-1-(4-fluorophenyl)prop-2-en-1-ones respectively in presence of excess of ammonium acetate.

Thus, new green chemistry approaches were developed for the synthesis of novel acridines and polyhydroquinolines leading to the improvement in the reaction time, yield and simplicity of work up procedure.

All the synthesized compounds were characterized by IR, Mass, ^{1}H NMR spectroscopy and elemental analyses.

Thus, 110 compounds are synthesized and characterized in entire thesis work. The synthesized compounds are screened for antimicrobial activity, results of which are incorporated in the thesis. Looking at the antimicrobial activity results (i.e. antibacterial and antifungal), remarkable number of compounds have demonstrated excellent antimicrobial activity as compared to the standard drugs.

All the newly synthesized compounds are also under antimycobacterial, anticancer and antiviral evaluation and their results are awaited.

Publications

1. Novel dihydropyrimidines as a potential new class of antitubercular agents, by Amit R. Trivedi, Vimal R. Bhuva, Bipin H. Dholariya, **Dipti K. Dodiya**, Vipul B. Kataria and Viresh H. Shah. *Bioorganic & Medicinal Chemistry Letters*, Accepted, In Press (DOI: 10.1016/j.bmcl.2010.08.046).
2. Synthesis and antimycobacterial evaluation of various 6-substituted pyrazolo[3,4-*d*]pyrimidine derivatives, by Amit R. Trivedi, Shailesh J. Vaghasiya, Bipin H. Dholariya, **Dipti K. Dodiya** and Viresh H. Shah. *Journal of Enzyme Inhibition and Medicinal Chemistry*, Accepted, In Press.
3. Synthesis, characterization and biological screening of some novel tetrahydroquinazoline derivatives, by S. J. Vaghasia, **D. K. Dodiya**, A. R. Trivedi, H. K. Ram and V. H. Shah. *Indian Journal of Chemistry (Section B)*, 49B, 802-806, 2010.
4. A new synthetic approach and biological evaluation of novel phenothiazines bearing *tert*-butyl group, by Amit R. Trivedi; Arif B. Siddiqui; **Dipti K. Dodiya**; Manish J. Soalnki; Viresh H. Shah. *Journal of Sulfur Chemistry*, 30(6), 590-595, 2009.
5. Facile one pot synthesis and antimycobacterial evaluation of pyrazolo[3,4-*d*]pyrimidines, by Amit Trivedi, **Dipti Dodiya**, Janak Surani, Samir Jarsania, Hitesh mathukia, Naresh Ravat, Viresh shah. *Archiv der Pharmazie,* 341(7), 435-439, 2008.
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7. Synthesis and biological screening of some novel pyrazolo[3',4':4,5]thieno[2,3-*d*]pyrimidin-8-ones via Gewald reaction, by Shailesh J. Vaghasiya, **Dipti K. Dodiya,** Amit R. Trivedi and Viresh H. Shah. *ARKIVOC*, 12, 1-8, 2008.
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9. Synthesis and biological screening of some novel pyrazolo[3,4-*d*]pyrimidines, by Vaghasia, S. J.; **Dodiya, D. K.**; Shah, V. H. *Indian Journal of Heterocyclic Chemistry*, 18(1), 9-12, 2008.
10. Simple and efficient synthetic routes to bioactive s-triazinyl dithiocarbamate derivatives, by R. M. Desai, **D. K. Dodiya**, A. R. Trivedi and V. H. Shah. *Medicinal Chemistry Research*, 17, 495. 2008.
11. Synthesis and biological evaluation of some novel N-aryl-1,4-dihydropyridines as potential antitubercular agents, by Amit Trivedi, Bipin Dholariya, **Dipti Dodiya**, Vipul Kataria, Vimal Bhuva, Viresh shah. *European journal of Medicinal Chemistry*, Submitted, Under Review.
12. Synthesis and antimicrobial evaluation of novel benzo[*b*]thiophenes comprising *β*-lactam nucleus, by Amit R. Trivedi, Jignesh M Desai, Bipin H. Dholariya, **Dipti Dodiya** and Viresh H. Shah. *Medicinal Chemistry Research*, Under Revision.
13. A simple and efficient microwave-assisted green access to 5-arylidene-2-thiohydantoins in aqueous media by **Dipti K. Dodiya** & Viresh H. Shah. *Chemical Monthly*, Under Review.
14. Microwave-assisted efficient one-pot synthesis of novel *5,6,7,8*-tetrahydroquinoline-3-carbonitriles by **Dipti K. Dodiya** & Viresh H. Shah. *Journal of the Serbian Chemical Society,* Under Review.
15. Recent Advances in the Synthesis of Pyrazolo[3,4-*b*]pyridines by **Dipti K. Dodiya** & Viresh H. Shah. *Current Organic Chemistry*, Under Review.

Conferences/Seminars participated

1. National seminar on "Recent Advances in Chemical Sciences & An Approach to Green Chemistry" jointly organized by Department of Chemistry & Gujarat Council on Science and Technology (GUJCOST)-Gandhinagar at Rajkot, India (October 11-13, 2006).
2. National Workshop on Management and Use of Chemistry Databases and Patent Literature jointly organized by Department of Chemistry & Gujarat Council on Science and Technology (GUJCOST)-Gandhinagar at Rajkot, India (February 27-29, 2008).
3. "National Workshop on Updates in Process & Medicinal Chemistry" jointly Organized by Department of Chemistry, Saurashtra University, Rajkot and National Facility for Drug Discovery Through NCE's Development & Instrumentation Support to Small Manufacturing Pharma Enterprises and Think Pharma-USA (March 3-4, 2009).
4. "National Conference on Spectroscopy & Stereochemistry" Organized by Department of Chemistry, Saurashtra University, Rajkot Sponsored by UGC, New Delhi and Gujarat Council on Science and Technology (GUJCOST)-Gandhinagar (March 18-20, 2009).
5. "Two Days National Workshop on Patents & IPR Related Updates" Organized by Technology Information, Forecasting Assessment Council (TIFAC)-New Delhi, Gujarat Council on Science and Technology (GUJCOST)-Gandhinagar and National Facility for Drug Discovery Through NCE's Development & Instrumentation Support to Small Manufacturing Pharma Enterprises at Department of Chemistry, Saurashtra University, Rajkot (September 19-20, 2009).
6. "International Seminar on Recent Developments in Structure and Ligand Based Drug Design" jointly organized by Schrodinger LLC, USA & Department of Chemistry and National Facility for Drug Discovery Through NCE's Development & Instrumentation Support to Small Manufacturing Pharma Enterprises at Department of Chemistry, Saurashtra University, Rajkot (December 23, 2009).

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