Synthesis of Heterocyclic Compounds of Therapeutic 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: 31/03/2011

Place: Rajkot (Bipin H. Dholariya)

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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 Mr. Bipin H. Dholariya has been the result of work carried out under my supervision and is a significant contribution in the field of synthetic organic chemistry.

Date: 31/03/2011
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It is not because things are difficult that we do not dare,

It is because we do not dare that they are difficult.

-Seneca

Table of Contents

List of Abbreviations

NCEs	New Chemical Entities
MAOS	Microwave Assisted Organic Synthesis
R & D	Research & Development
Da	Dalton
HTS	High Throughput Screening
HIV	Human Immunodeficiency Virus
NNRTIs	Non Nucleoside Reverse Transcriptase Inhibitors
PDE inhibitors	Phosphodiesterase inhibitors
cAMP	cyclic Adenosine Monophosphate
IAP	Inhibitors of Apoptosis
AMPK	Adenosine Monophosphate-activated Protein Kinase
DMF	Dimethylformamide
Et_3N	Triethylamine
AcOH	Acetic Acid
EtOH	Ethanol
t-BuOK	Potassium tertiary butoxide
DMSO	Dimethyl sulfoxide
DBU	Diaza(1,3)bicyclo[5.4.0]undecane
DMA	Dimethylacetal
DEEMM	Diethyl-2-(ethoxymethylene)malonate
m/e	Mass/charge ratio
C. cylindracea	Cylichna cylindracea
i-PrOH	Isopropyl alcohol
FT-IR	Fourier Transform- Infrared spectroscopy
^{1}H-NMR	^{1}H- Nuclear Magnetic Resonance spectroscopy
TLC	Thin Layer Chromatography
h.	Hours
GC-MS	Gas Chromatograph- Mass Spectrometry
MeOH	Methanol
KBr	Potassium bromide
mL	Milliliter

mp	Melting Point
Ms	Mass
IR	Infrared
TMS	Trimethylsilane
Anal. Calcd.	Analytical Calculated
MHz	Megahertz
R_f	Retardation factor
MIC	Minimum Inhibitory Concentration
MTCC	Microbial Type Culture Collection
NCCLS	National Committee for Clinical Laboratory Standards
HSG	Human Salivary Gland
mg	Miligram
µg	Microgram
cfu	colony forming unit
MCRs	Multicomponent Reactions
IMCRs	Isocyanide Based Multicomponent Reactions
HCN	Hydrogen cyanide
U-4CR	Ugi Four-component Reactions
TEBA	Triethyl Benzyl Ammonium chloride
rt	Room Tempurature
per-6-ABCD	per-6-amino-bcyclodextrin
TBAB	Tetra Butyl Ammonium Bromide
TMAH	Tetramethyl Ammonium Hydroxide
KDR	Kinase insert Domain Receptor
CDK-2	Cyclin-Dependent Kinase -2
Al_2O_3	Aluminium oxide
MgO	Magnasium Oxide
MW	Microwave
Min.	Minute
W	Watt

General remarks

1. ^{1}H NMR spectra were recorded on Bruker avance II 400 MHz NMR 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.

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).

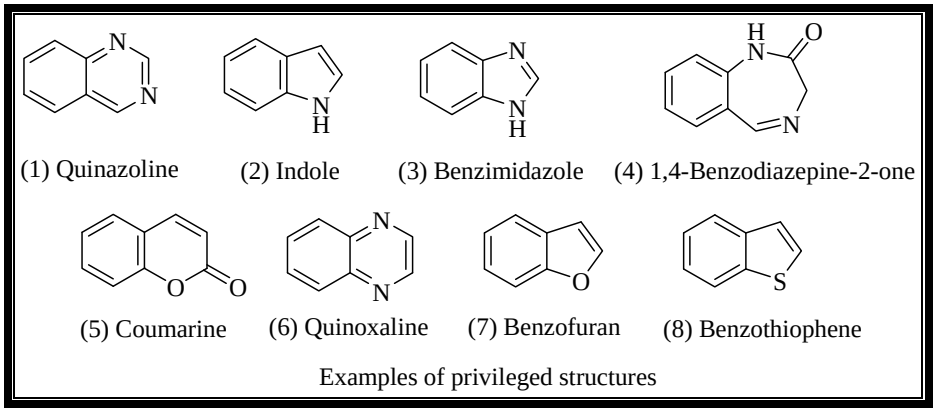
The cause of this innovation deficit is definitively not the biology. Decoding of the human genome has led to a wealth of drug targets. With more than 30,000 human genes, the assumption is that at least 1,000 are significantly involved in the emergence and course of disease. Furthermore, because each 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 (MAOS) and high-throughput purification [2]. Despite this steady increase in R & D, the number of NCEs reaching the market has actually decreased dramatically.

It seems clear that selecting 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 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, which have the potential to be biologically active [3].

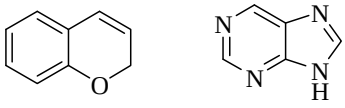
In order to start a new drug discovery project and to find biologically active compounds, different options are available. Hits can be obtained *via* a virtual screening approach or can be copied from scientific or patent literature. Very often, drug discovery projects start with a high-throughput screening campaign of commercially available compound libraries against the target of interest. It became clear in recent years that combinatorial libraries are not diverse enough. As the main interest of the laboratory of medicinal chemistry lays in the synthesis and biological evaluation of aromatic heterocycles, we performed a literature survey of commercially available combinatorial libraries. This search revealed that the number of available heterocycles is mainly limited to well-known nitrogen containing compounds, such as quinazolines (1), indoles (2) and benzimidazole (3).



Examples of privileged structures

These structural classes are considered to be privileged structures. The concept of “privileged structures” was first proposed by Evans *et al.* to describe selected structural types that bind to multiple, unrelated classes of protein receptors and enzymes as high affinity ligands [4]. These privileged structures are typically rigid, polycyclic heteroatomic systems capable of orienting the various substituents in a well-defined three-dimensional space. Well-known examples of privileged

substructures include benzodiazepines (4), coumarins (5), quinoxalines (6), benzofurans (7) and benzothiophenes (8) [5]. 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 and co-workers constructed a library based on the benzopyran (9) privileged scaffold [6], whereas Schultz and co-workers made use of the purine (10) scaffold [7].



(9) Benzopyran (10) Purine

Thr benzopyran and purine privileged scaffold

1.2 Nomenclature of the fused ring system

As the following chapters deal with the synthesis of bicyclic and tricyclic 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'.

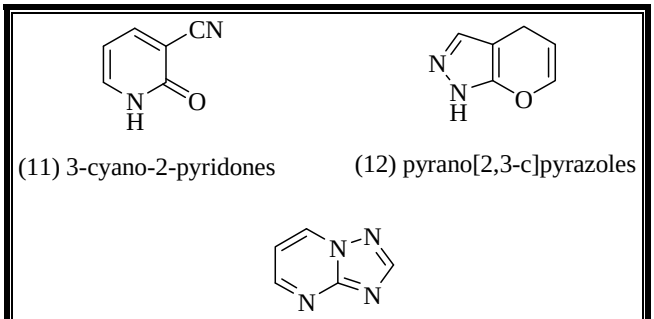
(4) The bonds of the parent component are indicated by a, b, c…starting with the bond normally occupying the 1,2 positions. The atoms of the attached

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 bicycles and the fact that some of these compounds 3-cyano-2-pyridones (11), pyrano[2,3-*c*]pyrazoles (12) and 1,2,4-triazolo[1,5-*a*]pyrimidines (13) are not frequently used in commercial compound libraries, prompted us to elaborate this type of chemistry and to synthesize three different heterocyclic scaffolds.



(11) 3-cyano-2-pyridones (12) pyrano[2,3-c]pyrazoles

(13) 1,2,4-triazolo[1,5-*a*]pyrimidine

1.4 References and notes

[1] (a) Drews, J. *Science* **2000**, *287*, 1960; (b) Wess, G.; Urmann M.; Sickenberger, B. *Angew. Chem. Int. Ed*. **2001**, *40*, 3341.

[2] Lombardino, J. G.; Lowe III, J. A. *Nat. Rev. Drug Discov*. **2004**, *3*, 853.

[3] Kolbn H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed*. **2001**, *40*, 2004.

[4] Evans, B. E.; Rittle, K. E.; Bock, M. G.; Dipardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. *J. Med. Chem*. **1988**, *31*, 2235.

[5] Horton, D. A.; Bourne, G. T.; Smythe, M. L. *Chem. Rev*. **2003**, *103*, 893.

[6] Nicolaou, K. C.; Pfefferkorn J. A.; Roecker, A. J.; Cao, G. Q.; Barluenga, S.; Mitchell, H. J. *J. Am. Chem. Soc*. **2000**, *122*, 9939.

[7] Ding, S.; Gray, N. S.; Ding, Q.; Schultz, P. G. *Tetrahedron Lett*. **2001**, *42*, 8751.

Chapter 2

Synthesis and biological evaluation of cyanopyridones

2.1 Introduction

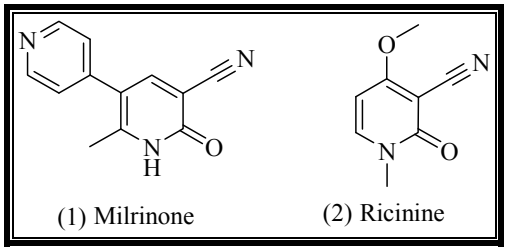
The pyridine skeleton is of great importance to chemists as well as to biologists as it is found in a large variety of naturally occurring compounds and also in clinically useful molecules having diverse biological activities. The pyridine ring systems have emerged as integral backbones of over 7000 existing drugs [1, 2]. The pyridine ring is also an integral part of anticancer and anti-inflammatory agents [3].

In association with those, Pyridone and their derivatives play an essential role in several biological processes and have considerable chemical and pharmacological importance [4-6]. 2-Pyridones represent a unique class of pharmacophore, which are observed in various therapeutic agents [7] and antibiotics [8]. These heterocycles attracted attention because of their applications as bioactive compounds for example as a promising class of HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) [9], as antibacterial [10], antifungal [11], sedative [12] and cardiotonic agents [13]. Moreover, such derivatives have recently become important due to their structural similarity to nucleosides [14]. Also, 2-pyridones were used as ligands for the late 3d-metals [15].

They are also versatile precursors for the construction of complex natural products [16], pyridines [17] and larger pyridone systems such as those found in the nitroguanidine insecticide Imidacloprid [18] and subtype selective GABAA receptor agonists [19]. Consequently, methodologies for the preparation of pyridones have attracted much attention from both industry and academia.

3-Cyano-2-Pyridones are much interest in the anticancer activity of these compounds owing to different types of biological targets they might interfere with for this effect to occur e.g. PDE3, PIM1 Kinase, and Survivin protein.

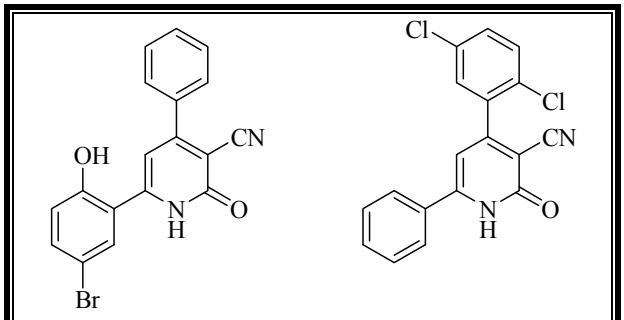
The 3-cyanopyridin-2-one nucleus is the structural basis of the alkaloid ricinine (2), the first known alkaloid containing a cyano-group.



Milrinone (1) is a 3-cyano-2-oxopyridine derivative that has been introduced to the clinic for the treatment of congestive heart failure. Its mechanism of action involves PDE3 inhibition, leading to high levels of cAMP and consequent inotropic effect. Recent studies showed that PDE3, PDE4 and PDE5 are over expressed in cancerous cells compared with normal cells. In addition, cross inhibition of PDE3 together with other PDEs may lead to inhibition of tumor cell growth and angiogenesis [20-24]. The inhibition of PDE3 was able to inhibit the growth and proliferation of the squamous cell carcinoma cell line HeLa, and in HSG cells and further studies revealed that the pyridone derivative, cilostamide- a selective PDE3 inhibitor- has synergism action to the anti-apoptotic action of PDE4 inhibitors in leukemia cells [23-25].

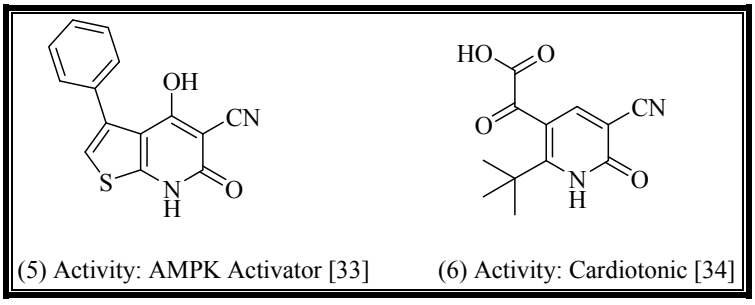
Cheney *et al.* reported 4,6-diaryl-2-oxo-1,2-dihydropyridine-3-carbonitriles (3), as inhibitors of the oncogenic serine/threonine kinase PIM-1, which plays a role in cancer cell survival, differentiation and proliferation. PIM-1 kinase has been shown to be over expressed in a variety of cancer cell lines [26].

Wendt *et al.* showed that several compounds with the same general formula as above but with higher lipophilic properties (4) can inhibit survivin which is a member of the inhibitor of apoptosis family (IAP) [27]. The level of expression of surviving in tumor cells is often associated with poor prognosis and shorter patient survival rates. Survivin is highly expressed in most human tumors and fetal tissue but undetectable in most terminally differentiated adult tissues. This fact therefore makes survivin an ideal target for cancer therapy [28, 29].



(3) PIM-1 kinase inhibition (4) Survivin inhibition

The thienopyridone agonist (5) is showed modest AMPK activity. AMPK (adenosine monophosphate-activated protein kinase), a heterotrimeric serine/ threonine kinase, is well established as a key sensor and regulator of intracellular and whole-body energy metabolism [30]. Activation of AMPK alters carbohydrate and lipid metabolism to increase fatty acid oxidation and glucose uptake and decrease fatty acid and cholesterol synthesis. Through its central role in the regulation of glucose and lipid metabolism, AMPK is emerging as an attractive molecular target for the treatment of diabetes, metabolic syndrome, and obesity [31, 32].



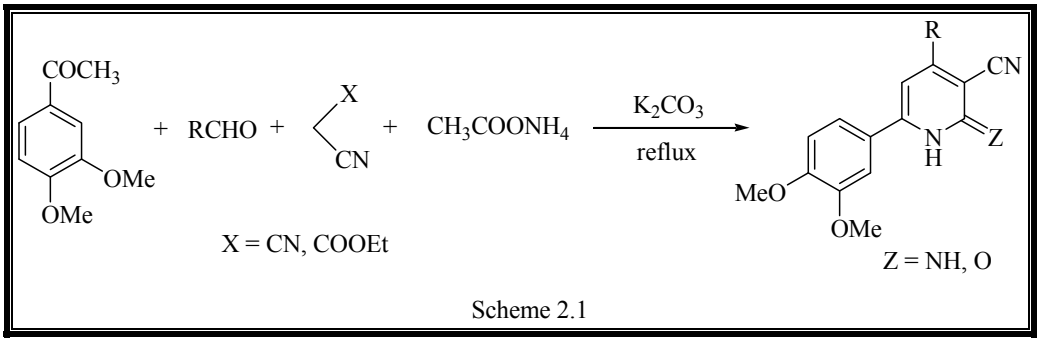
(5) Activity: AMPK Activator [33] (6) Activity: Cardiotonic [34]

2. 2 Reported synthetic strategies

2.2.1 One-pot multi-component synthesis of 3-cyano-2-pyridinones

Beheshtia *et al.* developing new selective and environmental friendly methodologies for the preparation of fine chemicals, they performed the synthesis of 4-alkyl(aryl)-6-aryl-3-cyano-2(1*H*)-pyridinones and their 2-imino isosteres [35, 36] through one-pot multi-component reaction of 3,4-dimethoxyacetophenone, malonitrile or ethyl cyanoacetate, an aldehyde and ammonium acetate in the presence of K_2CO_3 (Scheme 2.1).

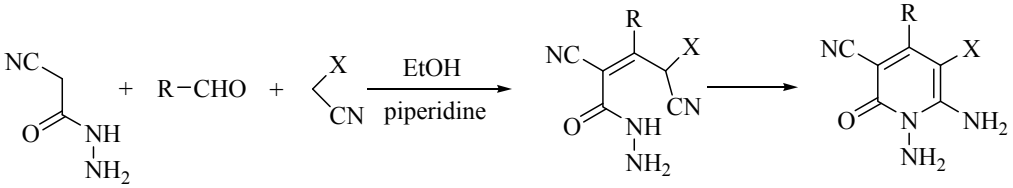
This reaction was carried out in various solvents such as water, DMF, chloroform, ethanol, CH_2Cl_2 and toluene. The best results in terms of yield and time were obtained in ethanol. By carrying out reactions with different amounts of ammonium acetate, it has been found that 8 mmol of the ammonium acetate furnished the maximum yield for 1 mmol of the reactants. When ethyl cyanoacetate was used instead of malononitrile, the corresponding 2-pyridone was obtained in good yield.



Scheme 2.1

Also, they have used Et_3N instead of K_2CO_3 in these reactions, but K_2CO_3 affords better yields. In addition, the time required for completion of the reaction was found to be less with K_2CO_3.

The one-pot reaction of 2-cyanoacetohydrazide (Scheme 2.2) with aldehyde and an activated nitrile in ethanol containing a catalytic amount of piperidine yielded pyridine-2-one derivative [37-39].



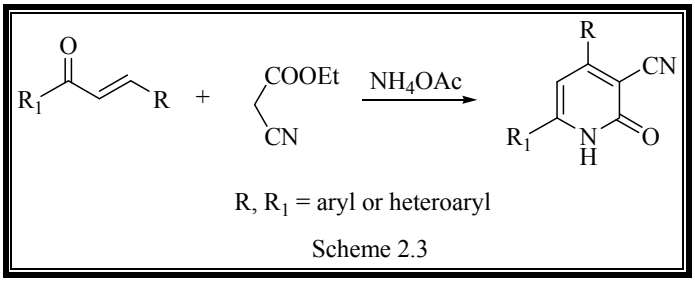
R = H, Me, aryl

X = CN, COPh, CO_2Ph

Scheme 2.2

2.2.2 From *α,β*-unsaturated reagents

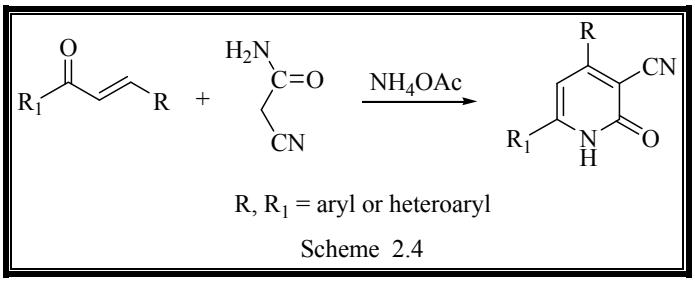
Condensation of ethyl cyanoacetate with *α,β*-unsaturated ketones in presence of excess ammonium acetate afforded 3-cyanopyridin-2-ones [40-43] (Scheme 2.3). Also, a green chemistry approach describing reaction of *α,β*-unsaturated ketones with ethyl cyanoacetate using samarium iodide as catalyst has been reported recently [44].



R, R_1 = aryl or heteroaryl

Scheme 2.3

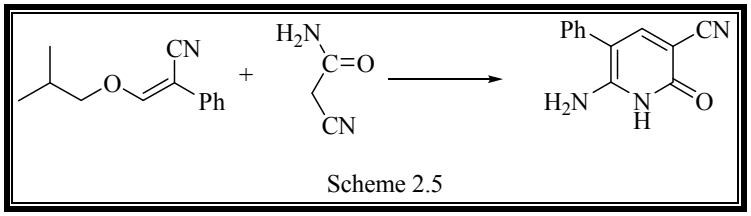
Barat reported first that condensation of cyanoacetamide with *α,β*-unsaturated ketones also affords 3-cyanopyridin-2-ones. Number of reports following this approach have been reported till date [45-47] (Scheme 2.4).



R, R_1 = aryl or heteroaryl

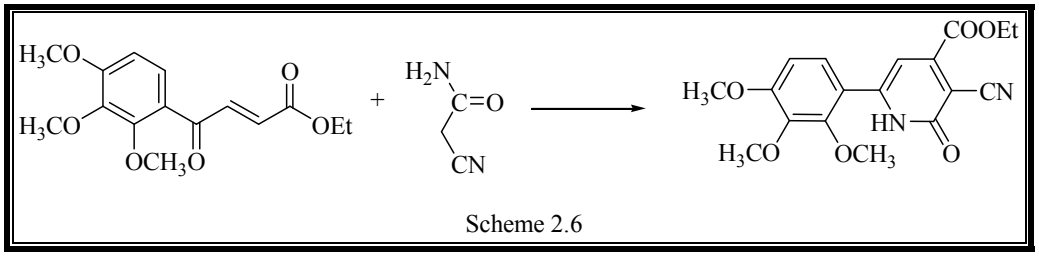
Scheme 2.4

Chase *et al.* have synthesized 6-amino-5-phenyl-3-cyanopyridin-2-one by the reaction of 3-isobutoxy-2-phenylacrylonitrile with cyanoacetamide [48] (Scheme 2.5).



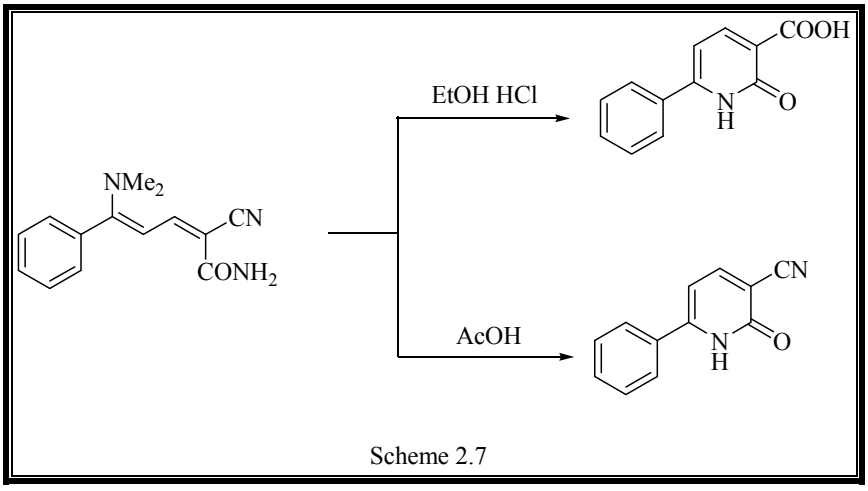
Scheme 2.5

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 [49] (Scheme 2.6).



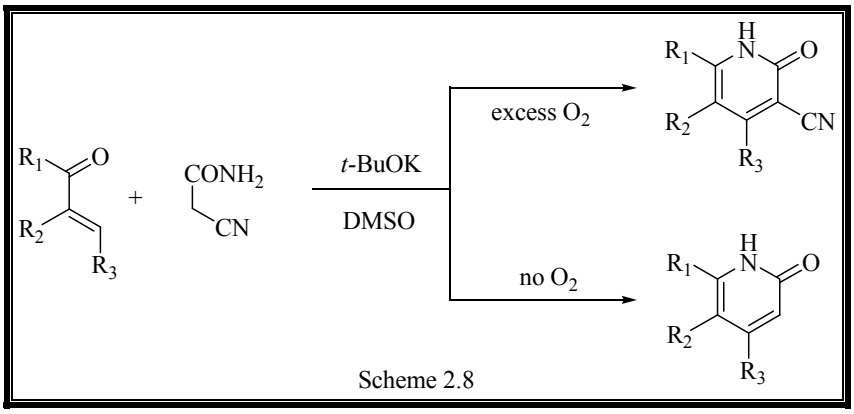
Scheme 2.6

Alnajjar *et al.* [50] reported the conversions of 2-cyano-5-(dimethylamino)-5-phenylpenta-2,4-dienamides into nicotinic acid derivatives by boiling in EtOH/HCl. But, When 2-cyano-5-(dimethylamino)-5-phenylpenta-2,4-dienamides are heated under reflux in AcOH, nicotinic nitrile derivatives are obtained (Scheme 2.7).



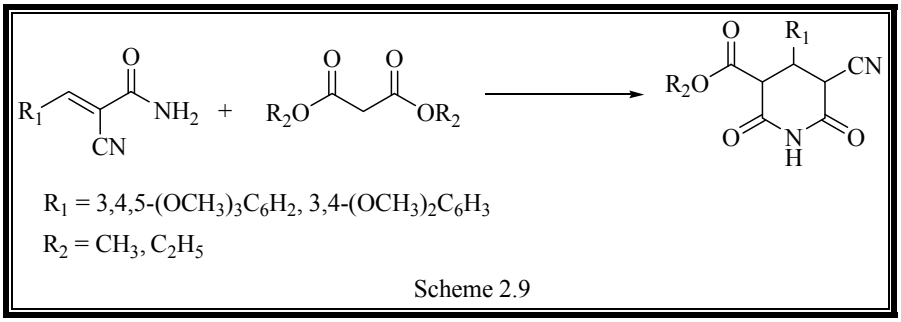
Scheme 2.7

The condensation of an enone or enal with cyanoacetamide derivatives and *t*-BuOK furnishes either 3-cyano-2-pyridones or 3-unsubstituted-2-pyridones (Scheme 2.8), depending on whether the reaction is carried out in the presence or in the absence of O_2. In the first case, *in situ* oxidation of Michael-type intermediates takes place; in the second case, a "decyanidative aromatization" of such intermediates occurs [51, 52].



Scheme 2.8

The synthesis of 2,6-piperidindione derivatives (Scheme 2.9) was achieved by the Michael addition of dialkylmalonates to benzylidene cyanoacetamide derivative. Here benzylidene cyanoacetamide derivative were obtained by reaction of cyano-acetamide with certain aromatic aldehydes according to the reported procedure [53].



R_1 = 3,4,5-$(OCH_3)_3C_6H_2$, 3,4-$(OCH_3)_2C_6H_3$

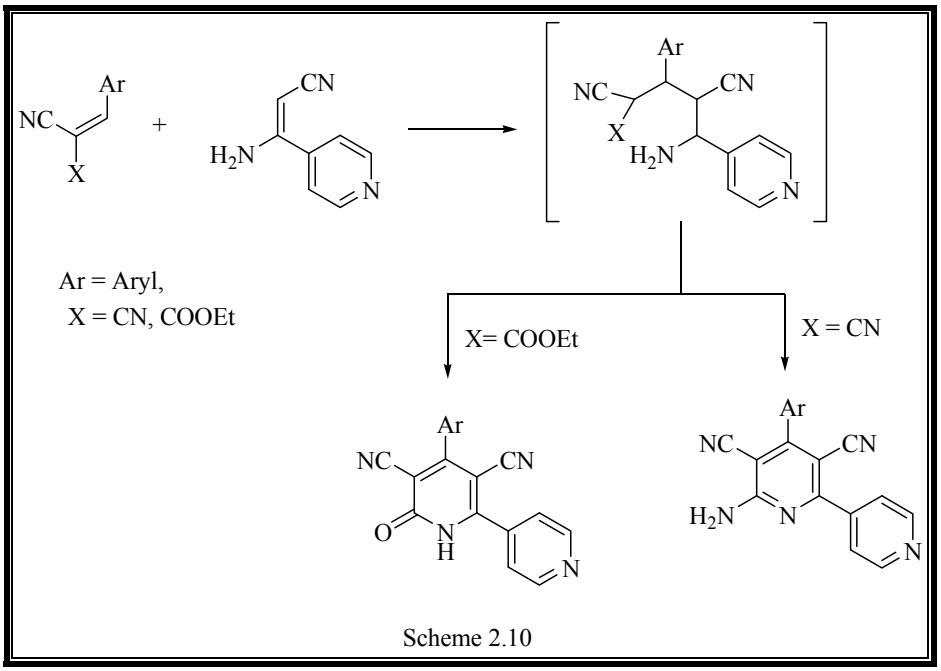
R_2 = CH_3, C_2H_5

Scheme 2.9

Enaminonitrile reacted with an equimolar amount of a-cinnamonitriles to provide pyridine and pyridinone derivatives [54], respectively. Formation of pyridine and pyridinone derivatives is assumed to proceed via an acyclic intermediate form followed by intramolecular cyclization and spontaneous autooxidation under the

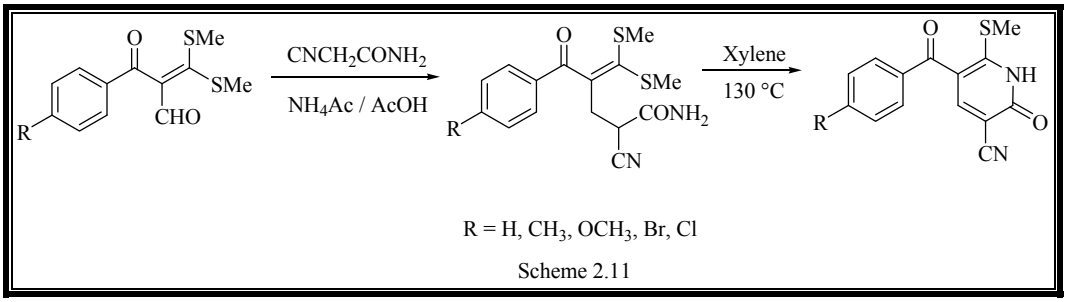
reaction conditions in the case of pyridine derivatives and elimination of ethanol in the case of pyridone derivatives (Scheme 2.10).

Here, the enaminonitrile can be readily prepared by reaction of acetonitrile with 4-cyanopyridine in the presence of potassium-*t*-butoxide [55].



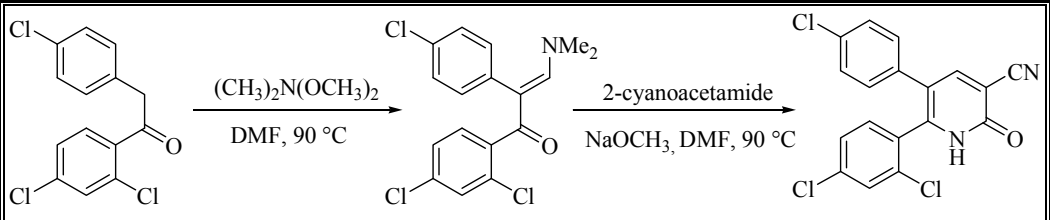
Scheme 2.10

Mathews *et al.* [56] attempted the reaction of 2-aroyl-3,3-bis(alkylsulfanyl) acrylaldehydes with cyanoacetamide in the presence of ammonium acetate/acetic acid at 80 °C, it afforded only the Knoevenagel condensation adduct, 4-aroyl-2-cyano-5,5-bis-(methylsulfanyl)-2,4-pentadien-amides and no 2-pyridone was formed. As the condensation product has the scope for cyclization to produce 2-pyridones by the elimination of an alkylsulfanyl group, they tried the thermal cyclization of the condensation products by heating in xylene at 130 °C (Scheme 2.11).



Scheme 2.11

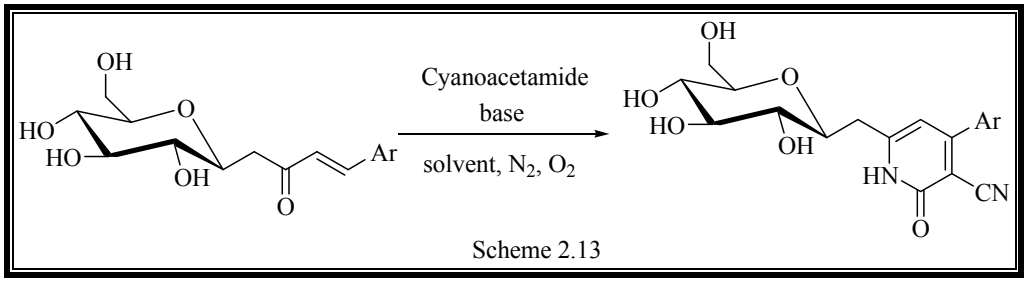
Condensation of ketone with dimethylformamide dimethylacetal afforded vinylogous amide, which in turn reacted with cyanoacetamide under basic conditions to generate the 5,6-diaryl-3-cyano-2-pyridones (Scheme 2.12) [57].



Scheme 2.12

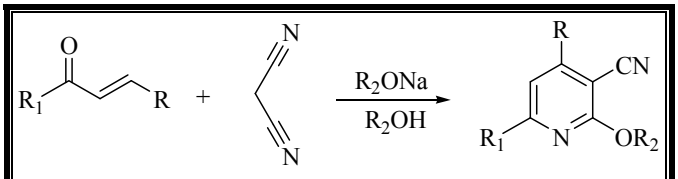
The reaction of *(E)*-1-(*β*-D-glucopyranosyl)-4-(aryl)but-3-en-2-ones and cyanoacetamide was carried out (Scheme 2.13) with *t*-BuOK in DMSO under N_2 atmosphere at ambient temperature to give the respective 3-cyano-4-phenyl-6-[(*β*-D-glucopyranosyl)methyl]pyridones [58]. The reaction mixture was brought under the influence of an O_2 atmosphere to carry out oxidative aromatization.

Here, the starting butenonyl C-glycosides were prepared from commercially available D-glucose following earlier reported protocols [59-62].



Scheme 2.13

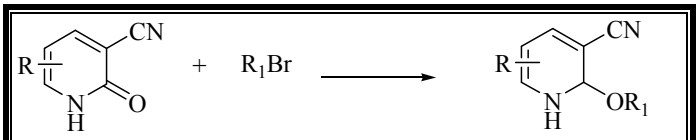
2-alkoxy-3-cyanopyridines is achieved by the reaction of chalcones with malononitrile in corresponding sodium alkoxide [63-66] (Scheme 2.14).



R, R_1 = aryl or heteroaryl

Scheme 2.14

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 [67, 68] (Scheme 2.15).

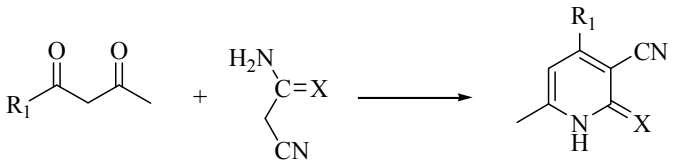


R = substituent/substituents on 4, 5 and/or 6 position of pyridine
R_1 = alkyl or aryl

Scheme 2.15

2.2.3 From 1,3-dicarbonyl reagents

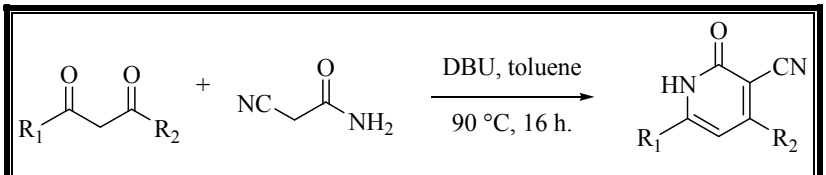
Literature survey revealed many reports on synthesis of 3-cyanopyridin-2-one and 3-cyanopyridin-2-thione by reaction of 1,3-dicarbonyl compounds with cyanoacetamide [69-72] and cyanothioacetamide [73-75] respectively (Scheme 2.16).



R_1 = H [67, 68], R_1 = $COOCH_3$ [69], R_1 = heteroaryl [70], X = O [67-70], S [71-73]
R_1 = benzoyl [71], R_1 = CH_3, OC_2H_5 [72], R_1 = OC_2H_5 [73]

Scheme 2.16

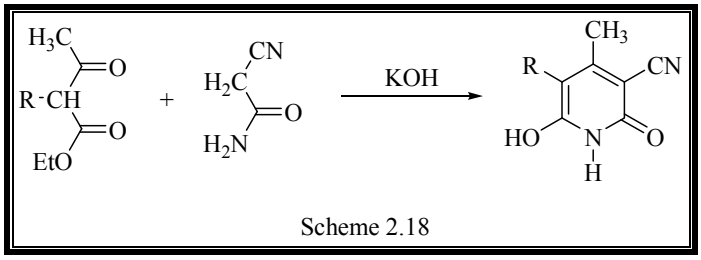
The pyridone derivatives were also obtained from a cyclo-condensation reaction in one step (Scheme 2.17) [28]. Reaction of commercially available diketone with cyanoacetamide, in the presence of DBU in toluene at 90 °C for 16 h, readily afforded the title compounds.



R_1, R_2 = CF_3, thiophene, aryl

Scheme 2.17

5-Substituted-4-methyl-3-cyano-6-hydroxy-2-pyridones were synthesized from cyanoacetamide and the corresponding alkyl ethyl acetoacetate in methanol in the presence of potassium hydroxide at 60 ºC. Cyclization of cyanoacetamide with an alkyl ethyl acetoacetate belongs to a 3-2 type of condensation where the pyridine nucleus is formed (Scheme 2.18) [76].

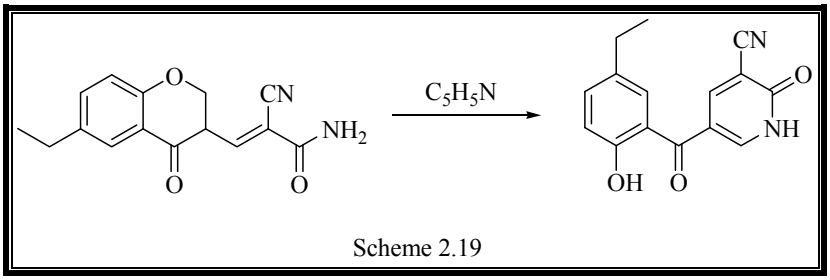


Scheme 2.18

2.2.4 Miscellaneous

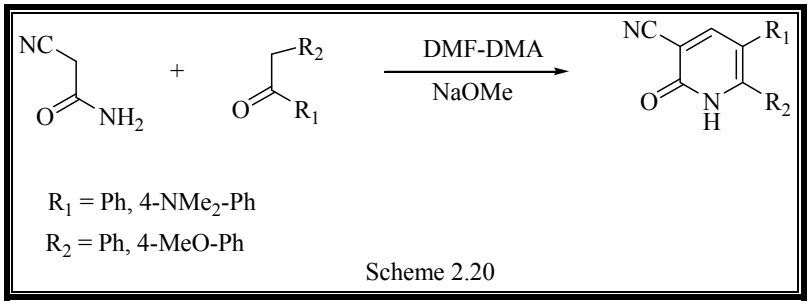
Literature survey also revealed a few ring transformation reactions of 4-amino-1*H*-1,5-benzodiazepine-3-carbonitrile [77] and substituted uracil [78] yielding 3-cyano-pyridones.

Nohara *et al.* have reported that heating 2-cyano-3-(6-ethyl-4-oxo-4*H*-1-benzopyran-3-yl) acrylamide in pyridine yields 3-cyano-5-(5-ethyl-2-hydroxy-benzoyl)-2(1*H*)-pyridones [79] (Scheme 2.19).



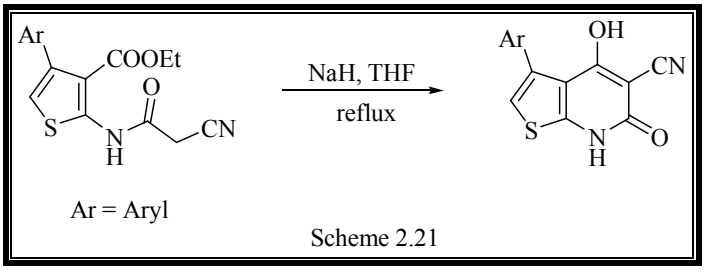
Scheme 2.19

Treatment of the ketones with dimethylformamide-dimethylacetal (DMF-DMA) gave the corresponding 3-dimethylaminopropen-2-ones, and generally without purification, these were condensed with 2-cyanoacetamide under basic condition give the 3-cyano-2-pyridones**,** reported by Collins *et al.* [12] (Scheme 2.20).



Scheme 2.20

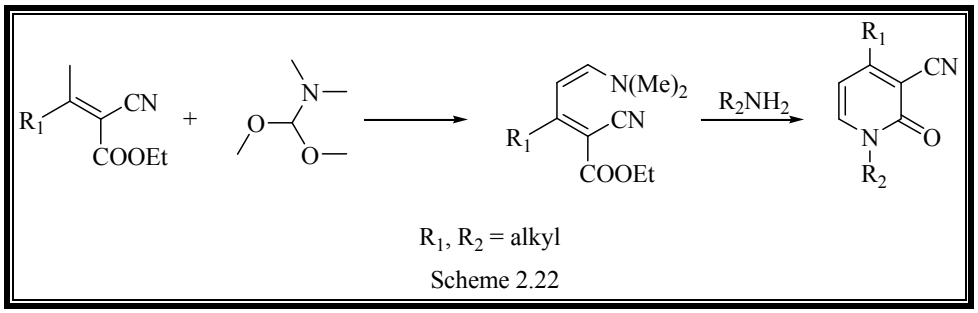
The 2-(2-cyano-acetylamino)-4-aryl-thiophene-3-carboxylic acid ethyl esters were treated with sodium hydride in tetrahydrofuran at temperatures ranging from 25 to 80 °C to provide the thienopyridone compounds [35] (Scheme 2.21).



Scheme 2.21

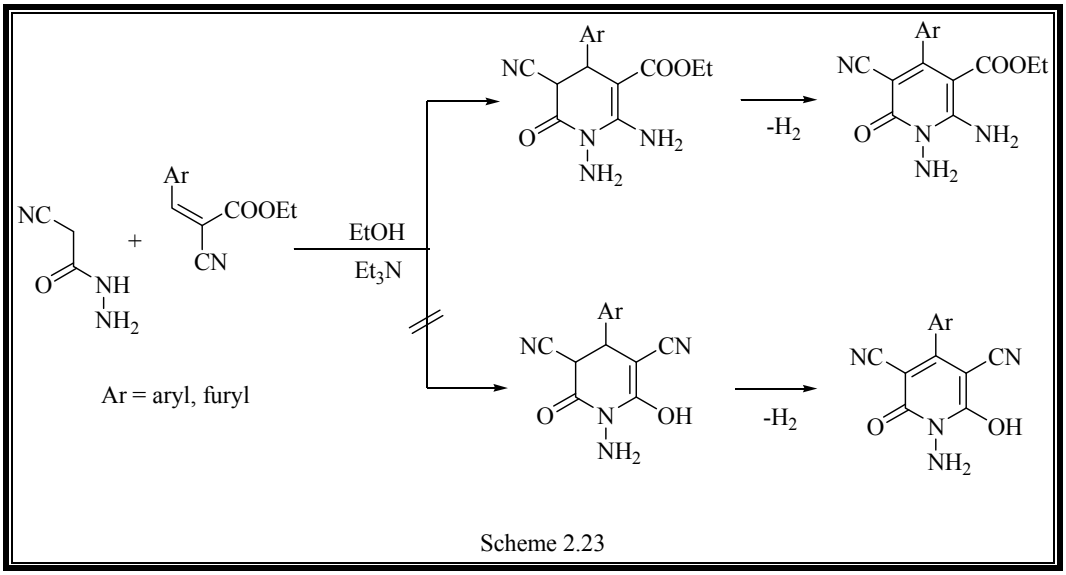
2.2.5 Synthesis of *N*-substituted-cyanopyridones

Melikyan *et al.* have reported synthesis of novel *N*-substituted-3-cyanopyridin-2-ones from ylidenecyanoacetic acid ethyl esters in two steps [80] (Scheme 2.22).



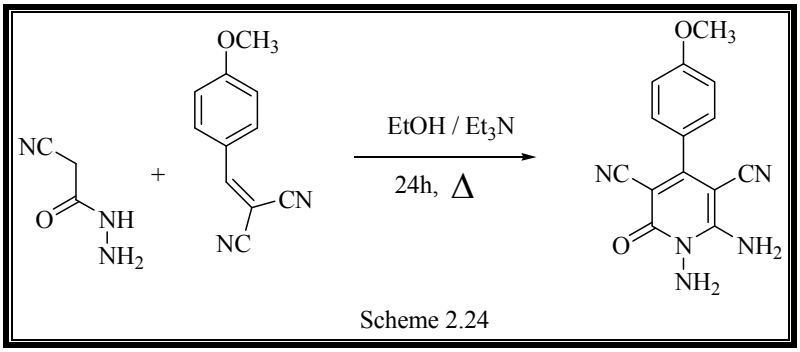
Scheme 2.22

On heating 2-cyanoacetohydrazide and arylidene of ethyl cyanoacetate in ethanol containing triethyl amine under reflux afforded diaminopyridine derivative rather than aminopyridine derivative [81, 82] (Scheme 2.23).



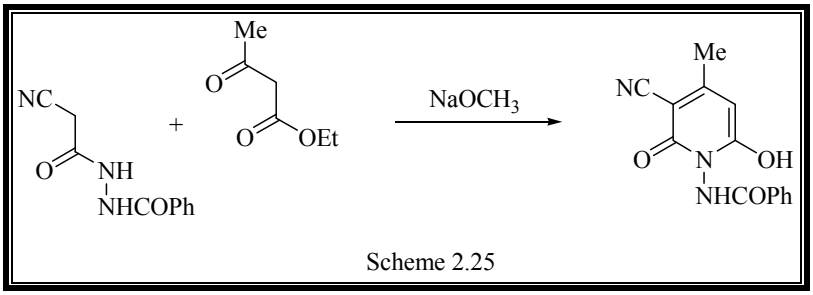
Scheme 2.23

Martin and coworkers reinvestigated the cyclocondensation of 2-cyanoaceto-hydrazide with (4-methoxybenzylidene)malononitrile (Scheme 2.24). They have found that prolonged heating lead only to the formation of 1,6-diamino-4-(4-methoxy-phenyl) -3,5-dicyano-2-pyridone [83].



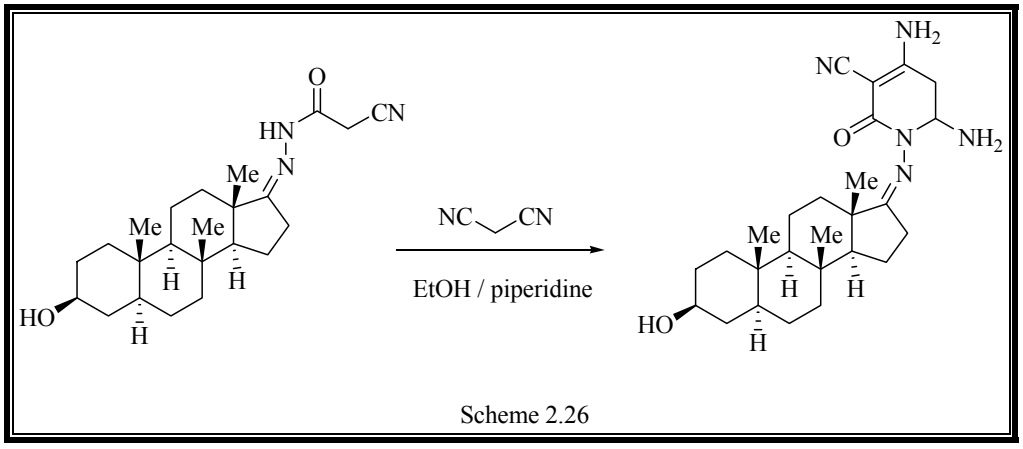
Scheme 2.24

Substituted *N*-benzoylaminopyridone was prepared by cyclocondensation of *N*-benzoylcyano-acetohydrazide with ethyl acetoacetate in presence of sodium methoxide [84] (Scheme 2.25).



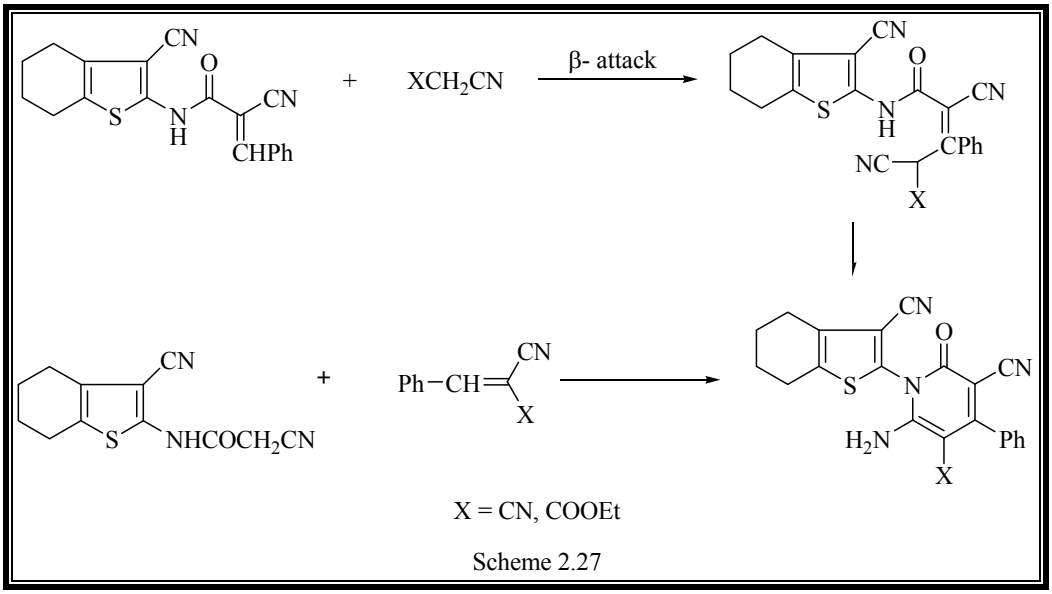
Scheme 2.25

The reaction of *N*-cyanoacetylhydrazone of epiandrosterone with malononitrile in ethanol in the presence of a catalytic amount of piperidine afforded pyridine-2-one derivative [85] (Scheme 2.26).



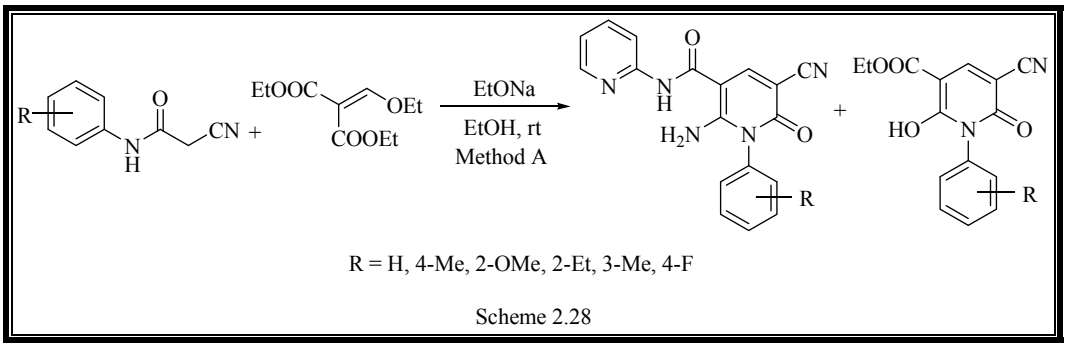
Scheme 2.26

Shams *et al.* [86] reported the reaction of the benzylidene derivative with methylene carbonitrile reagents (XCH_2CN; X = CN, X = CO_2Et) afforded the respective pyridone derivatives (Scheme 2.4). The reaction took place via *β*-attack on the benzylidene moiety followed by 1,6-intramolecular dipolar cyclization with concomitant aromatization. Further confirmation of the reaction products was achieved through an alternative synthetic route involving treatment of the starting compound cyano acetamide with benzylidene carbonitrile reagents (PhCH=C(CN)X; X = CN; X = COOEt) to afford the same pyridone derivatives with better yields (80%, 86%) than in their formation by the reaction of benzylidene derivative and either malononitrile (75% yield) or ethyl cyanoacetate (73% yield) (Scheme 2.27).



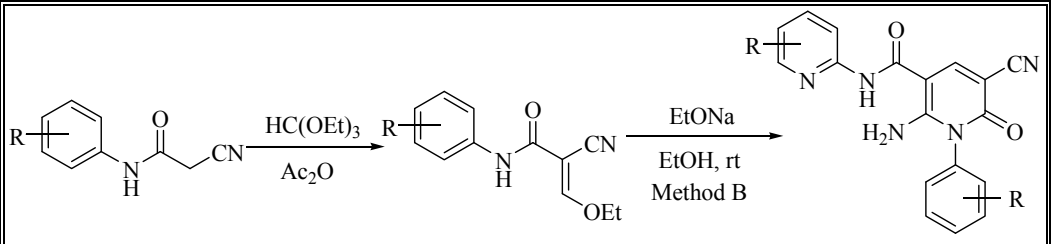
Scheme 2.27

Reactions of *N*-substituted cyanoacetamides with diethyl-2-(ethoxymethylene) malonate (DEEMM) [87] in EtOH in the presence of EtONa at room temperature resulted in the isolation of diaryl derivatives as major products (Method A) instead of the expected ethyl carboxylates (Scheme 2.28).



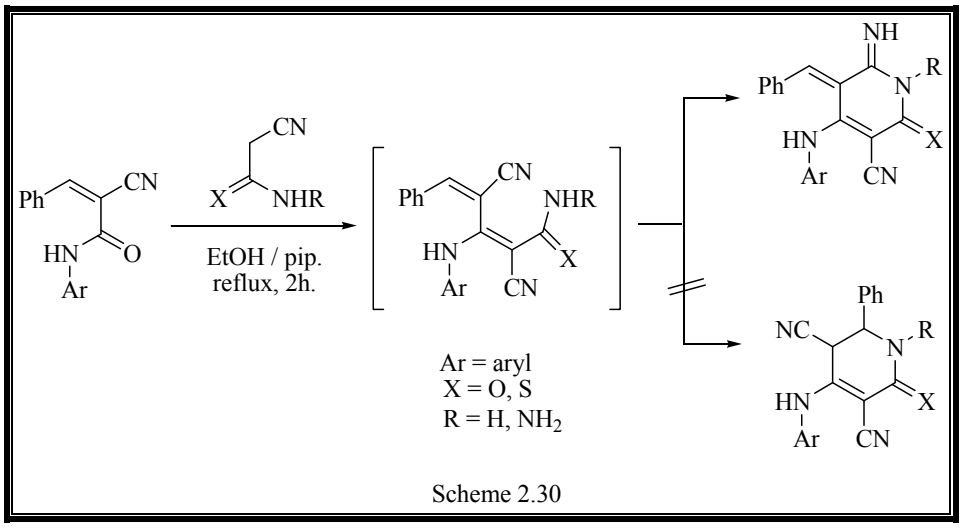
Scheme 2.28

Thus, to examine a possible alternative synthetic route to such molecules, and also to model one of the possible reaction stages of their formation from cyanoacetamides and DEEMM (Scheme 2.28), ethoxymethylene derivatives were used (Scheme 2.29). Thus, their reaction with cyanoacetamides under the same conditions resulted in formation of the desired derivatives (Method B) [88].



Scheme 2.29

El-Rady *et al.* [89] reported the synthesis of 5-cyano-3-phenylmethylene-pyridines by the condensation of 2-cyano-3-phenyl-*N*-*p*-tolylacrylamide with cyanoacetamide or cyanothioacet-amide or cyanoacetic acid hydrazide in ethanolic solution at reflux in the presence of drops of piperidine (Scheme 2.30). The isomers 3,5-dicyano-2,3-dihydropyridines which have the same *m/e* are ruled out based mainly on the spectral analysis.

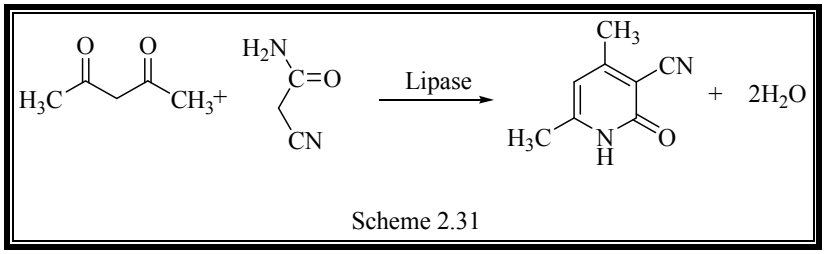


Scheme 2.30

2.2.6 Enzymatic synthesis of 3-cyano-2-pyridones

Lipases, including *Candida rugosa,* formerly *C. cylindracea,* were used to synthesize the substituted 3-cyano-2-pyridones [90-93]. Unlike conventional syntheses that require heating in the presence of various catalysts and usually polar organic solvents, enzymatic synthesis was achieved under mild reaction conditions. Under such

conditions, various 4,6-disubstituted-3-cyano-2-pyridones were obtained, including aryl-substituted-3-cyano-2-pyridones (Scheme 2.31).



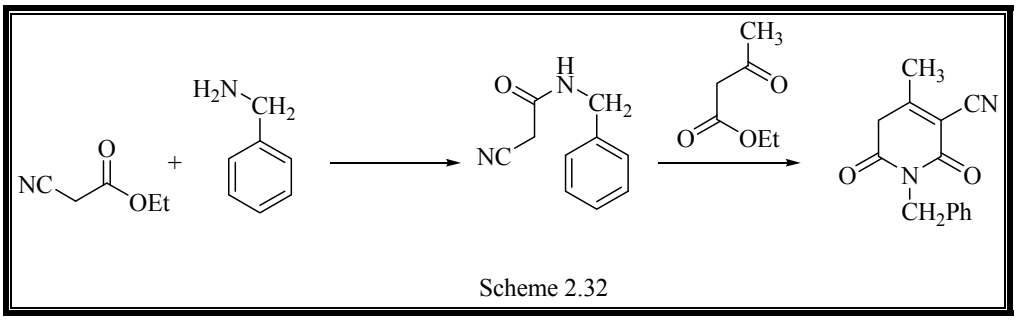
Scheme 2.31

Prlainovic *et al.* [94] studies the kinetics of the enzyme-catalyzed synthesis of 4,6-disubstituted-3-cyano-2-pyridones showed that the behavior of the lipase used as the catalyst in the synthesis of 4,6-dimethyl-3-cyano-2-pyridone is more complicated than that of chemical catalysts [92].

2.2.7 Cyanopyridones via Ultrasound and Microwave irradiation

Al-Zaydi [95] reported the synthesis of cyanopyridones *via* heating cyano-acetamide derivative with ethyl acetoacetate in absence of solvent under reflux conventionally or ultrasound irradiation or in a microwave oven.

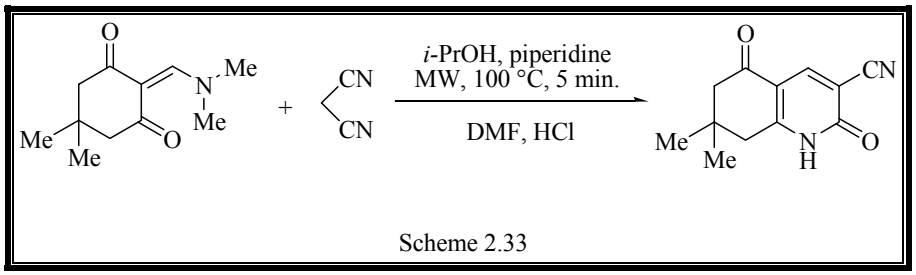
They have prepared Several cyanoacetamides *via* treatment of ethylcyano-acetate with primary amines either at room temperature for a longer time or *via* irradiation with microwave for 1 min. at 100 W or with ultrasound for 2 min. at 40 ºC. Then cyanoacetamides was reacted with an ethyl acetoacetate also either *via* a longer time using reflux of neat reagents and by a short time microwave or by ultrasound to afford cyanopyridones (Scheme 2.32).



Scheme 2.32

Also, Gorobets *et al.* [96] develop a rapid microwave assisted protocol for the solution phase synthesis of highly substituted 2-pyridone derivatives (Scheme 2.33). They introduce general microwave-assisted [97] three component one-pot synthesis of highly substituted 2-pyridones, utilizing CH-acidic substrates, dimethylformamide dimethylacetal (DMF-DMA) and diverse methylene active nitriles [98].

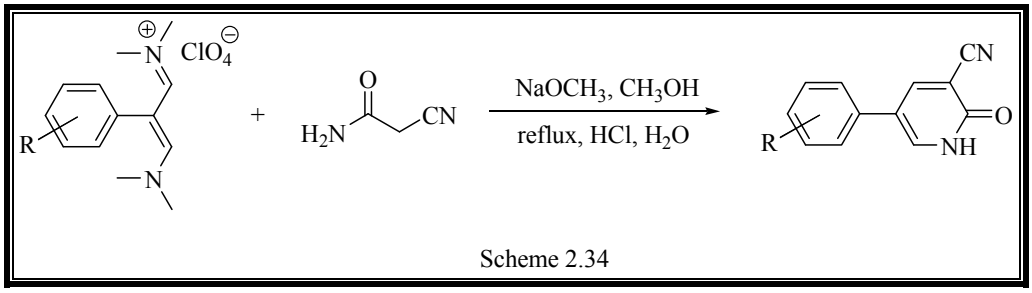
The substituted 2-pyridone derivatives were obtained when a mixture of enamine, malononitrile and catalytic amounts of piperidine in *i*-PrOH was irradiated at 100 °C for 5 min.



Scheme 2.33

2.2.8 Synthesis of 3-cyano-2-pyridones by using vinamidinium salts

5-Aryl-3-cyano-2-pyridones have been prepared by the cyclization of cyanoacetamide condensated with 2-aryl-3-dimethylamino-2-propenals. As shown in Scheme 2.34, the 2-arylvinamidinium salts were condensed with cyanoacetamide in refluxing methanol that contained sodium methoxide to give the desired 5-aryl-3-cyano-2-pyridones [99]. The vinamidinium salts were prepared by the standard Vilsmeier-Haack reaction from the appropriate aryl acetic acid.

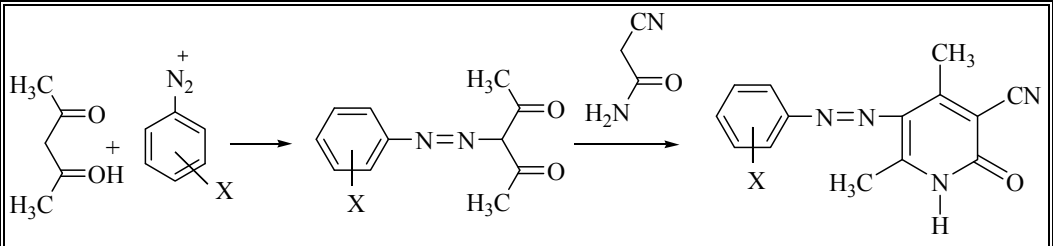


Scheme 2.34

2.2.9 Synthesis of azo-cyanopyridones

Azo pyridines were synthesized by the diazotization-coupling reaction, as shown in Scheme 2.35. In this procedure acetylacetone was coupled with diazotised substituted

aniline to give an intermediate (3-(substituted-phenylazo)-2,4-pentanedione), which was then cyclized with cyanoacetamide to yield an azo-pyridones [100].



X = H, 4-CN, 4-Cl, 4-NO2, etc.

Scheme 2.35

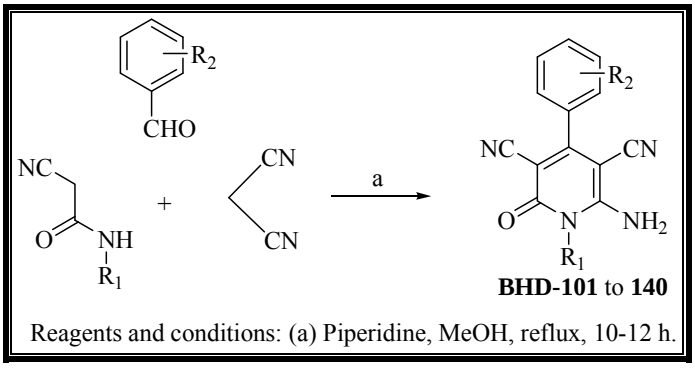
2.3 Current work

The chemistry of pyridine and its derivatives has been studied for over a century due to their diverse biological activities. 3-cyano-2-pyridone 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.

Keeping in mind, various biomedical applications and with a view to further assess the pharmacological profile of this class of compounds, four novel series of 3-cyano-2-pyridones (**BHD-101** to **BHD-140**) have been synthesized.

The synthesis of 3-cyano-2-pyridones was achieved by the reaction of an appropriate aromatic aldehydes, 2-cyano-*N*-(substituted)acetamides and malononitrile by using methanol as a solvent and piperidine as a catalyst. 2-cyano-*N*-(substituted) acetamides were prepared by the reaction of substituted anilines with ethyl cyanoacetate [101]. The products were characterized by FT-IR, mass, ^{1}H NMR spectroscopy and elemental analyses. The newly synthesized compounds were subjected to various biological activities *viz.,* antimicrobial, antimycobacterial, anticancer and antiviral.

2.4 Reaction scheme

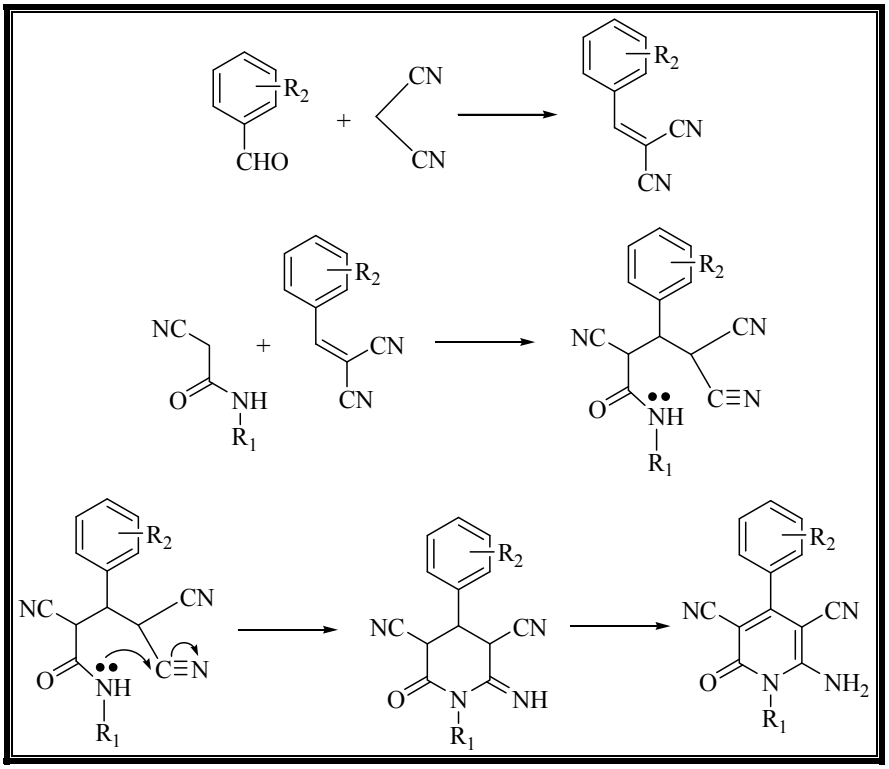


Reagents and conditions: (a) Piperidine, MeOH, reflux, 10-12 h.

Code	R_1	R_2	M.F.	M.W.	M.P. °C	Yield %	R_{f1}	R_{f2}
BHD-101	pyridin-2-yl	H	$C_{18}H_{11}N_5O$	313	240-242	71	0.52	0.70
BHD-102	pyridin-2-yl	4-F	$C_{18}H_{10}FN_5O$	331	189-191	66	0.50	0.69
BHD-103	pyridin-2-yl	4-Cl	$C_{18}H_{10}ClN_5O$	347	245-247	75	0.48	0.65
BHD-104	pyridin-2-yl	4-NO_2	$C_{18}H_{10}N_6O_3$	358	270-272	74	0.45	0.68
BHD-105	pyridin-2-yl	4-CH_3	$C_{19}H_{13}N_5O$	327	261-263	78	0.53	0.72
BHD-106	pyridin-2-yl	4-OCH_3	$C_{19}H_{13}N_5O_2$	343	204-206	72	0.44	0.64
BHD-107	pyridin-2-yl	3,4-OCH_3	$C_{20}H_{15}N_5O_3$	373	244-246	65	0.55	0.73
BHD-108	pyridin-2-yl	3-NO_2	$C_{18}H_{10}N_6O_3$	358	231-233	61	0.50	0.67
BHD-109	pyridin-2-yl	3-Cl	$C_{18}H_{10}ClN_5O$	347	269-271	70	0.41	0.60
BHD-110	pyridin-2-yl	2-Cl	$C_{18}H_{10}ClN_5O$	347	199-201	72	0.46	0.66
BHD-111	3-Cl-4-F-C_6H_3	H	$C_{19}H_{10}ClFN_4O$	364	219-221	70	0.52	0.69
BHD-112	3-Cl-4-F-C_6H_3	4-F	$C_{19}H_9ClF_2N_4O$	382	227-229	79	0.56	0.74
BHD-113	3-Cl-4-F-C_6H_3	4-Cl	$C_{19}H_9Cl_2FN_4O$	399	190-192	75	0.50	0.66
BHD-114	3-Cl-4-F-C_6H_3	4-NO_2	$C_{19}H_9ClFN_5O_3$	409	247-249	68	0.52	0.69
BHD-115	3-Cl-4-F-C_6H_3	4-CH_3	$C_{20}H_{12}ClFN_4O$	378	239-241	76	0.61	0.77
BHD-116	3-Cl-4-F-C_6H_3	4-OCH_3	$C_{20}H_{12}ClFN_4O_2$	394	215-217	77	0.54	0.68
BHD-117	3-Cl-4-F-C_6H_3	3,4-OCH_3	$C_{21}H_{14}ClFN_4O_3$	424	233-235	69	0.50	0.70
BHD-118	3-Cl-4-F-C_6H_3	3-NO_2	$C_{19}H_9ClFN_5O_3$	409	190-192	66	0.64	0.78
BHD-119	3-Cl-4-F-C_6H_3	3-Cl	$C_{19}H_9Cl_2FN_4O$	399	262-264	70	0.48	0.64
BHD-120	3-Cl-4-F-C_6H_3	2-Cl	$C_{19}H_9Cl_2FN_4O$	399	260-262	75	0.51	0.69
BHD-121	4-CH_3-C_6H_4	H	$C_{20}H_{14}N_4O$	326	256-258	75	0.45	0.59
BHD-122	4-CH_3-C_6H_4	4-F	$C_{20}H_{13}FN_4O$	344	230-232	74	0.55	0.68
BHD-123	4-CH_3-C_6H_4	4-Cl	$C_{20}H_{13}ClN_4O$	360	215-217	80	0.52	0.70
BHD-124	4-CH_3-C_6H_4	4-NO_2	$C_{20}H_{13}N_5O_3$	371	270-272	70	0.50	0.66
BHD-125	4-CH_3-C_6H_4	4-CH_3	$C_{21}H_{16}N_4O$	340	203-205	79	0.58	0.73
BHD-126	4-CH_3-C_6H_4	4-OCH_3	$C_{21}H_{16}N_4O_2$	356	186-188	82	0.43	0.59
BHD-127	4-CH_3-C_6H_4	3,4-OCH_3	$C_{22}H_{18}N_4O_3$	386	264-266	75	0.53	0.69
BHD-128	4-CH_3-C_6H_4	3-NO_2	$C_{20}H_{13}N_5O_3$	371	218-220	72	0.52	0.67
BHD-129	4-CH_3-C_6H_4	3-Cl	$C_{20}H_{13}ClN_4O$	360	258-260	85	0.50	0.63
BHD-130	4-CH_3-C_6H_4	2-Cl	$C_{20}H_{13}ClN_4O$	360	220-222	71	0.48	0.58
BHD-131	3-CF_3-C_6H_4	H	$C_{20}H_{11}F_3N_4O$	380	228-230	68	0.51	0.65
BHD-132	3-CF_3-C_6H_4	4-F	$C_{20}H_{10}F_4N_4O$	398	239-241	71	0.45	0.61
BHD-133	3-CF_3-C_6H_4	4-Cl	$C_{20}H_{10}ClF_3N_4O$	414	201-203	74	0.47	0.60
BHD-134	3-CF_3-C_6H_4	4-NO_2	$C_{20}H_{10}F_3N_5O_3$	425	266-268	65	0.57	0.74
BHD-135	3-CF_3-C_6H_4	4-CH_3	$C_{21}H_{13}F_3N_4O$	394	257-259	69	0.49	0.62
BHD-136	3-CF_3-C_6H_4	4-OCH_3	$C_{21}H_{13}F_3N_4O_2$	410	230-232	74	0.52	0.65
BHD-137	3-CF_3-C_6H_4	3,4-OCH_3	$C_{22}H_{15}F_3N_4O_3$	440	250-252	70	0.48	0.65
BHD-138	3-CF_3-C_6H_4	3-NO_2	$C_{20}H_{10}F_3N_5O_3$	425	270-272	75	0.43	0.59
BHD-139	3-CF_3-C_6H_4	3-Cl	$C_{20}H_{10}ClF_3N_4O$	414	221-223	68	0.50	0.64
BHD-140	3-CF_3-C_6H_4	2-Cl	$C_{20}H_{10}ClF_3N_4O$	414	217-219	72	0.54	0.68

TLC Solvent system R_{f1}: Hexane: Ethyl acetate - 6:4; TLC Solvent system R_{f2}: Chloroform: Methanol - 9:1

2.4.1 Plausible Reaction Mechanism



2.5 Experimental

2.5.1 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 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.

2.5.2 Synthesis of 2-cyano-N-(substituted)acetamides

Synthesis of 2-cyano-*N*-(substituted)acetamides was achieved using previously published methods [101].

2.5.3 General procedure for the synthesis of 6-amino-1,2-dihydro-4-(aryl)-2-oxo-1-(pyridin-2-yl)pyridine-3,5-dicarbonitriles (BHD 101-110)

A mixture of 2-cyano-*N*-(pyridin-2-yl)acetamide (0.01 mol), appropriate aromatic aldehydes (0.01 mol), malononitrile (0.01 mol) and catalytical amount of piperidine in methanol (20 ml) was heated under reflux condition for 10-12 h. The reaction mixture was kept at room temperature for 2-4 h. The solid product obtained was isolated and recrystallized from ethanol.

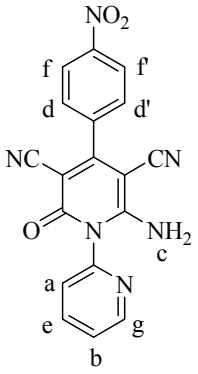
2.5.3.1 6-amino-1,2-dihydro-2-oxo-4-phenyl-1-(pyridin-2-yl)pyridine-3,5-dicarbonitrile (BHD-101) Yield: 71%; mp 240-242 ºC; IR (cm^{-1}): 3466 and 3331 (N-H stretching of primary amine), 3064 (C-H stretching of aromatic ring), 2220 and 2206 (C≡N stretching of nitrile group), 1656 (C=O stretching of pyridone ring), 1606 (N-H deformation of NH_2 group), 1629 (C=N stretching of pyridine ring), 1546 and 1518 (C=C stretching of aromatic ring), 999 (C-H in plane bending for aromatic ring); 802 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 7.47-7.49 (d, 1H, H_a, J = 7.8 Hz), 7.55 (s, 6H, $H_{b\text{-}ee'}$), 7.58 (s, 2H, H_f), 8.02-8.06 (t, 1H, H_g, J = 7.7 Hz), 8.71-8.72 (d, 1H, H_h); MS: *m/z* 313; Anal. Calcd. for $C_{18}H_{11}N_5O$: C, 69.00; H, 3.54; N, 22.35. Found: C, 68.80; H, 3.50; N, 22.27%.

2.5.3.2 6-amino-4-(4-fluorophenyl)-1,2-dihydro-2-oxo-1-(pyridin-2-yl)pyridine-3,5-dicarbonitrile (BHD-102) Yield: 66%; mp 189-191 ºC; MS: *m/z* 331; Anal. Calcd. for $C_{18}H_{10}FN_5O$: C, 65.26; H, 3.04; N, 21.14. Found: C, 65.11; H, 3.00; N, 21.09%.

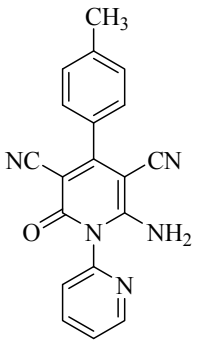
2.5.3.3 6-amino-4-(4-chlorophenyl)-1,2-dihydro-2-oxo-1-(pyridin-2-yl)pyridine-3,5-dicarbonitrile (BHD-103) Yield: 75%; mp 245-247 ºC; IR (cm^{-1}): 3485 and 3321 (N-H stretching of primary amine), 3072 (C-H stretching of aromatic ring), 2216 (C≡N stretching of nitrile group), 1672 (C=O stretching of pyridone ring), 1643 (C=N stretching of pyridine ring), 1595 (N-H deformation of NH_2 group), 1531 and 1498 (C=C stretching of aromatic ring), 1352 (C-N stretching for carbon bonded to amino group), 1014 (C-H in plane bending for aromatic ring), 839 (C-H out of plane bending for 1,4-disubstituted aromatic ring), 771 (C-Cl stretching); ^{1}H NMR (DMSO-

d$_6$) δ ppm: 7.39-7.42 (d, 1H, H_a, *J* = 7.1 Hz), 7.46-7.59 (m, 5H, $H_{b\text{-}dd'}$), 7.65 (s, 2H, H_e), 8.03-8.07 (t, 1H, H_f, *J* = 7.7 Hz), 8.73-8.74 (d, 1H, H_g); MS: *m/z* 347; Anal. Calcd. for $C_{18}H_{10}ClN_5O$: C, 62.17; H, 2.90; N, 20.14. Found: C, 61.98; H, 2.84; N, 20.11%.

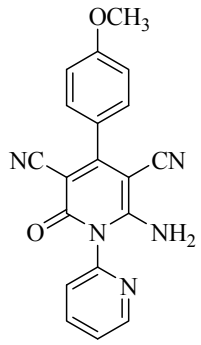
2.5.3.4 6-amino-1,2-dihydro-4-(4-nitrophenyl)-2-oxo-1-(pyridin-2-yl)pyridine-3,5-dicarbonitrile (BHD-104) Yield: 74%; mp 270-272 ºC; IR (cm^{-1}): 3296 and 3269 (N-H stretching of primary amine), 3082 (C-H stretching of aromatic ring), 2218 (C≡N stretching of nitrile group), 1664 (C=O stretching of pyridone ring), 1647 (C=N stretching of pyridine ring), 1602 (N-H deformation of NH_2 group), 1552 (NO_2 asymmetrical stretching), 1533 and 1516 (C=C stretching of aromatic ring), 1346 (C-N stretching for carbon bonded to amino group), 1290 (NO_2 symmetrical stretching), 852 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-*d*$_6$) δ ppm: 7.47-7.49 (d, 1H, H_a, *J* = 8.0 Hz), 7.56-7.59 (t, 3H, H_{bc}), 7.77-7.79 (d, 2H, $H_{dd'}$, *J* = 8.6 Hz), 8.03-8.07 (t, 1H, H_e, *J* = 7.3 Hz), 8.39-8.41 (d, 2H, $H_{ff'}$, *J* = 8.6 Hz); 8.73-8.74 (d, 1H, H_g); MS: *m/z* 358; Anal. Calcd. for $C_{18}H_{10}N_6O_3$: C, 60.34; H, 2.81; N, 23.45. Found: C, 60.19; H, 2.75; N, 23.42%.



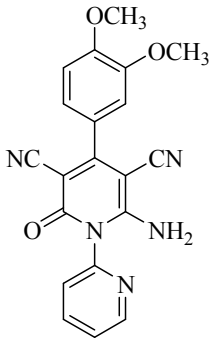
2.5.3.5 6-amino-1,2-dihydro-2-oxo-1-(pyridin-2-yl)-4-p-tolylpyridine-3,5-dicarbonitrile (BHD-105) Yield: 78%; mp 261-263 ºC; MS: *m/z* 327; Anal. Calcd. for $C_{19}H_{13}N_5O$: C, 69.71; H, 4.00; N, 21.39. Found: C, 69.57; H, 3.99; N, 21.31%.



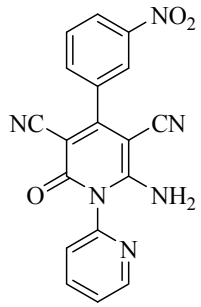
2.5.3.6 6-amino-1,2-dihydro-4-(4-methoxyphenyl)-2-oxo-1-(pyridin-2-yl)pyridine-3,5-dicarbonitrile (BHD-106) Yield: 72%; mp 204-206 ºC; MS: *m/z* 343; Anal. Calcd. for $C_{19}H_{13}N_5O_2$: C, 66.47; H, 3.82; N, 20.40. Found: C, 66.31; H, 3.80; N, 20.32%.



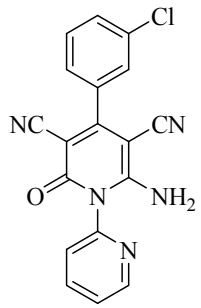
2.5.3.7 6-amino-1,2-dihydro-4-(3,4-dimethoxyphenyl)-2-oxo-1-(pyridin-2-yl)pyridine-3,5-dicarbonitrile (BHD-107) Yield: 65%; mp 244-246 ºC; MS: *m/z* 373; Anal. Calcd. for $C_{20}H_{15}N_5O_3$: C, 64.34; H, 4.05; N, 18.76. Found: C, 64.20; H, 4.01; N, 18.69%.



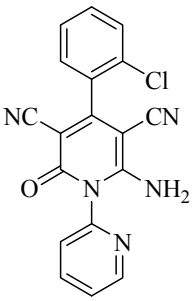
2.5.3.8 6-amino-1,2-dihydro-4-(3-nitrophenyl)-2-oxo-1-(pyridin-2-yl)pyridine-3,5-dicarbonitrile (BHD-108) Yield: 61%; mp 231-233 ºC; MS: *m/z* 358; Anal. Calcd. for $C_{18}H_{10}N_6O_3$: C, 60.34; H, 2.81; N, 23.45. Found: C, 60.16; H, 2.77; N, 23.40%.



2.5.3.9 6-amino-4-(3-chlorophenyl)-1,2-dihydro-2-oxo-1-(pyridin-2-yl)pyridine-3,5-dicarbonitrile (BHD-109) Yield: 70%; mp 269-271 ºC; MS: *m/z* 347; Anal. Calcd. for $C_{18}H_{10}ClN_5O$: C, 62.17; H, 2.90; N, 20.14. Found: C, 62.01; H, 2.86; N, 20.08%.



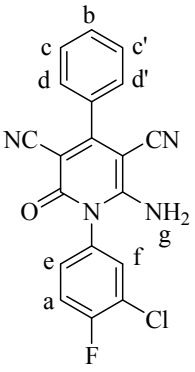
2.5.3.10 6-amino-4-(2-chlorophenyl)-1,2-dihydro-2-oxo-1-(pyridin-2-yl)pyridine-3,5-dicarbonitrile (BHD-110) Yield: 72%; mp 199-201 ºC; MS: *m/z* 347; Anal. Calcd. for $C_{18}H_{10}ClN_5O$: C, 62.17; H, 2.90; N, 20.14. Found: C, 62.00; H, 2.87; N, 20.10%.



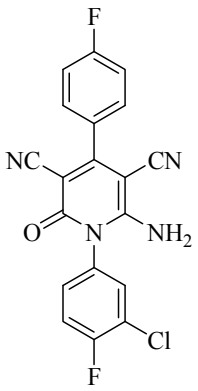
2.5.4 General procedure for the synthesis of 6-amino-1-(3-chloro-4-fluorophenyl)-1,2-dihydro-4-(aryl)-2-oxopyridine-3,5-dicarbonitrile (BHD 111-120)

A mixture of *N*-(3-chloro-4-fluorophenyl)-2-cyano-acetamide (0.01 mol), appropriate aromatic aldehydes (0.01 mol), malononitrile (0.01 mol) and catalytical amount of piperidine in methanol (20 ml) was heated under reflux condition for 10-12 h. The reaction mixture was kept at room temperature for 2-4 h. The solid product obtained was isolated and recrystallized from ethanol.

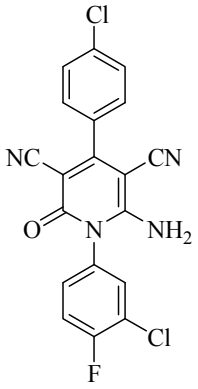
2.5.4.1 6-amino-1-(3-chloro-4-fluorophenyl)-1,2-dihydro-2-oxo-4-phenylpyridine-3,5-dicarbonitrile (BHD-111) Yield: 70%; mp 219-221 ºC; IR (cm^{-1}): 3444 and 3304 (N-H stretching of primary amine), 3109 (C-H stretching of aromatic ring), 2224 (C≡N stretching of nitrile group), 1660 (C=O stretching of pyridone ring), 1629 (N-H deformation of NH_2 group), 1527 and 1494 (C=C stretching of aromatic ring), 1300 (C-N stretching for carbon bonded to amino group), 1078 (C-F stretching), 1058 (C-H in plane bending for aromatic ring), 833 (C-H out of plane bending for 1,4-disubstituted aromatic ring), 773 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 7.27-7.29 (d, 1H, H_a), 7.41-7.45 (t, 1H, H_b), 7.48-7.56 (m, 6H, $H_{cc'-f}$), 7.77 (s, 1H, H_g); MS: *m/z* 364; Anal. Calcd. for $C_{19}H_{10}ClFN_4O$: C, 62.56; H, 2.76; N, 15.36. Found: C, 62.40; H, 2.73; N, 15.29%.



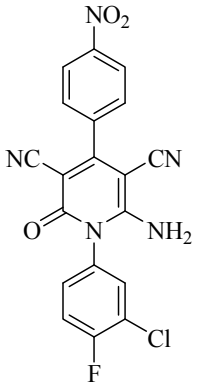
2.5.4.2 6-amino-1-(3-chloro-4-fluorophenyl)-4-(4-fluorophenyl)-1,2-dihydro-2-oxo-pyridine-3,5-dicarbonitrile (BHD-112) Yield: 79%; mp 227-229 ºC; MS: *m/z* 382; Anal. Calcd. for $C_{19}H_9ClF_2N_4O$: C, 59.62; H, 2.37; N, 14.64. Found: C, 59.49; H, 2.31; N, 14.54%.



2.5.4.3 6-amino-1-(3-chloro-4-fluorophenyl)-4-(4-chlorophenyl)-1,2-dihydro-2-oxo-pyridine-3,5-dicarbonitrile (BHD-113) Yield: 75%; mp 190-192 ºC; MS: *m/z* 399; Anal. Calcd. for $C_{19}H_9Cl_2FN_4O$: C, 57.16; H, 2.27; N, 14.03. Found: C, 57.01; H, 2.21; N, 13.97%.



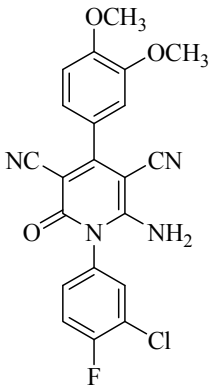
2.5.4.4 6-amino-1-(3-chloro-4-fluorophenyl)-1,2-dihydro-4-(4-nitrophenyl)-2-oxo-pyridine-3,5-dicarbonitrile (BHD-114) Yield: 68%; mp 247-249 ºC; MS: *m/z* 409; Anal. Calcd. for $C_{19}H_9ClFN_5O_3$: C, 55.69; H, 2.21; N, 17.09. Found: C, 55.53; H, 2.20; N, 16.98%.



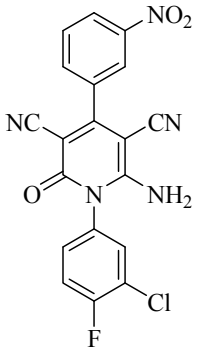
2.5.4.5 6-amino-1-(3-chloro-4-fluorophenyl)-1,2-dihydro-2-oxo-4-p-tolylpyridine-3,5-dicarbonitrile (BHD-115) Yield: 76%; mp 239-241 ºC; IR (cm^{-1}): 3423 and 3315 (N-H stretching of primary amine), 3041 (C-H stretching of aromatic ring), 2949 (C-H symmetrical stretching of CH_3 group), 2864 (C-H asymmetrical stretching of CH_3 group), 2224 and 2206 (C≡N stretching of nitrile group), 1662 (C=O stretching of pyridone ring), 1645 (N-H deformation of NH_2 group), 1533 and 1465 (C=C stretching of aromatic ring), 1300 (C-N stretching for carbon bonded to amino group), 1093 (C-F stretching), 1064 (C-H in plane bending for aromatic ring), 817 (C-H out of plane bending for 1,4-disubstituted aromatic ring), 773 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.43 (s, 3H, H_a), 7.23-7.27 (m, 1H, H_b), 7.33-7.35 (d, 2H, $H_{cc'}$), 7.39-7.47 (m, 4H, $H_{dd'-f}$), 7.53 (s, 2H, H_g); MS: *m/z* 378; Anal. Calcd. for $C_{20}H_{12}ClFN_4O$: C, 63.42; H, 3.19; N, 14.79. Found: C, 63.27; H, 3.15; N, 14.70%.

2.5.4.6 6-amino-1-(3-chloro-4-fluorophenyl)-1,2-dihydro-4-(4-methoxyphenyl)-2-oxopyridine-3,5-dicarbonitrile (BHD-116) Yield: 77%; mp 215-217 ºC; MS: *m/z* 394; Anal. Calcd. for $C_{20}H_{12}ClFN_4O_2$: C, 60.85; H, 3.06; N, 14.19. Found: C, 60.69; H, 3.02; N, 14.14%.

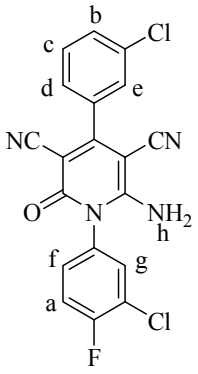
2.5.4.7 6-amino-1-(3-chloro-4-fluorophenyl)-1,2-dihydro-4-(3,4-dimethoxyphenyl)-2-oxopyridine-3,5-dicarbonitrile (BHD-117) Yield: 69%; mp 233-235 ºC; MS: *m/z* 424; Anal. Calcd. for $C_{21}H_{14}ClFN_4O_3$: C, 59.37; H, 3.32; N, 13.19. Found: C, 59.21; H, 3.27; N, 13.10%.



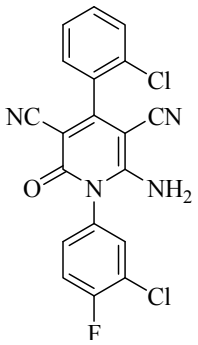
2.5.4.8 6-amino-1-(3-chloro-4-fluorophenyl)-1,2-dihydro-4-(3-nitrophenyl)-2-oxo-pyridine-3,5-dicarbonitrile (BHD-118) Yield: 66%; mp 190-192 ºC; MS: *m/z* 409; Anal. Calcd. for $C_{19}H_9ClFN_5O_3$: C, 55.69; H, 2.21; N, 17.09. Found: C, 55.50; H, 2.17; N, 17.03%.



2.5.4.9 6-amino-1-(3-chloro-4-fluorophenyl)-4-(3-chlorophenyl)-1,2-dihydro-2-oxo-pyridine-3,5-dicarbonitrile (BHD-119) Yield: 70%; mp 262-264 ºC; IR (cm^{-1}): 3419 and 3331 (N-H stretching of primary amine), 3068 (C-H stretching of aromatic ring), 2218 (C≡N stretching of nitrile group), 1691 (C=O stretching of pyridone ring), 1631 (N-H deformation of NH_2 group), 1531 and 1465 (C=C stretching of aromatic ring), 1300 (C-N stretching for carbon bonded to amino group), 1074 (C-F stretching), 802 (C-H out of plane bending for 1,3-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 7.26-7.30 (m, 1H, H_a), 7.42-7.55 (m, 6H, H_{b-g}), 7.80 (s, 2H, H_h); MS: *m/z* 399; Anal. Calcd. for $C_{19}H_9Cl_2FN_4O$: C, 57.16; H, 2.27; N, 14.03. Found: C, 57.01; H, 2.21; N, 13.97%.



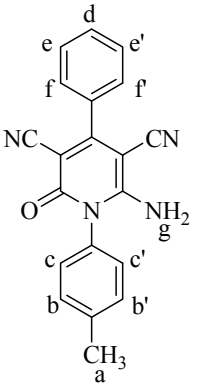
2.5.4.10 6-amino-1-(3-chloro-4-fluorophenyl)-4-(2-chlorophenyl)-1,2-dihydro-2-oxo-pyridine-3,5-dicarbonitrile (BHD-120) Yield: 75%; mp 260-262 ºC; MS: *m/z* 399; Anal. Calcd. for $C_{19}H_9Cl_2FN_4O$: C, 57.16; H, 2.27; N, 14.03. Found: C, 56.98; H, 2.20; N, 13.99%.



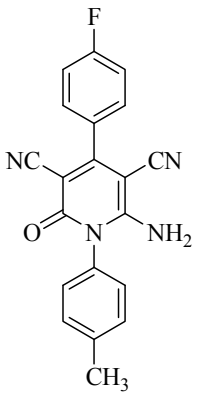
2.5.5 General procedure for the synthesis of 6-amino-1,2-dihydro-4-(aryl)-2-oxo-1-p-tolylpyridine-3,5- dicarbonitrile (BHD 121-130)

A mixture of 2-cyano-*N*-*p*-tolylacetamide (0.01 mol), appropriate aromatic aldehydes (0.01 mol), malononitrile (0.01 mol) and catalytical amount of piperidine in methanol (20 ml) was heated under reflux condition for 10-12 h. The reaction mixture was kept at room temperature for 2-4 h. The solid product obtained was isolated and recrystallized from ethanol.

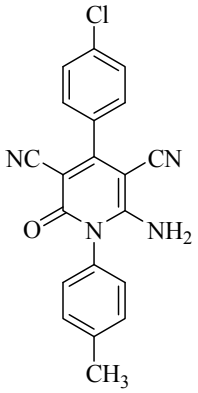
2.5.5.1 6-amino-1,2-dihydro-2-oxo-4-phenyl-1-p-tolylpyridine-3,5-dicarbonitrile (BHD-121) Yield: 75%; mp 256-258 ºC; IR (cm^{-1}): 3429 and 3275 (N-H stretching of primary amine), 3064 (C-H stretching of aromatic ring), 2229 and 2214 (C≡N stretching of nitrile group), 1647 (C=O stretching of pyridone ring), 1597 (N-H deformation of NH_2 group), 1560 and 1514 (C=C stretching of aromatic ring), 825 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.46 (s, 3H, H_a), 7.18-7.20 (d, 2H, $H_{bb'}$, J = 8.1 Hz), 7.42-7.44 (d, 2H, $H_{cc'}$, J = 7.9 Hz), 7.54-7.55 (d, 5H, $H_{d-ff'}$), 7.71 (s, 2H, H_g); MS: *m/z* 326; Anal. Calcd. for $C_{20}H_{14}N_4O$: C, 73.61; H, 4.32; N, 17.17. Found: C, 73.47; H, 4.25; N, 17.10%.



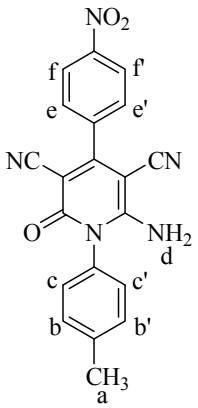
2.5.5.2 6-amino-4-(4-fluorophenyl)-1,2-dihydro-2-oxo-1-p-tolylpyridine-3,5-dicarbonitrile (BHD-122) Yield: 74%; mp 230-232 ºC; MS: *m/z* 344; Anal. Calcd. for $C_{20}H_{13}FN_4O$: C, 69.76; H, 3.81; N, 16.27. Found: C, 69.63; H, 3.80; N, 16.24%.



2.5.5.3 6-amino-4-(4-chlorophenyl)-1,2-dihydro-2-oxo-1-p-tolylpyridine-3,5-dicarbonitrile (BHD-123) Yield: 80%; mp 215-217 ºC; MS: *m/z* 360; Anal. Calcd. for $C_{20}H_{13}ClN_4O$: C, 66.58; H, 3.63; N, 15.53. Found: C, 66.41; H, 3.60; N, 15.42%.

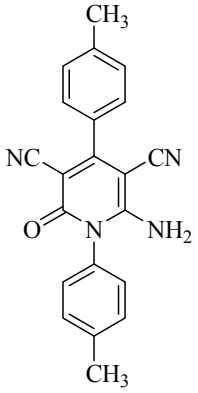


2.5.5.4 6-amino-1,2-dihydro-4-(4-nitrophenyl)-2-oxo-1-p-tolylpyridine-3,5-dicarbonitrile (BHD-124) Yield: 70%; mp 270-272 ºC; IR (cm^{-1}): 3429 and 3267 (N-H stretching of primary amine), 3064 (C-H stretching of aromatic ring), 2229 and 2214 (C≡N stretching of nitrile group), 1649 (C=O stretching of pyridone ring), 1599 (N-H deformation of NH_2 group), 1558 and 1516 (C=C stretching of aromatic ring), 1290 (C-N stretching for carbon bonded to amino group), 1070 (C-H in plane bending for aromatic ring), 823 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.47 (s, 3H, H_a), 7.18-7.20 (d, 2H, $H_{bb'}$, J = 8.2 Hz), 7.43-7.45 (d, 2H, $H_{cc'}$, J = 8.1 Hz), 7.75 (s, 2H, H_d), 7.78-7.81 (d, 2H, $H_{ee'}$), 7.39-7.42 (d, 2H, $H_{ff'}$); MS: *m/z* 371;

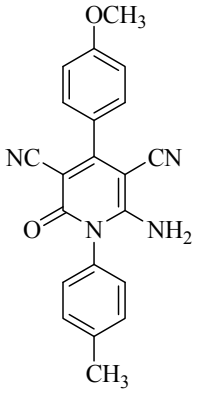


Anal. Calcd. for $C_{20}H_{13}N_5O_3$: C, 64.69; H, 3.53; N, 18.86. Found: C, 64.54; H, 3.49; N, 18.79%.

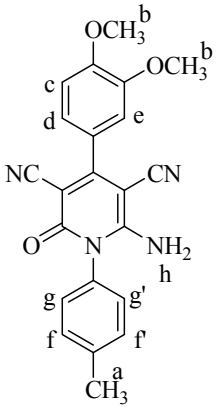
2.5.5.5 6-amino-1,2-dihydro-2-oxo-1,4-dip-tolylpyridine-3,5-dicarbonitrile (BHD-125) Yield: 79%; mp 203-205 ºC; MS: *m/z* 340; Anal. Calcd. for $C_{21}H_{16}N_4O$: C, 74.10; H, 4.74; N, 16.46. Found: C, 73.97; H, 4.69; N, 16.40%.



2.5.5.6 6-amino-1,2-dihydro-4-(4-methoxyphenyl)-2-oxo-1-p-tolylpyridine-3,5-dicarbonitrile (BHD-126) Yield: 82%; mp 186-188 ºC; MS: *m/z* 356; Anal. Calcd. for $C_{21}H_{16}N_4O_2$: C, 70.77; H, 4.53; N, 15.72. Found: C, 70.60; H, 4.47; N, 15.61 %.

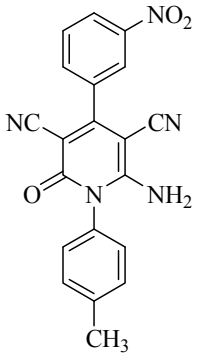


2.5.5.7 6-amino-1,2-dihydro-4-(3,4-dimethoxyphenyl)-2-oxo-1-p-tolylpyridine-3,5-dicarbonitrile (BHD-127) Yield: 75%; mp 264-266 ºC; IR (cm^{-1}): 3435 and 3365 (N-H stretching of primary amine), 3068 (C-H stretching of aromatic ring), 2997 (C-H symmetrical stretching of CH_3 group), 2839 (C-H asymmetrical stretching of CH_3 group), 2212 (C≡N stretching of nitrile group), 1660 (C=O stretching of pyridone ring), 1600 (N-H deformation of NH_2 group), 1560 and 1518 (C=C stretching of aromatic ring), 1325 (C-N stretching for carbon bonded to amino group), 1265 (C-O-C asymmetrical stretching

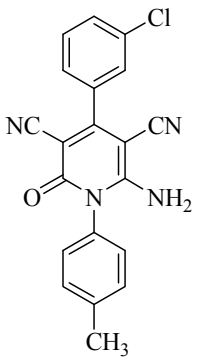


of OCH_3 group), 1145 (C-O-C symmetrical stretching OCH_3 group), 1022 (C-H in plane bending for aromatic ring), 877 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 2.46 (s, 3H, H_a), 3.93 (s, 6H, H_b), 7.01-7.21 (m, 5H, $H_{c\text{-}ff'}$), 7.42-7.44 (d, 2H, $H_{gg'}$, J = 7.9 Hz), 7.71 (s, 2H, H_h); MS: *m/z* 386; Anal. Calcd. for $C_{22}H_{18}N_4O_3$: C, 68.38; H, 4.70; N, 14.50. Found: C, 68.21; H, 4.67; N, 14.43 %.

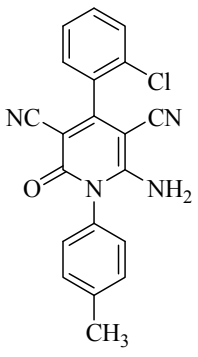
2.5.5.8 6-amino-1,2-dihydro-4-(3-nitrophenyl)-2-oxo-1-p-tolylpyridine-3,5-dicarbonitrile (BHD-128) Yield: 72%; mp 218-220 ºC; MS: *m/z* 371; Anal. Calcd. for $C_{20}H_{13}N_5O_3$: C, 64.69; H, 3.53; N, 18.86. Found: C, 64.51; H, 3.47; N, 18.81%.



2.5.5.9 6-amino-4-(3-chlorophenyl)-1,2-dihydro-2-oxo-1-p-tolylpyridine-3,5-dicarbonitrile (BHD-129) Yield: 85%; mp 258-260 ºC; MS: *m/z* 360; Anal. Calcd. for $C_{20}H_{13}ClN_4O$: C, 66.58; H, 3.63; N, 15.53. Found: C, 66.45; H, 3.58; N, 15.43%.



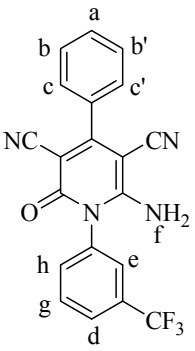
2.5.5.10 6-amino-4-(2-chlorophenyl)-1,2-dihydro-2-oxo-1-p-tolylpyridine-3,5-dicarbonitrile (BHD-130) Yield: 71%; mp 220-222 ºC; MS: *m/z* 360; Anal. Calcd. for $C_{20}H_{13}ClN_4O$: C, 66.58; H, 3.63; N, 15.53. Found: C, 66.40; H, 3.60; N, 15.46%.



2.5.6 General procedure for the synthesis of 6-amino-1-(3-(trifluoromethyl)phenyl)-1,2-dihydro-4-(aryl)-2-oxopyridine-3,5-dicarbonitrile (BHD 131-140)

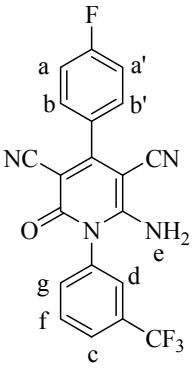
A mixture of 2-cyano-*N*-(3-(trifluoromethyl)phenyl)acetamide (0.01 mol), appropriate aromatic aldehydes (0.01 mol), malononitrile (0.01 mol) and catalytical amount of piperidine in methanol (20 ml) was heated under reflux condition for 10-12 h. The reaction mixture was kept at room temperature for 2-4 h. The solid product obtained was isolated and recrystallized from ethanol.

2.5.6.1 6-amino-1-(3-(trifluoromethyl)phenyl)-1,2-dihydro-2-oxo-4-phenylpyridine-3,5-dicarbonitrile (BHD-131)



Yield: 68%; mp 228-230 ºC; IR (cm^{-1}): 3456 and 3311 (N-H stretching of primary amine), 3066 (C-H stretching of aromatic ring), 2218 (C≡N stretching of nitrile group), 1674 (C=O stretching of pyridone ring), 1631 (N-H deformation of NH_2 group), 1525 and 1464 (C=C stretching of aromatic ring), 1329 (C-N stretching for carbon bonded to amino group), 1001 (C-H in plane bending for aromatic ring), 781 (C-H out of plane bending for 1,3-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 7.53-7.57 (t, 7H, $H_{a\text{-}e}$), 7.64 (s, 2H, H_f), 7.77-7.86 (m, 2H, H_{gh}); MS: *m/z* 380; Anal. Calcd. for $C_{20}H_{11}F_3N_4O$: C, 63.16; H, 2.92; N, 14.73. Found: C, 63.02; H, 2.90; N, 14.65%.

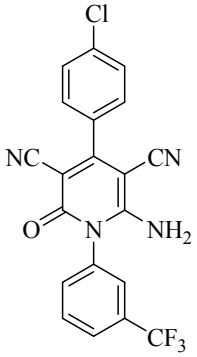
2.5.6.2 6-amino-1-(3-(trifluoromethyl)phenyl)-4-(4-fluorophenyl)-1,2-dihydro-2-oxopyridine-3,5-dicarbonitrile (BHD-132)



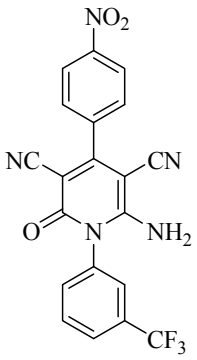
Yield: 71%; mp 239-241 ºC; IR (cm^{-1}): 3462 and 3311 (N-H stretching of primary amine), 3076 (C-H stretching of aromatic ring), 2225 and 2212 (C≡N stretching of nitrile group), 1687 (C=O stretching of pyridone ring), 1629 (C=N stretching of pyridine ring), 1600 (N-H deformation of NH_2 group), 1550 and 1465 (C=C stretching of aromatic ring), 1330 (C-N stretching for carbon bonded to amino group), 1070 (C-F stretching), 842 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 7.25-7.27 (d, 2H, $H_{aa'}$), 7.54-7.62 (m, 6H, $H_{bb'\text{-}e}$), 7.78-7.86 (m, 2H, H_{fg}); MS: *m/z* 398;

Anal. Calcd. for $C_{20}H_{10}F_4N_4O$: C, 60.31; H, 2.53; N, 14.07. Found: C, 60.25; H, 2.51; N, 13.97%.

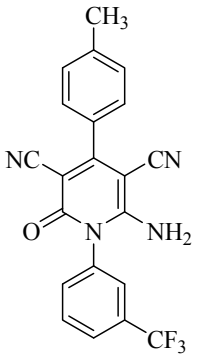
2.5.6.3 6-amino-4-(4-chlorophenyl)-1-(3-(trifluoromethyl)phenyl)-1,2-dihydro-2-oxopyridine-3,5-dicarbonitrile (BHD-133) Yield: 74%; mp 201-203 ºC; MS: *m/z* 414; Anal. Calcd. for $C_{20}H_{10}ClF_3N_4O$: C, 57.92; H, 2.43; N, 13.51. Found: C, 57.80; H, 2.40; N, 13.47%.



2.5.6.4 6-amino-1-(3-(trifluoromethyl)phenyl)-1,2-dihydro-4-(4-nitrophenyl)-2-oxo-pyridine-3,5-dicarbonitrile (BHD-134) Yield: 65%; mp 266-268 ºC; MS: *m/z* 425; Anal. Calcd. for $C_{20}H_{10}F_3N_5O_3$: C, 56.48; H, 2.37; N, 16.47. Found: C, 56.40; H, 2.33; N, 16.41%.



2.5.6.5 6-amino-1-(3-(trifluoromethyl)phenyl)-1,2-dihydro-2-oxo-4-p-tolylpyridine-3,5-dicarbonitrile (BHD-135) Yield: 69%; mp 257-259 ºC; MS: *m/z* 394; Anal. Calcd. for $C_{21}H_{13}F_3N_4O$: C, 63.96; H, 3.32; N, 14.21. Found: C, 63.82; H, 3.28; N, 14.14%.

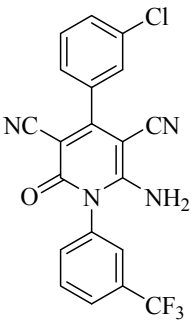


2.5.6.6 6-amino-1-(3-(trifluoromethyl)phenyl)-1,2-dihydro-4-(4-methoxyphenyl)-2-oxopyridine-3,5-dicarbonitrile (BHD-136) Yield: 74%; mp 230-232 ºC; IR (cm^{-1}): 3460 and 3292 (N-H stretching of primary amine), 3078 (C-H stretching of aromatic ring), 2224 and 2212 (C≡N stretching of nitrile group), 1689 (C=O stretching of pyridone ring), 1629 (C=N stretching of pyridine ring), 1593 (N-H deformation of NH_2 group), 1516 and 1460 (C=C stretching of aromatic ring), 1330 (C-N stretching for carbon bonded to amino group), 1232 (C-O-C asymmetrical stretching of OCH_3 group), 1070 (C-O-C symmetrical stretching OCH_3 group), 1020 (C-F stretching), 837 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 3.88 (s, 3H, H_a), 7.05-7.07 (d, 2H, $H_{bb'}$), 7.51-7.57 (m, 4H, $H_{cc'-e}$), 7.64 (s, 2H, H_f), 7.76-7.85 (m, 2H, H_{gh}); MS: *m/z* 410; Anal. Calcd. for $C_{21}H_{13}F_3N_4O_2$: C, 61.47; H, 3.19; N, 13.65. Found: C, 61.39; H, 3.17; N, 13.59%.

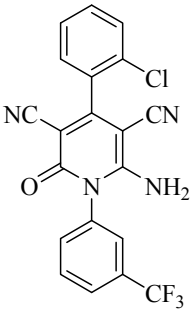
2.5.6.7 6-amino-1-(3-(trifluoromethyl)phenyl)-1,2-dihydro-4-(3,4-dimethoxyphenyl)-2-oxopyridine-3,5-dicarbonitrile (BHD-137) Yield: 70%; mp 250-252 ºC; MS: *m/z* 440; Anal. Calcd. for $C_{22}H_{15}F_3N_4O_3$: C, 60.00; H, 3.43; N, 12.72. Found: C, 59.87; H, 3.40; N, 12.65%.

2.5.6.8 6-amino-1-(3-(trifluoromethyl)phenyl)-1,2-dihydro-4-(3-nitrophenyl)-2-oxo-pyridine-3,5-dicarbonitrile (BHD-138) Yield: 75%; mp 270-272 ºC; MS: *m/z* 425; Anal. Calcd. for $C_{20}H_{10}F_3N_5O_3$: C, 56.48; H, 2.37; N, 16.47. Found: C, 56.36; H, 2.35; N, 16.39%.

2.5.6.9 6-amino-4-(3-chlorophenyl)-1-(3-(trifluoromethyl)phenyl)-1,2-dihydro-2-oxopyridine-3,5-dicarbonitrile (BHD-139) Yield: 68%; mp 221-223 ºC; MS: *m/z* 414; Anal. Calcd. for $C_{20}H_{10}ClF_3N_4O$: C, 57.92; H, 2.43; N, 13.51. Found: C, 57.77; H, 2.37; N, 13.45%.



2.5.6.10 6-amino-4-(2-chlorophenyl)-1-(3-(trifluoromethyl)phenyl)-1,2-dihydro-2-oxopyridine-3,5-dicarbonitrile (BHD-140) Yield: 72%; mp 217-219 ºC; MS: *m/z* 414; Anal. Calcd. for $C_{20}H_{10}ClF_3N_4O$: C, 57.92; H, 2.43; N, 13.51. Found: C, 57.75; H, 2.39; N, 13.41%.

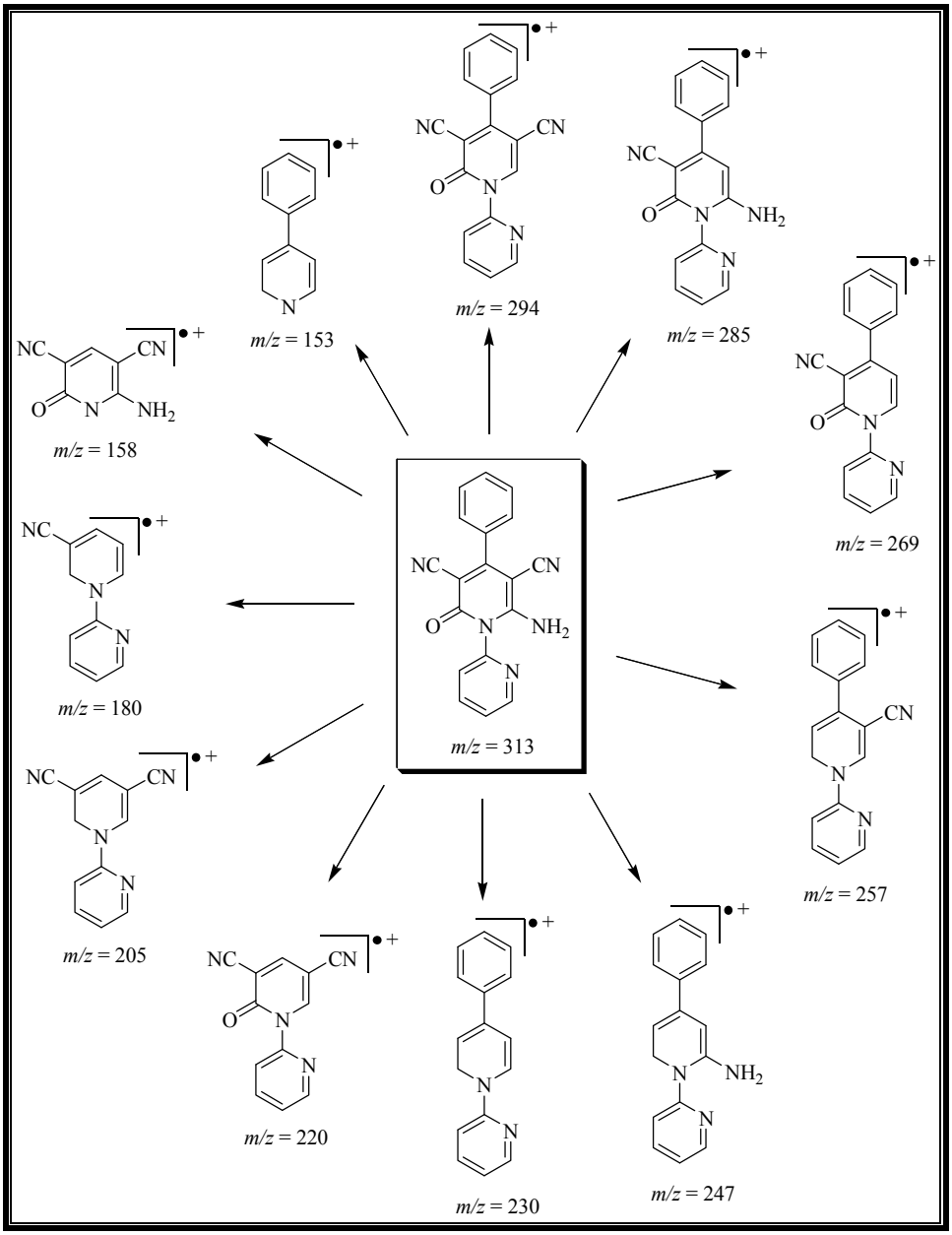


2.6 Spectral discussion

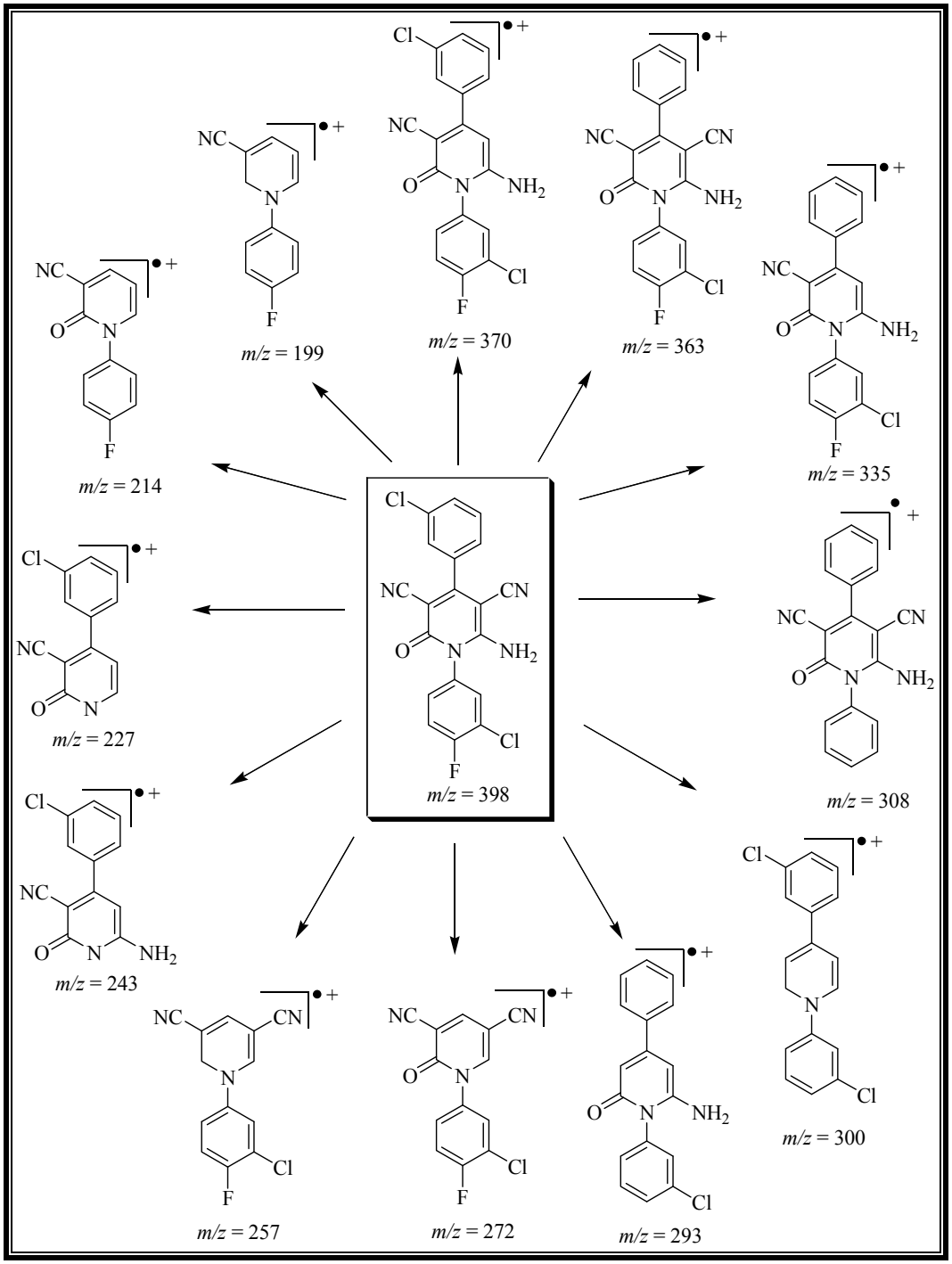
2.6.1 Mass spectral study

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.

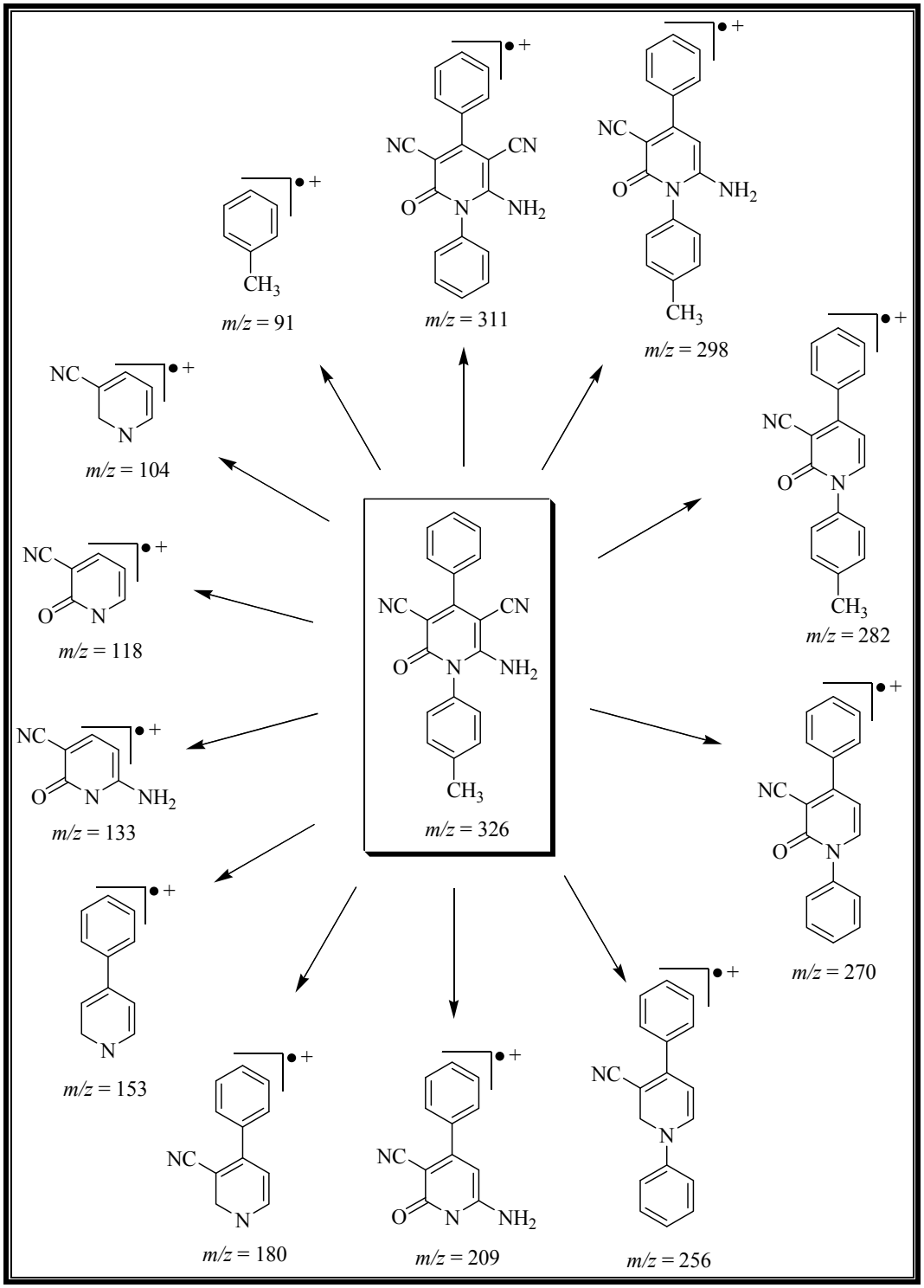
2.6.1.1 Plausible mass fragmentation pattern for BHD-101



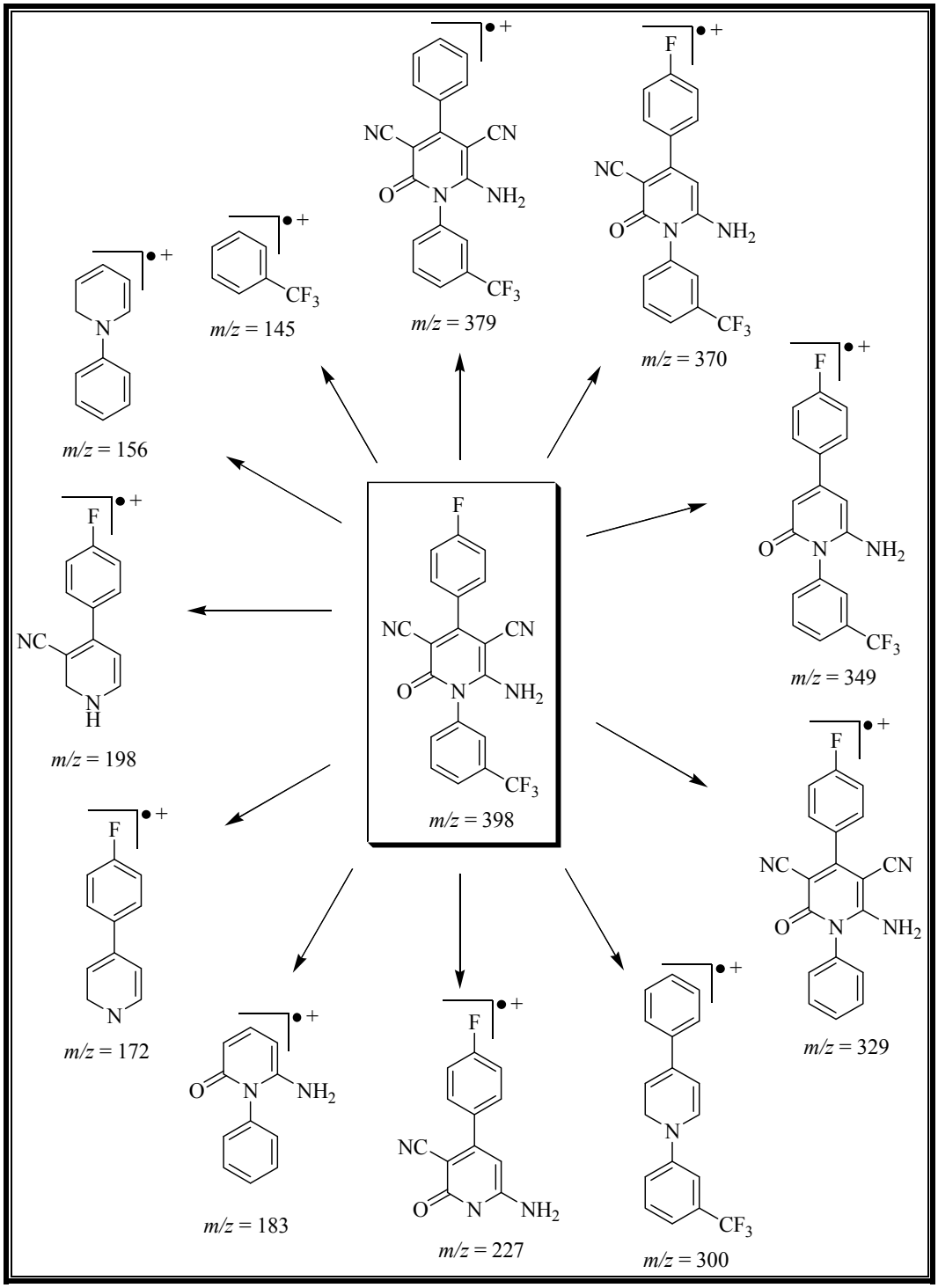
2.6.1.2 Plausible mass fragmentation pattern for BHD-119



2.6.1.3 Plausible mass fragmentation pattern for BHD-121



2.6.1.4 Plausible mass fragmentation pattern for BHD-132



2.6.2 IR spectral study

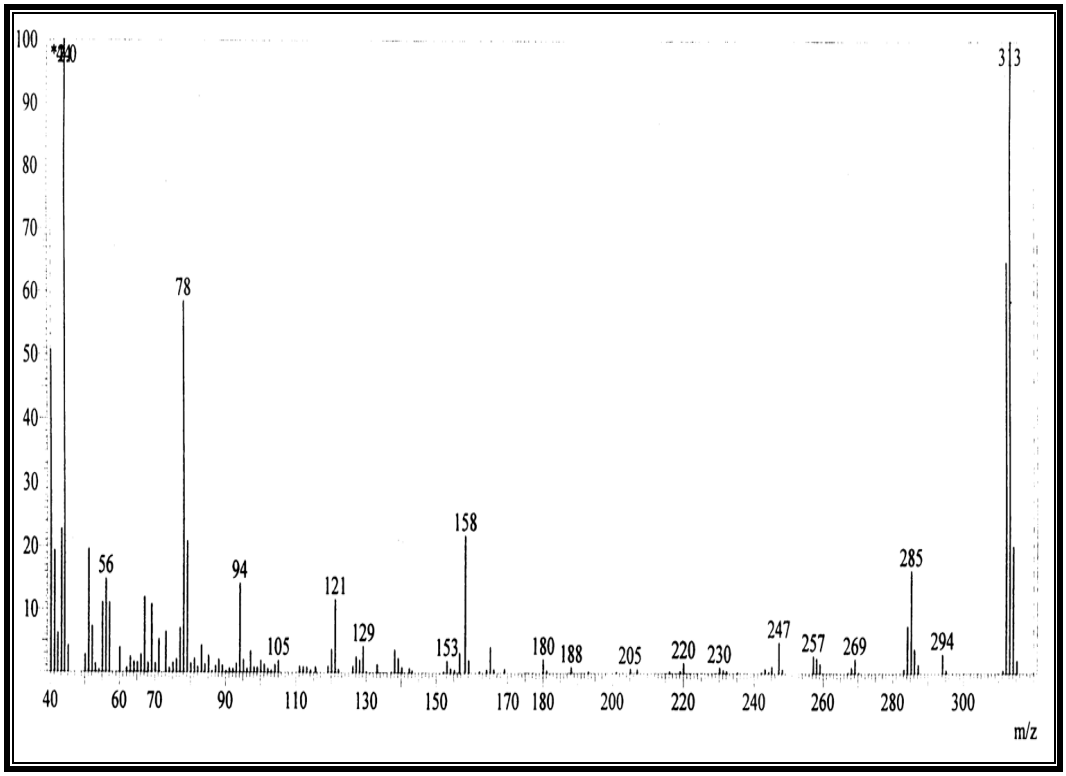
IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For 3-cyano-2-pyridones (BHD-101 to 140), confirmatory bands for primary amine ($-NH_2$), carbonyl (C=O) and nitrile (C≡N) stretching band was observed at 3250-3500 cm^{-1}, 1650-1700 cm^{-1} and 2200-2230 cm^{-1} respectively. Another characteristic band for N-H deformation and C-N stretching were observed at 1595-1650 cm^{-1} and 1290-1360 cm^{-1} respectively, which suggested the formation of pyridone ring.

2.6.3 ^{1}H NMR spectral study

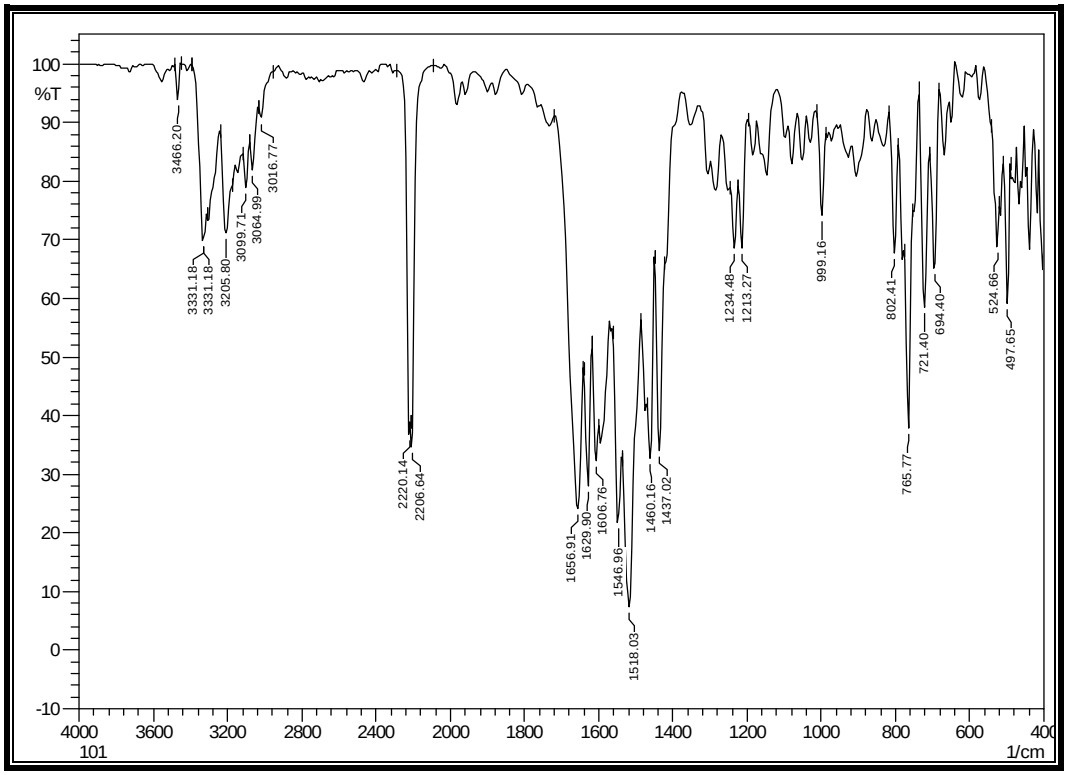
^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

^{1}H NMR spectra confirmed the structures of 3-cyano-2-pyridones (BHD-101 to 140) on the basis of following signals: singlet for primary amino group proton was observed at 7.50-7.90 δ ppm. The aromatic ring protons and *J* value were found to be in accordance with substitution pattern on phenyl ring.

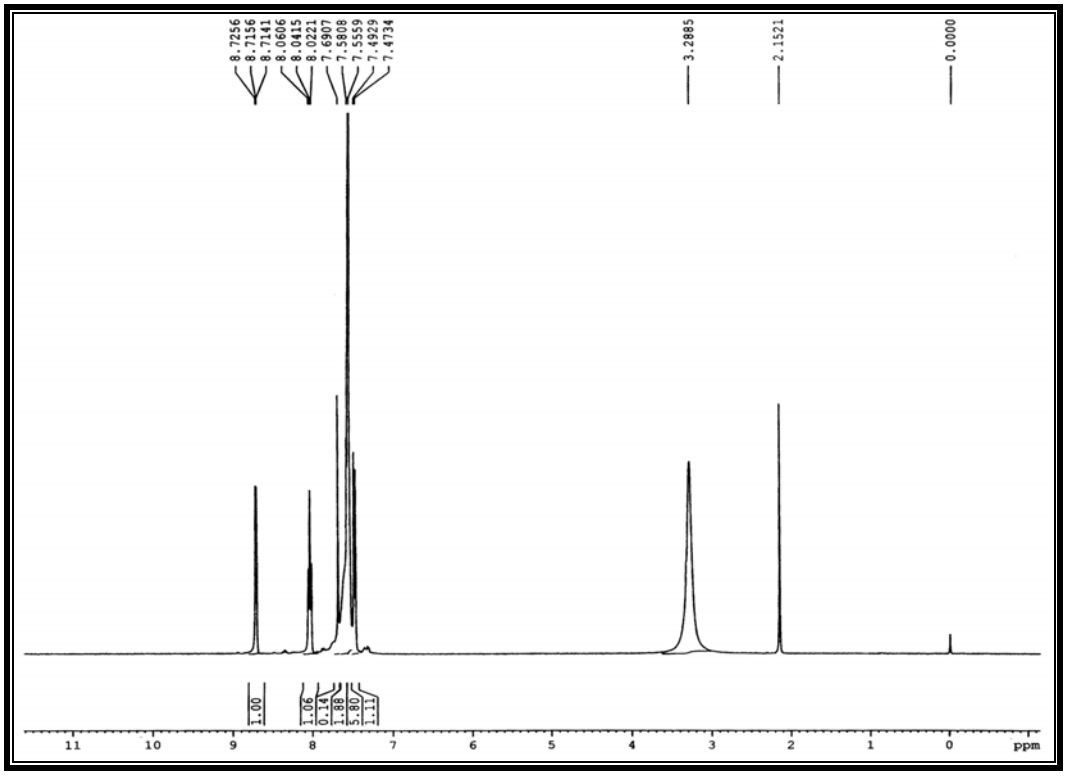
Mass spectrum of BHD-101



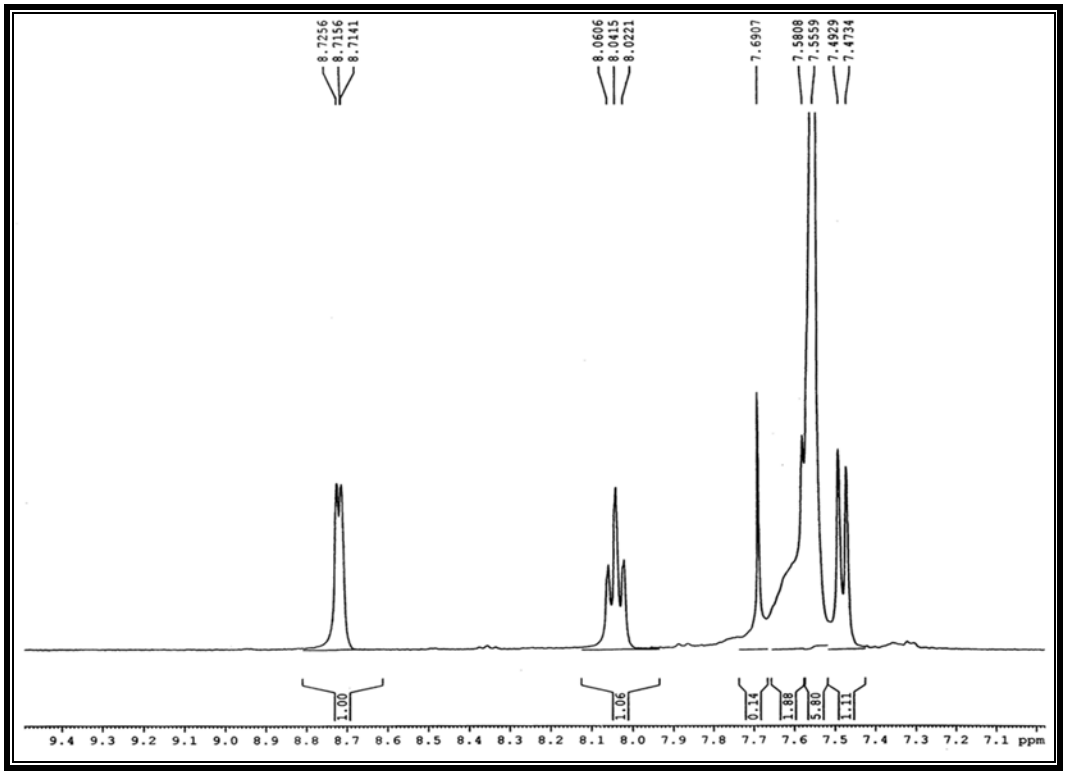
IR spectrum of BHD-101



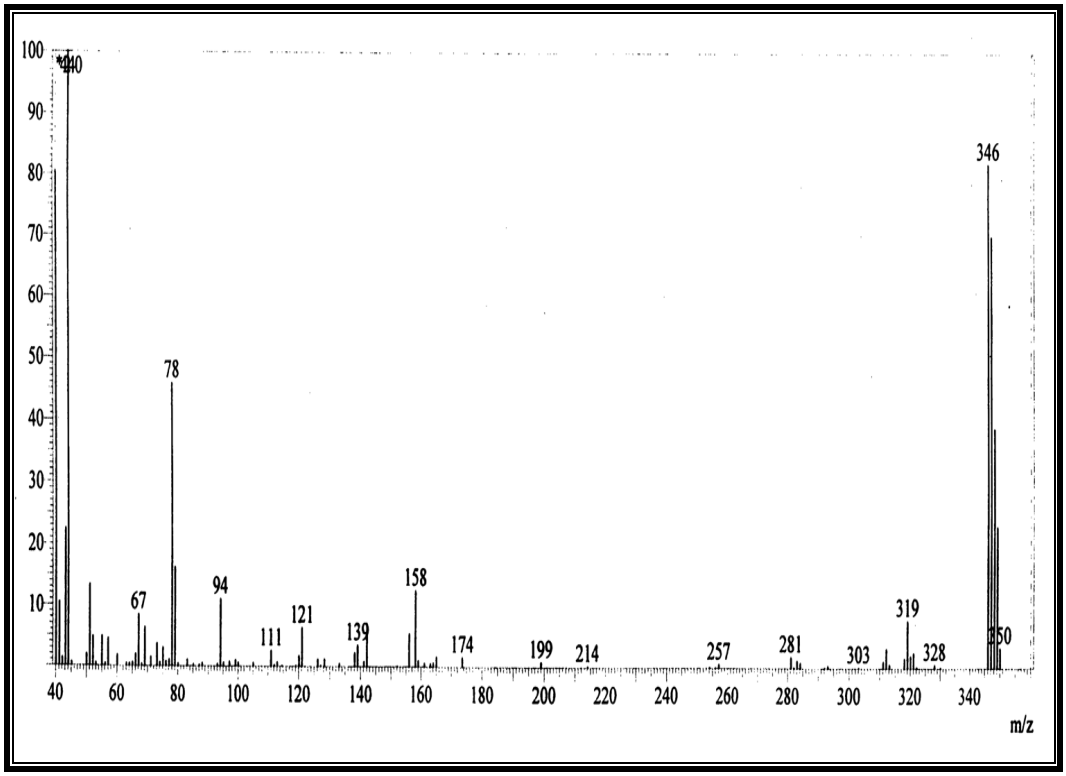
^{1}H NMR spectrum of BHD-101



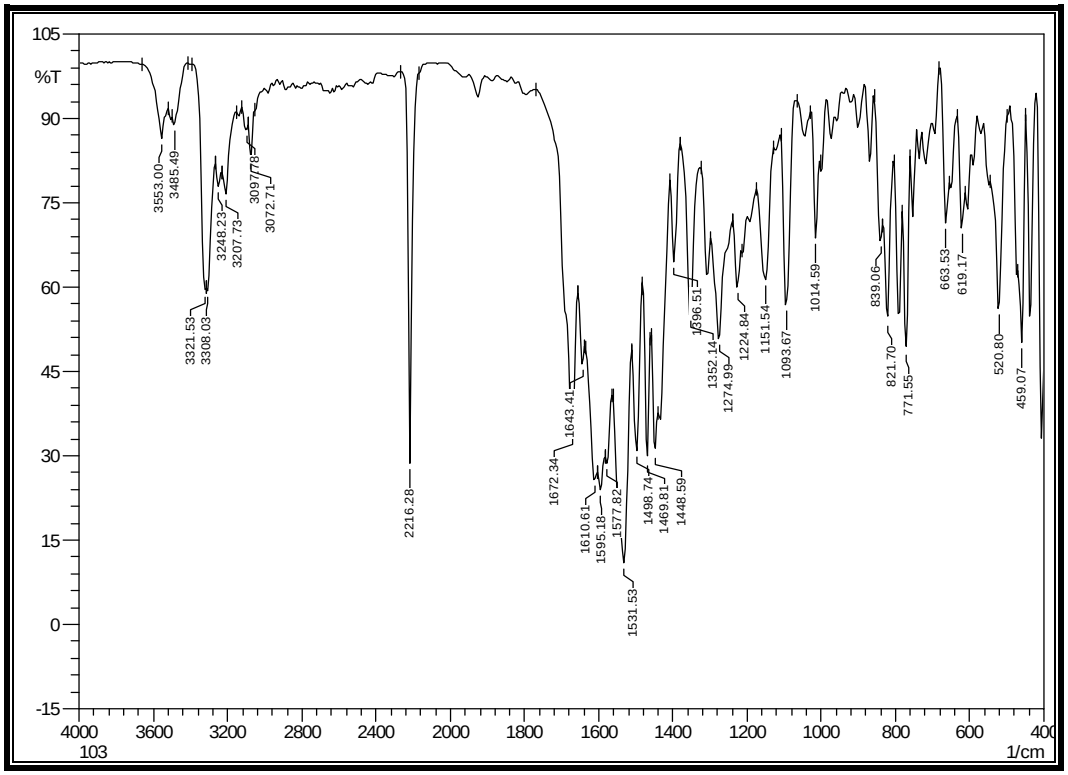
Expanded ^{1}H NMR spectrum of BHD-101



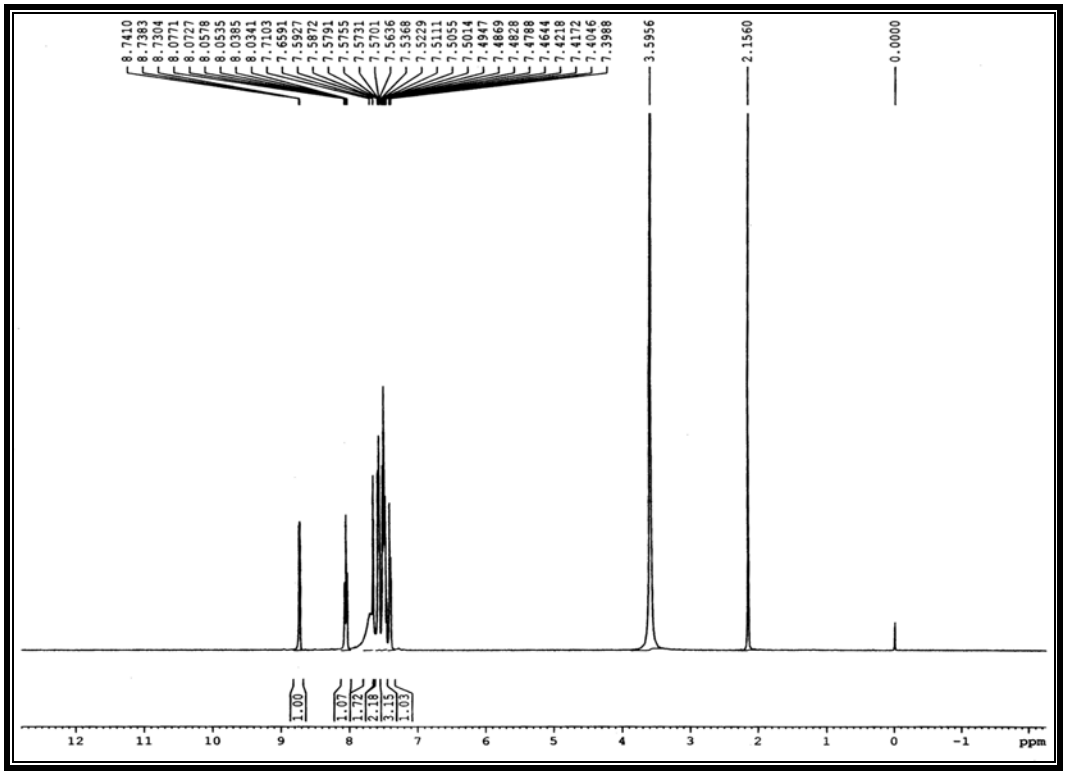
Mass spectrum of BHD-103



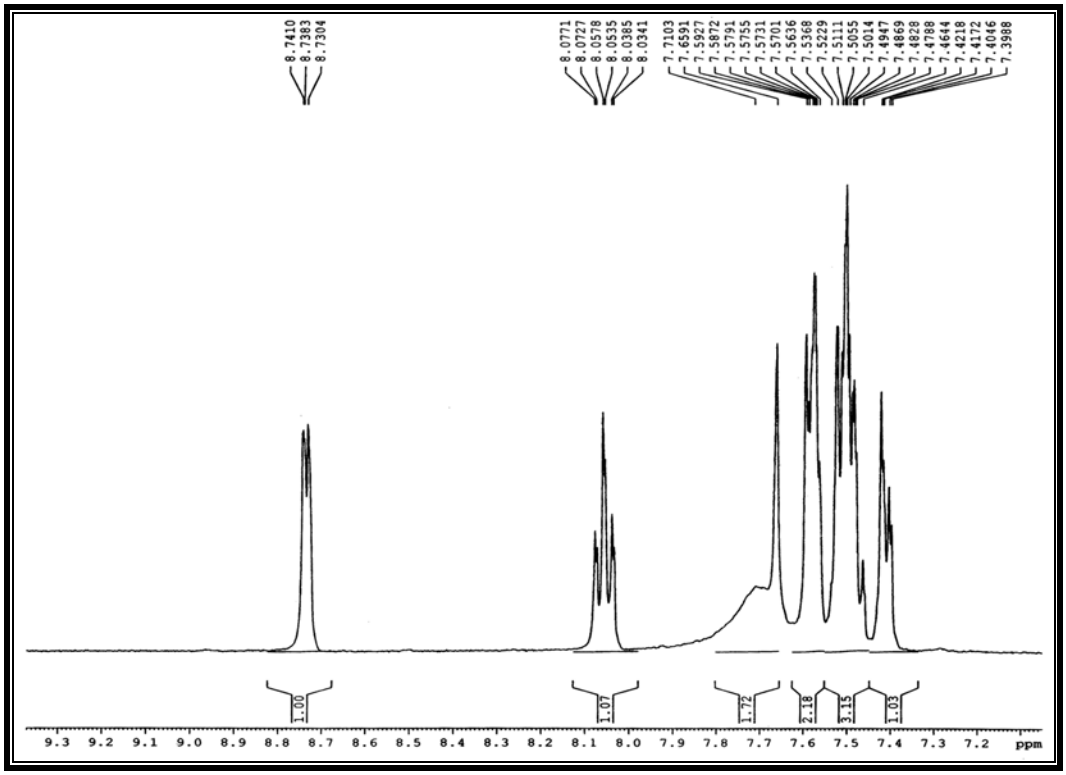
IR spectrum of BHD-103



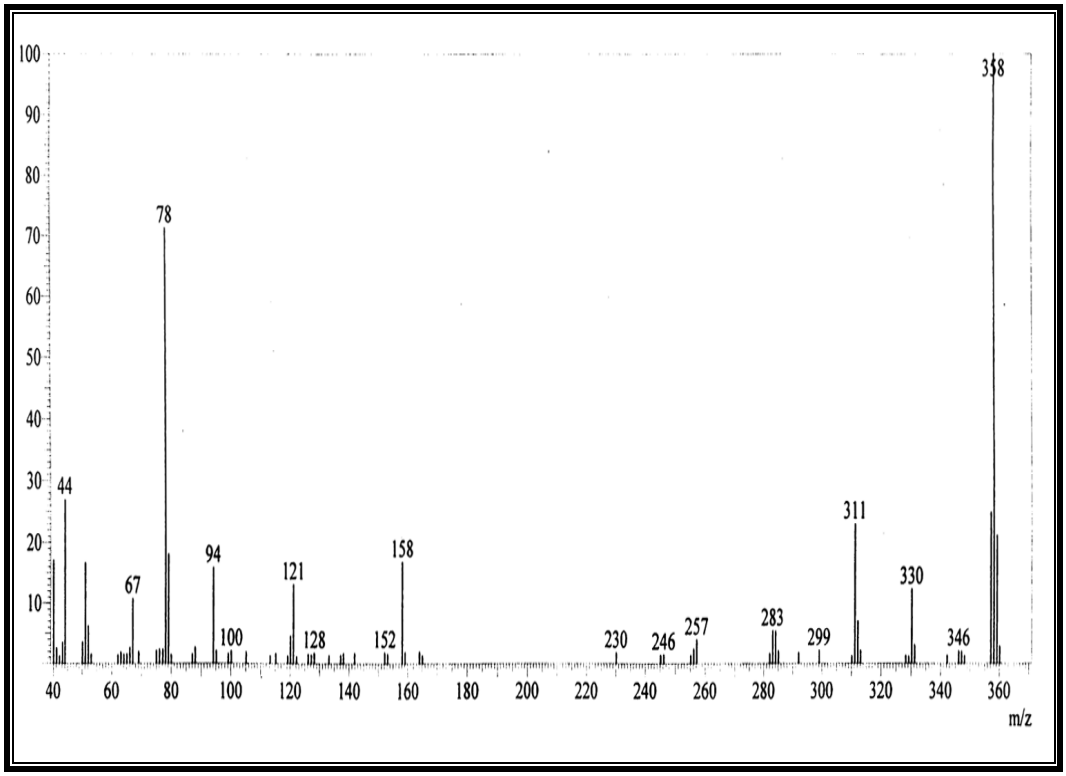
^{1}H NMR spectrum of BHD-103



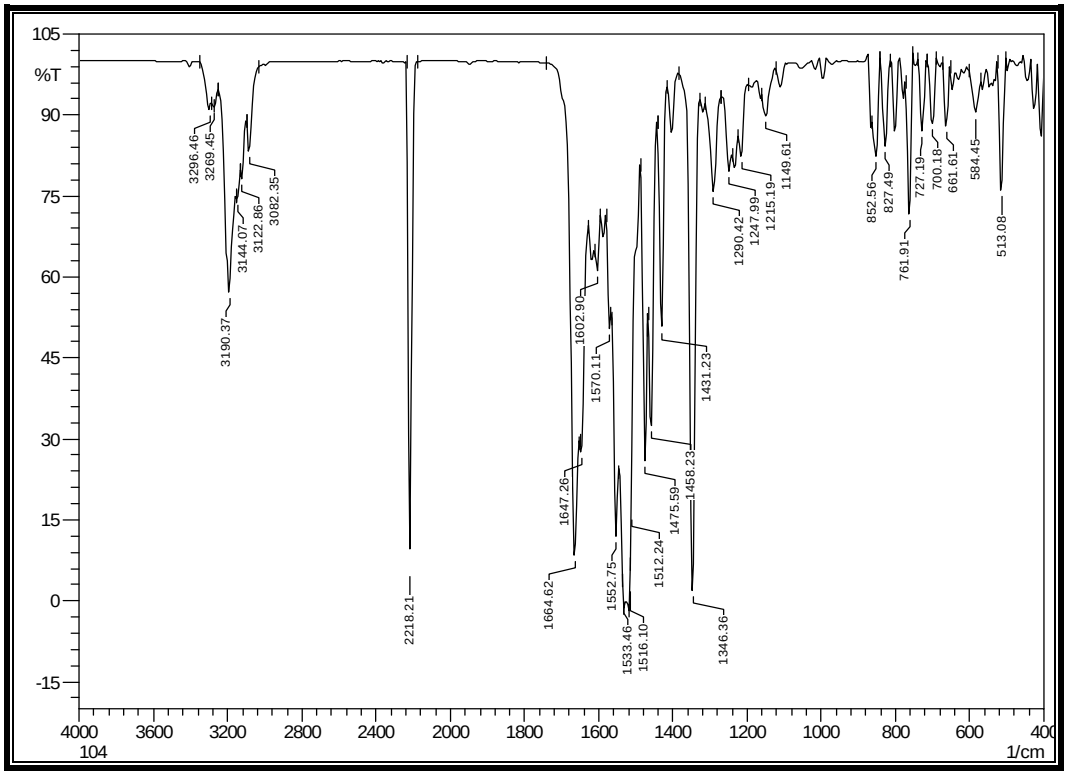
Expanded ^{1}H NMR spectrum of BHD-103



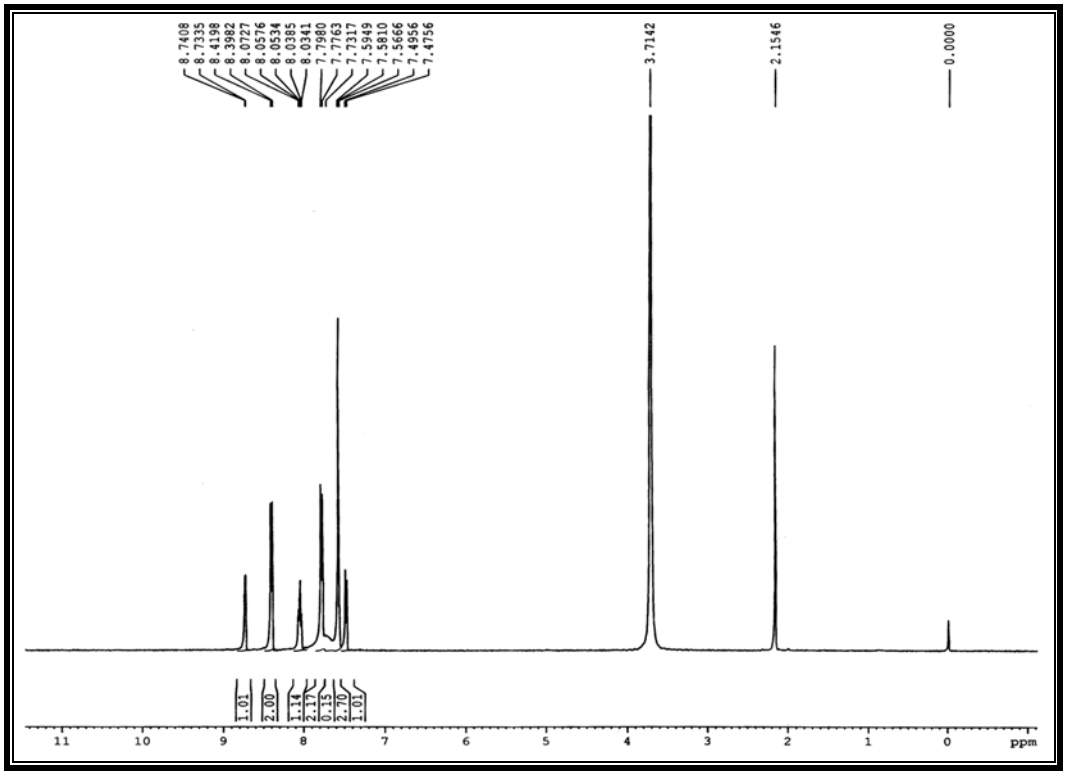
Mass spectrum of BHD-104



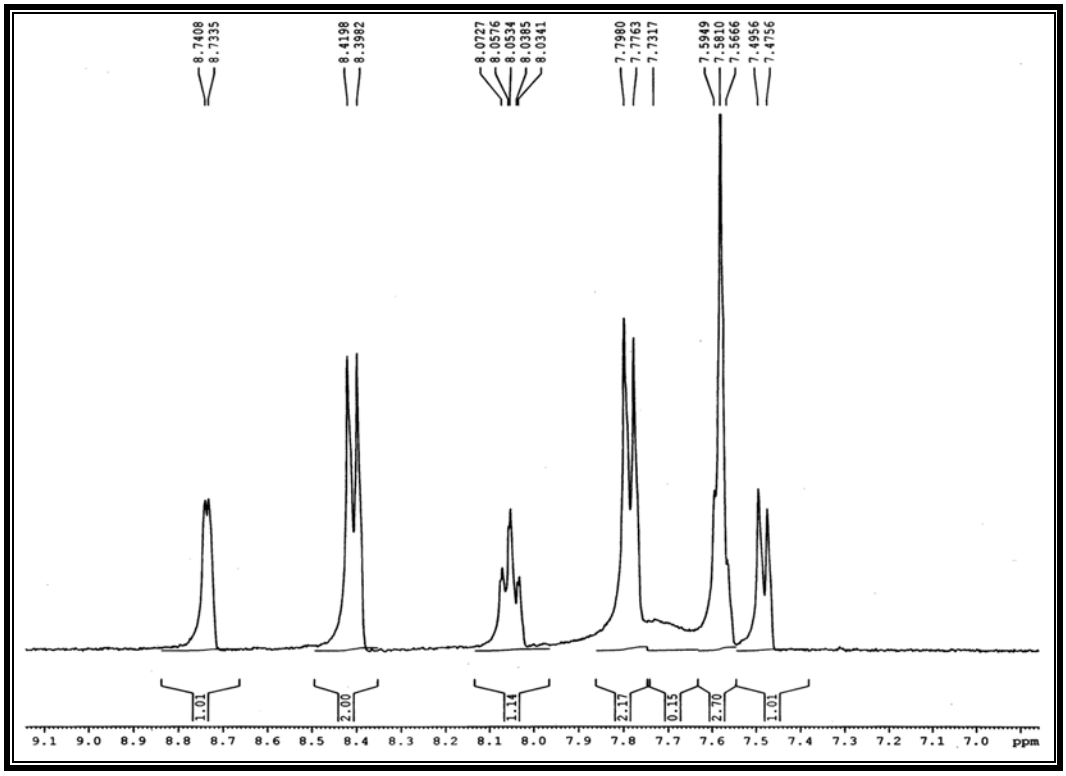
IR spectrum of BHD-104



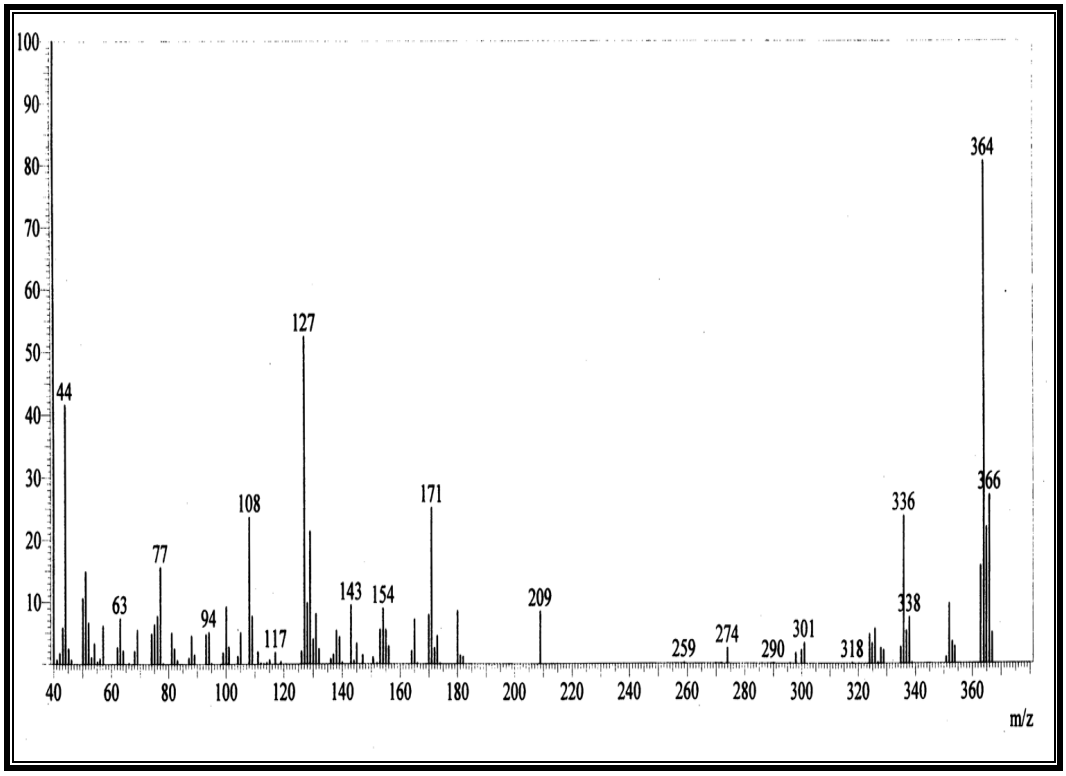
^{1}H NMR spectrum of BHD-104



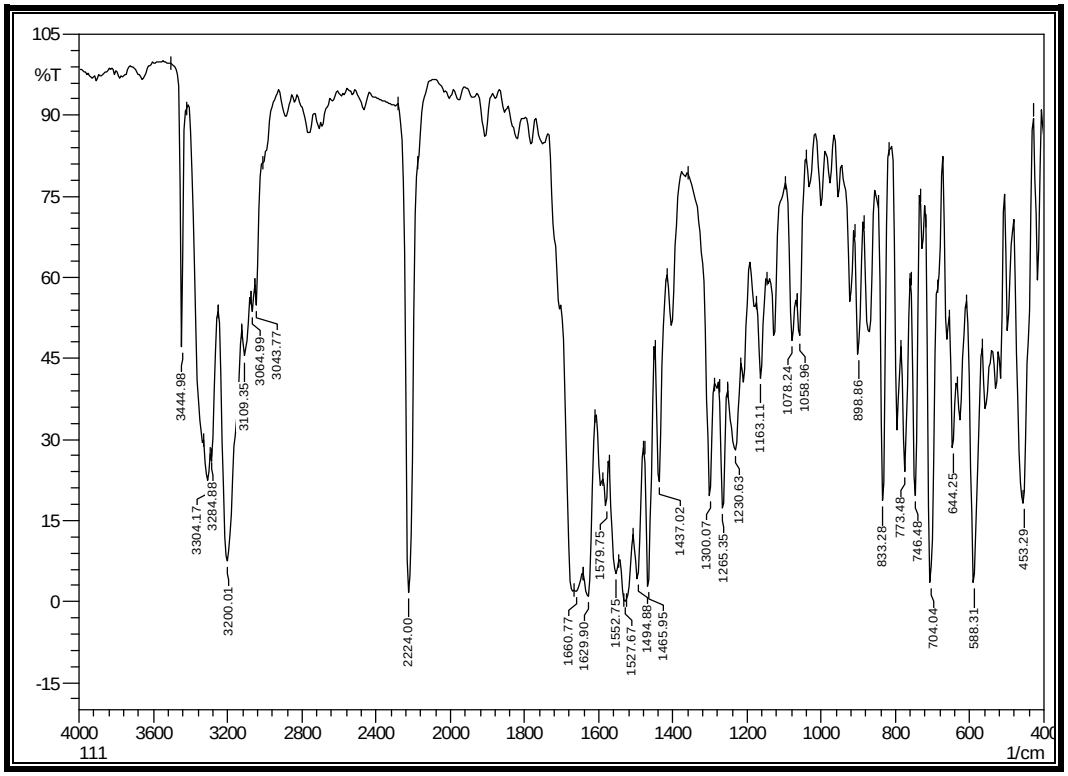
Expanded ^{1}H NMR spectrum of BHD-104



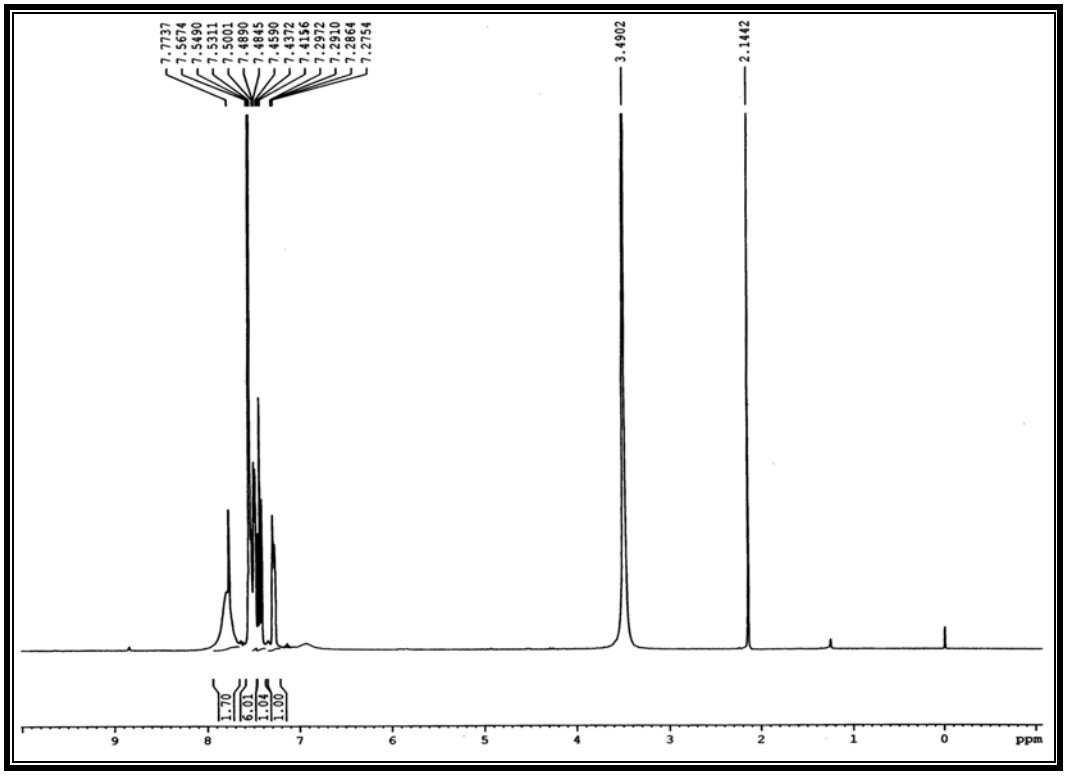
Mass spectrum of BHD-111



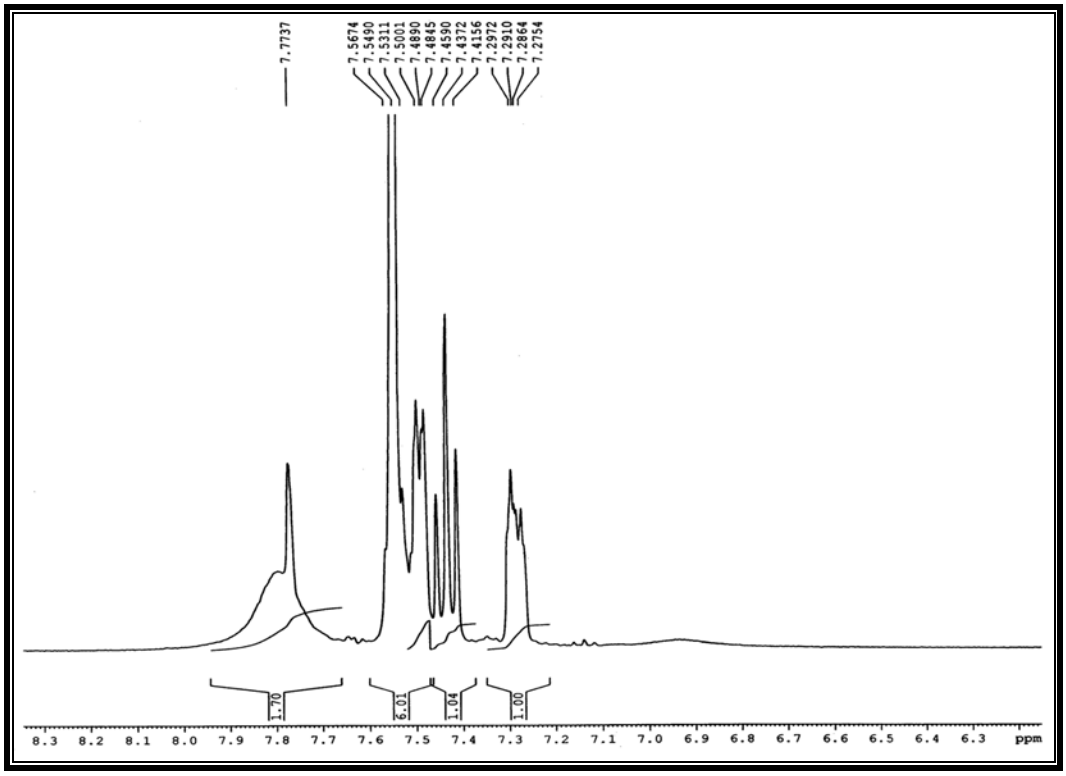
IR spectrum of BHD-111



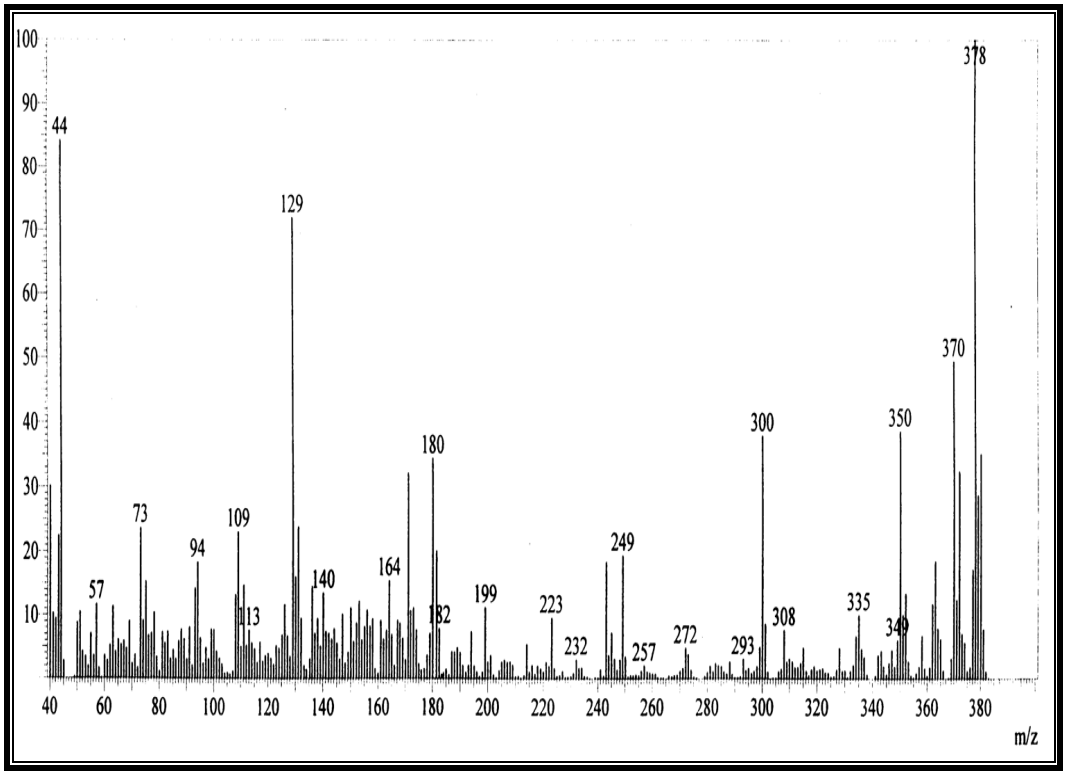
^{1}H NMR spectrum of BHD-111



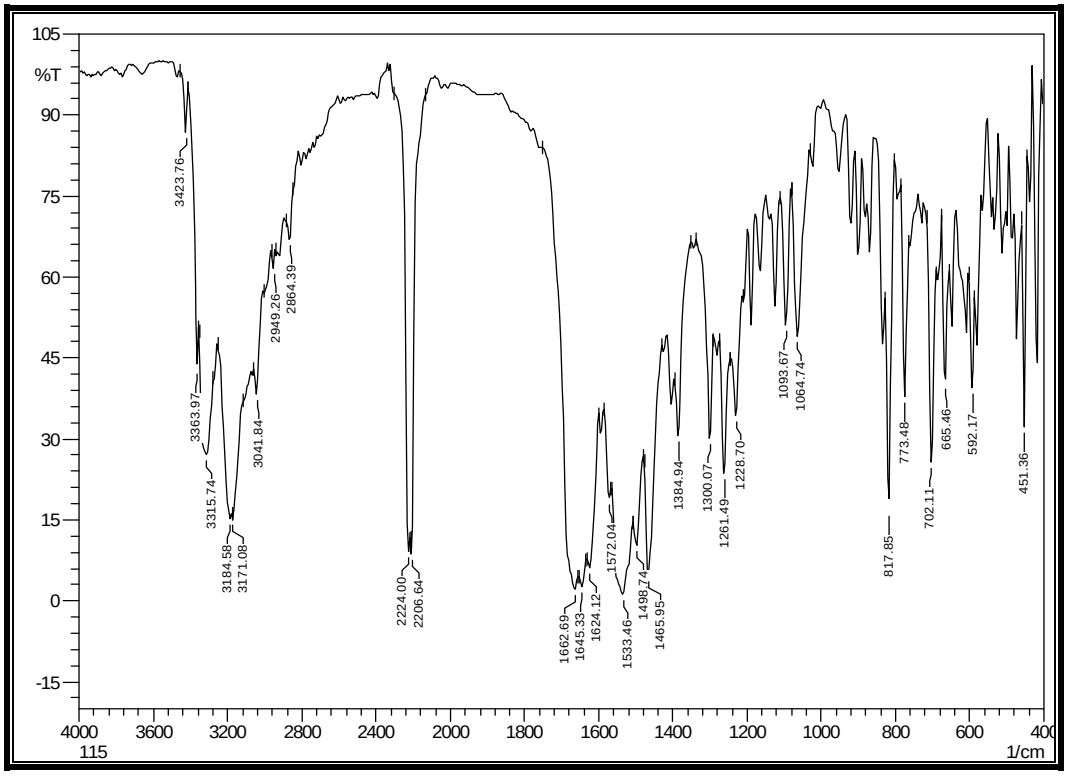
Expanded ^{1}H NMR spectrum of BHD-111



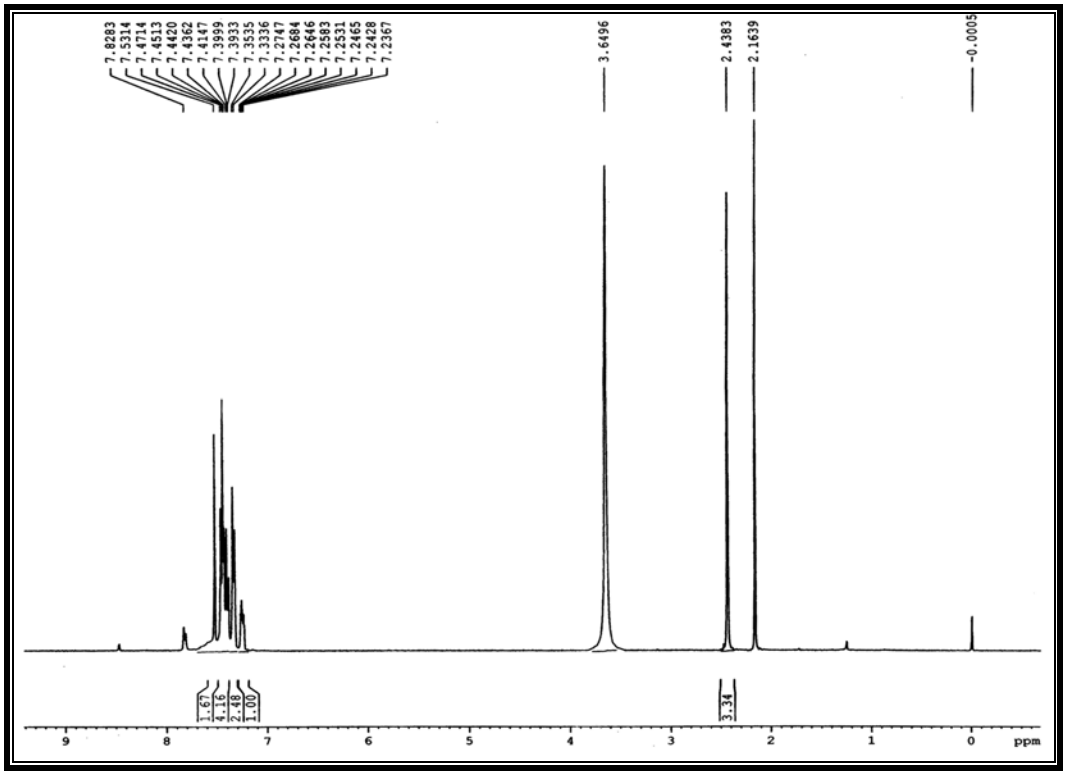
Mass spectrum of BHD-115



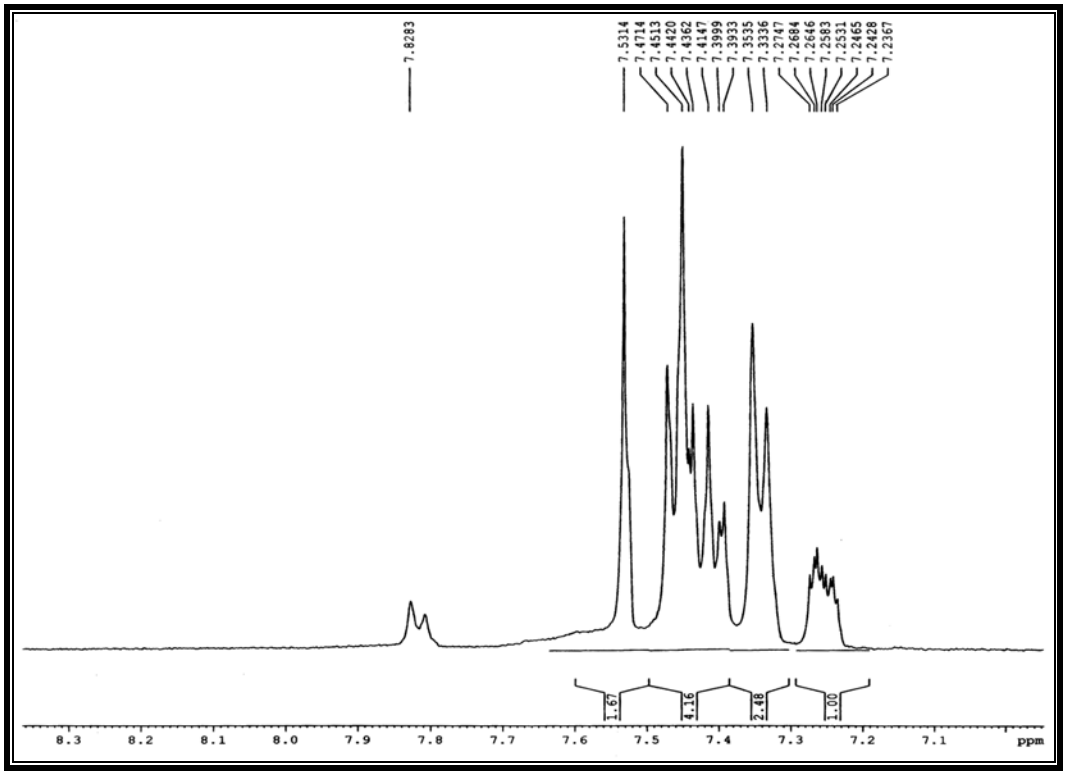
IR spectrum of BHD-115



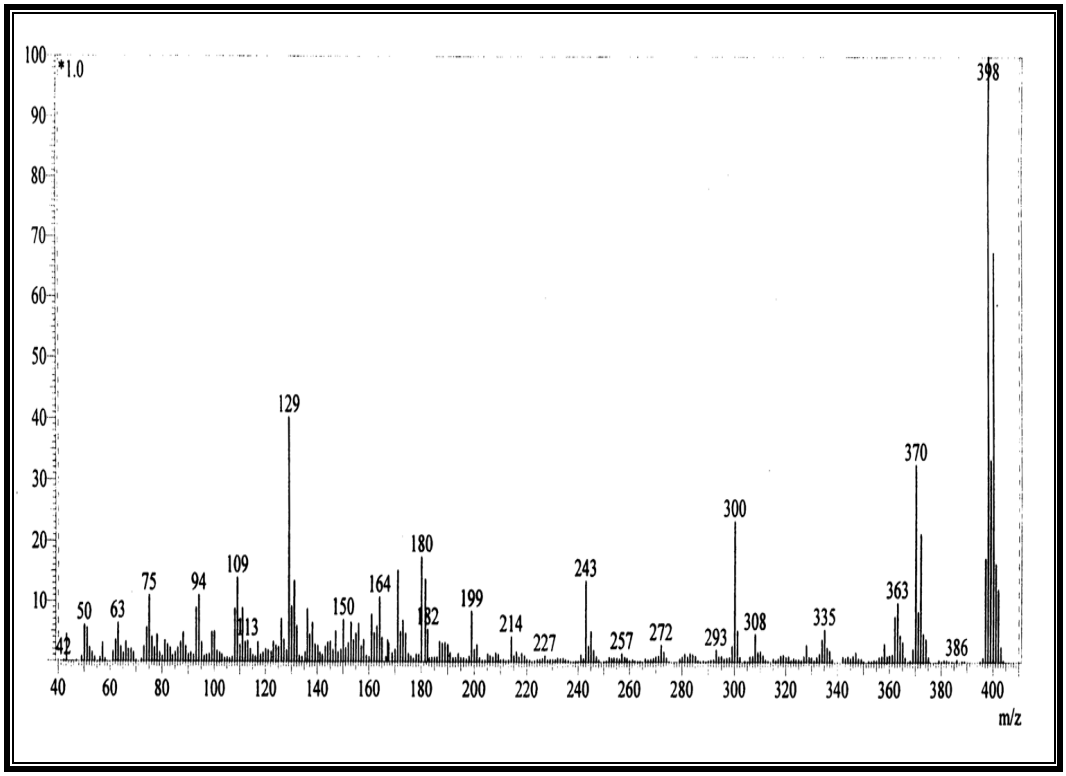
^{1}H NMR spectrum of BHD-115



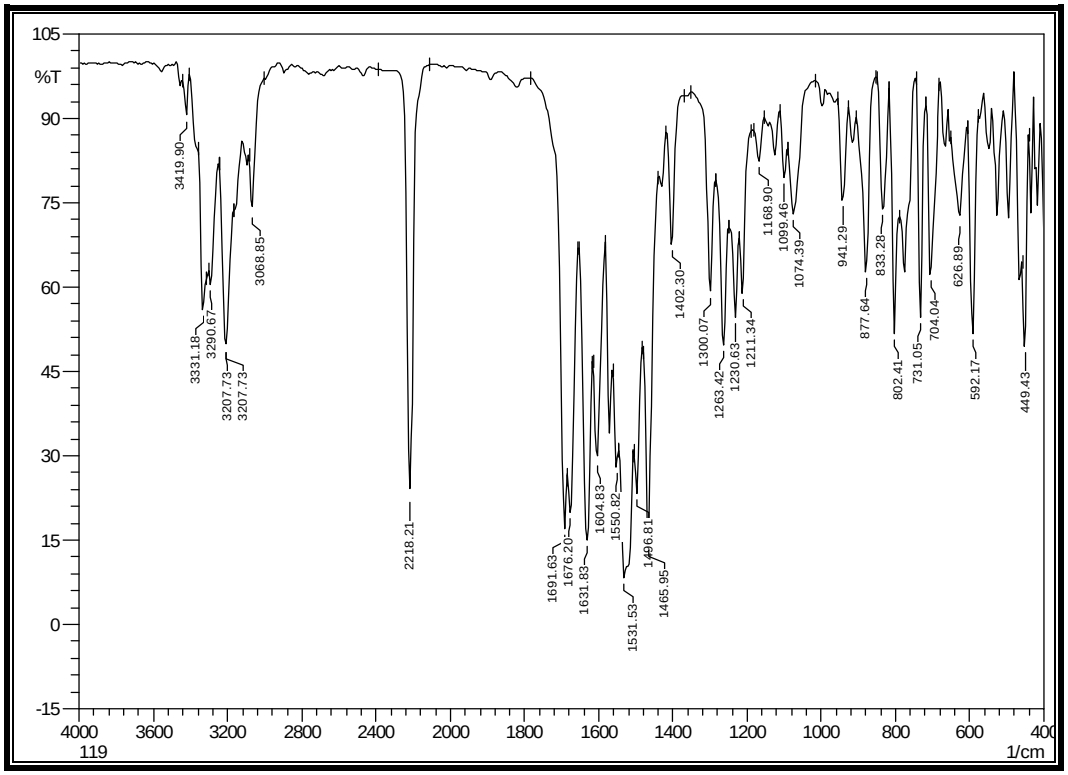
Expanded ^{1}H NMR spectrum of BHD-115



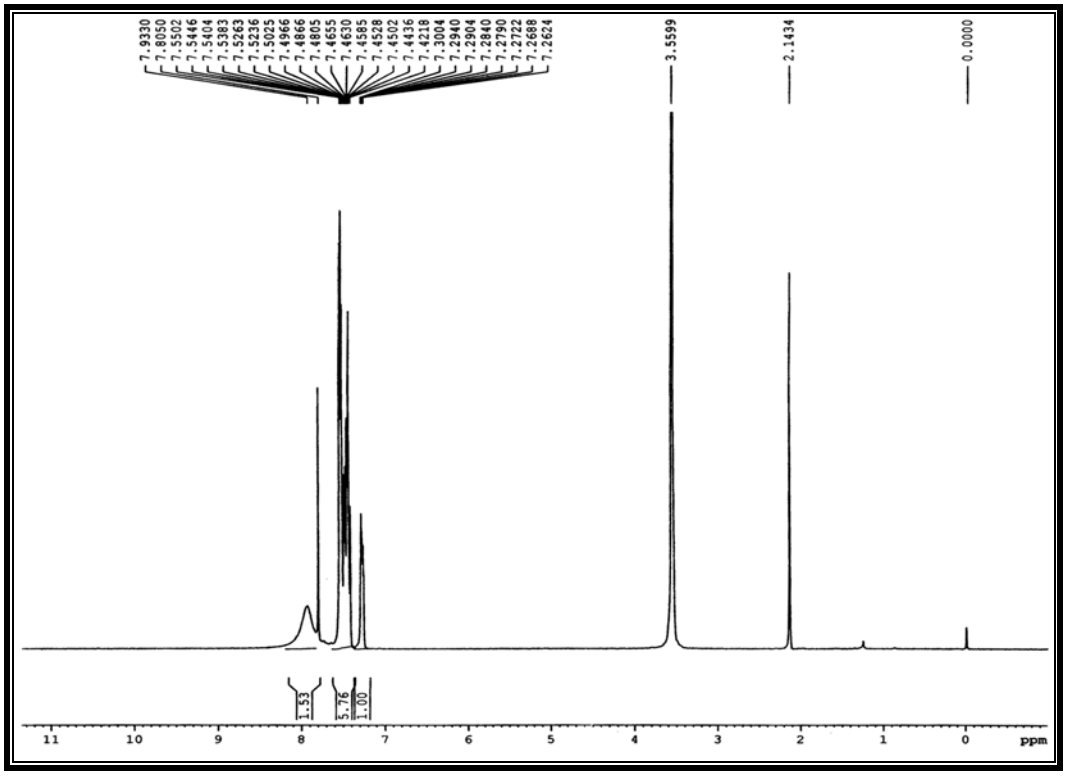
Mass spectrum of BHD-119



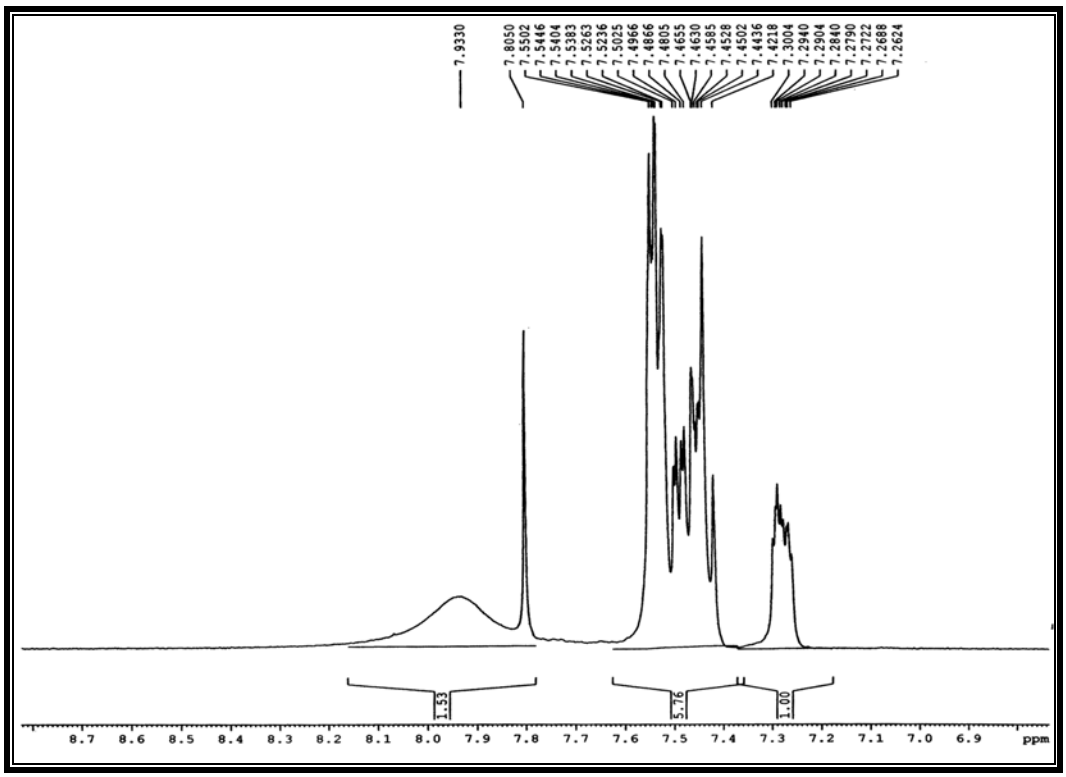
IR spectrum of BHD-119



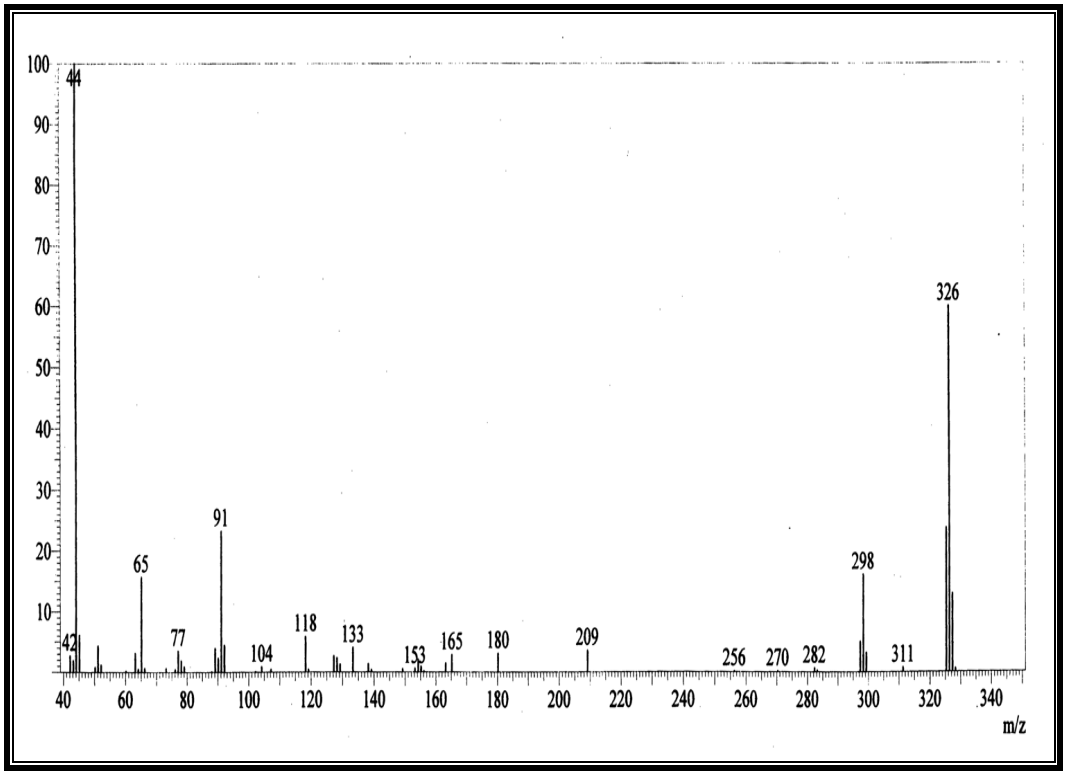
^{1}H NMR spectrum of BHD-119



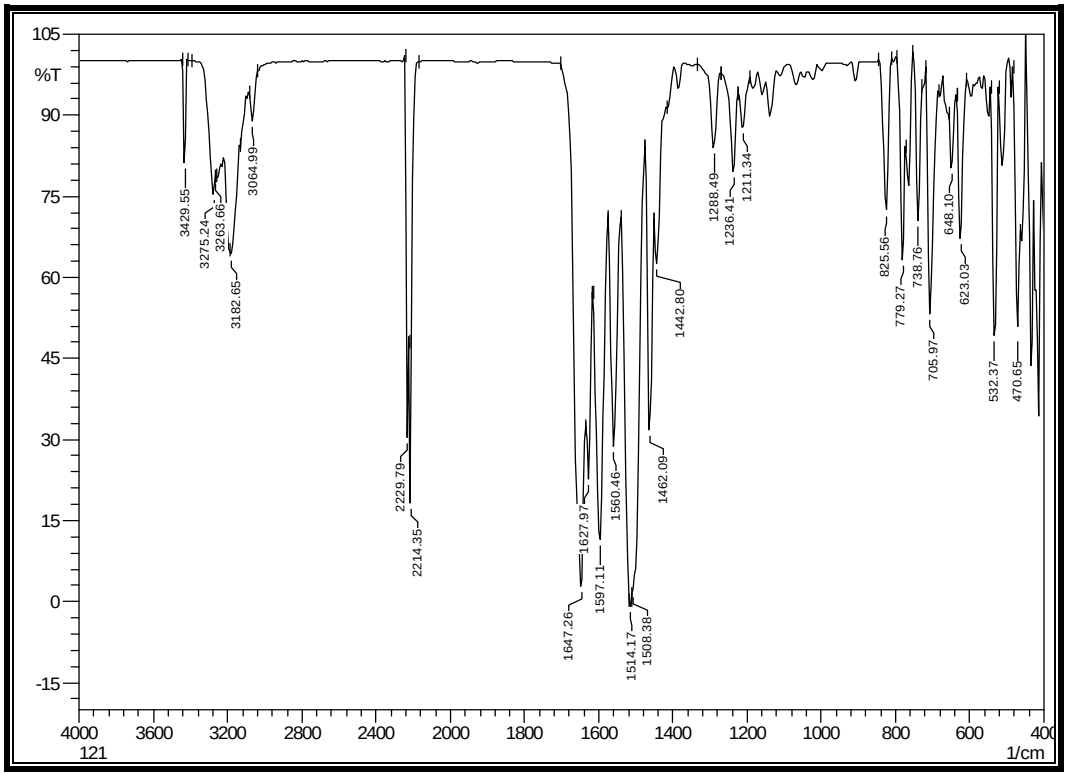
Expanded ^{1}H NMR spectrum of BHD-119



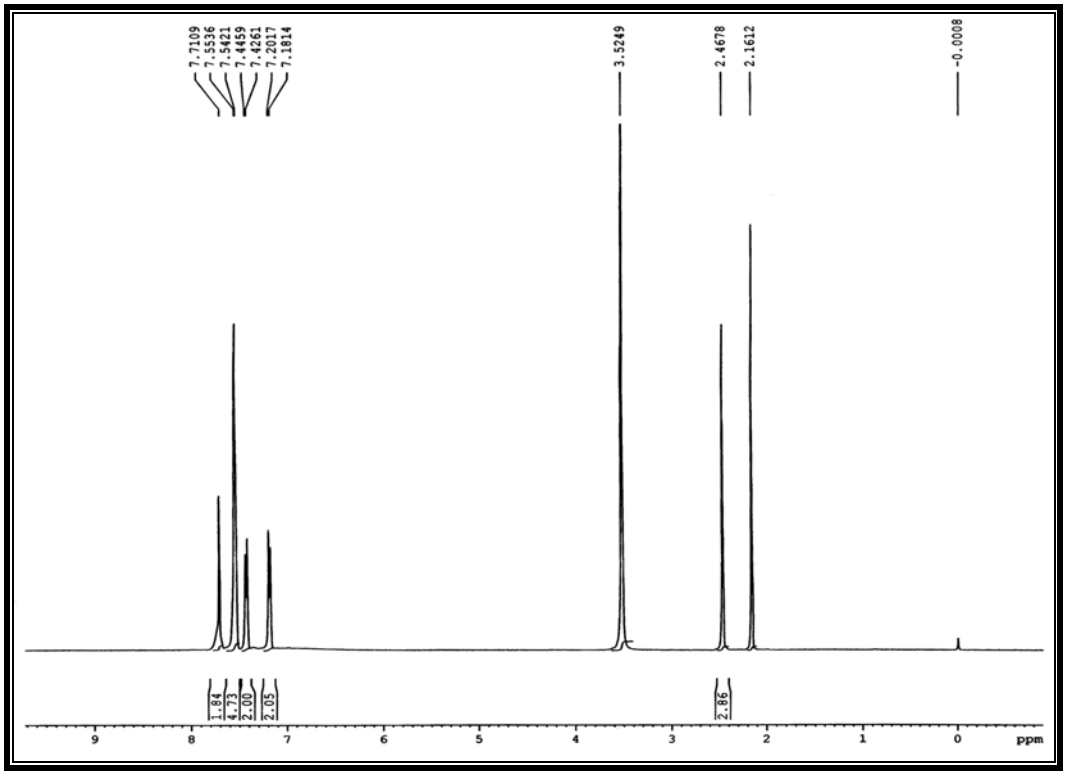
Mass spectrum of BHD-121



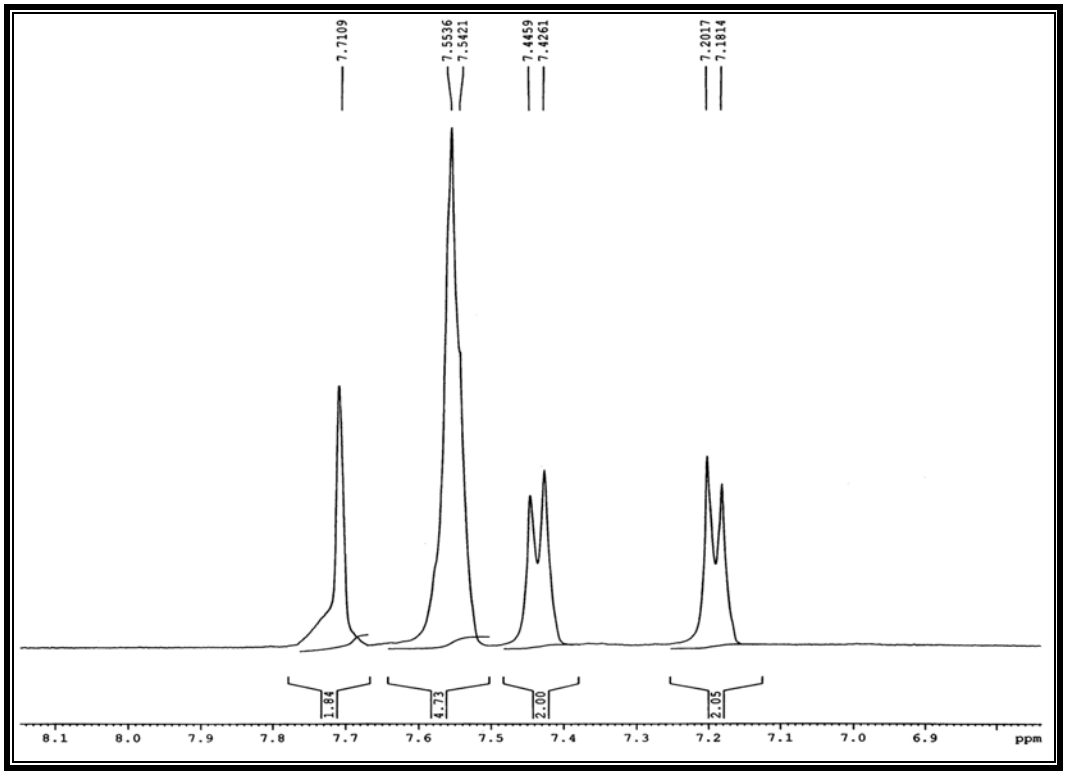
IR spectrum of BHD-121



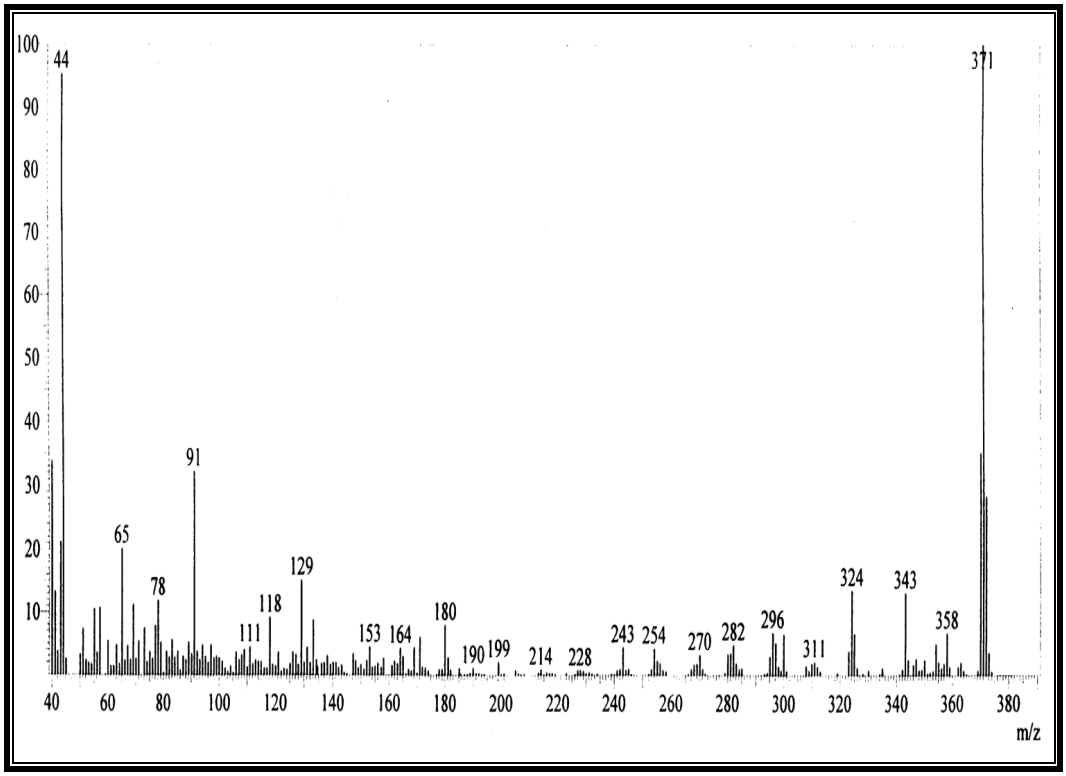
^{1}H NMR spectrum of BHD-121



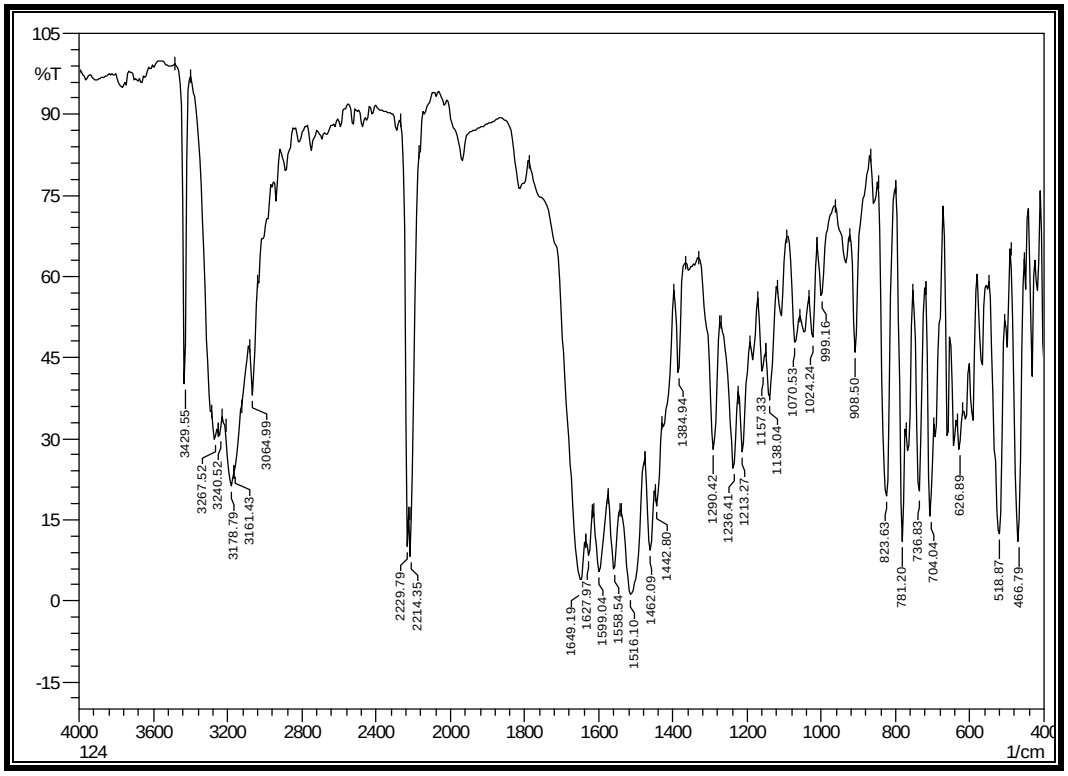
Expanded ^{1}H NMR spectrum of BHD-121



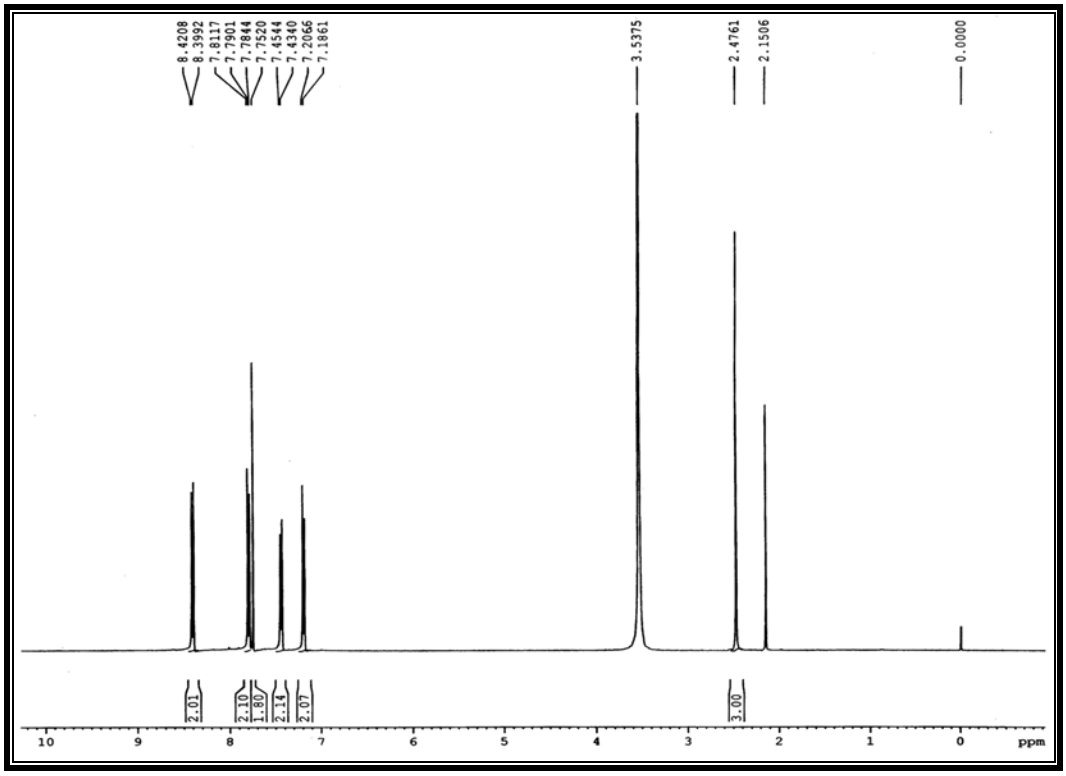
Mass spectrum of BHD-124



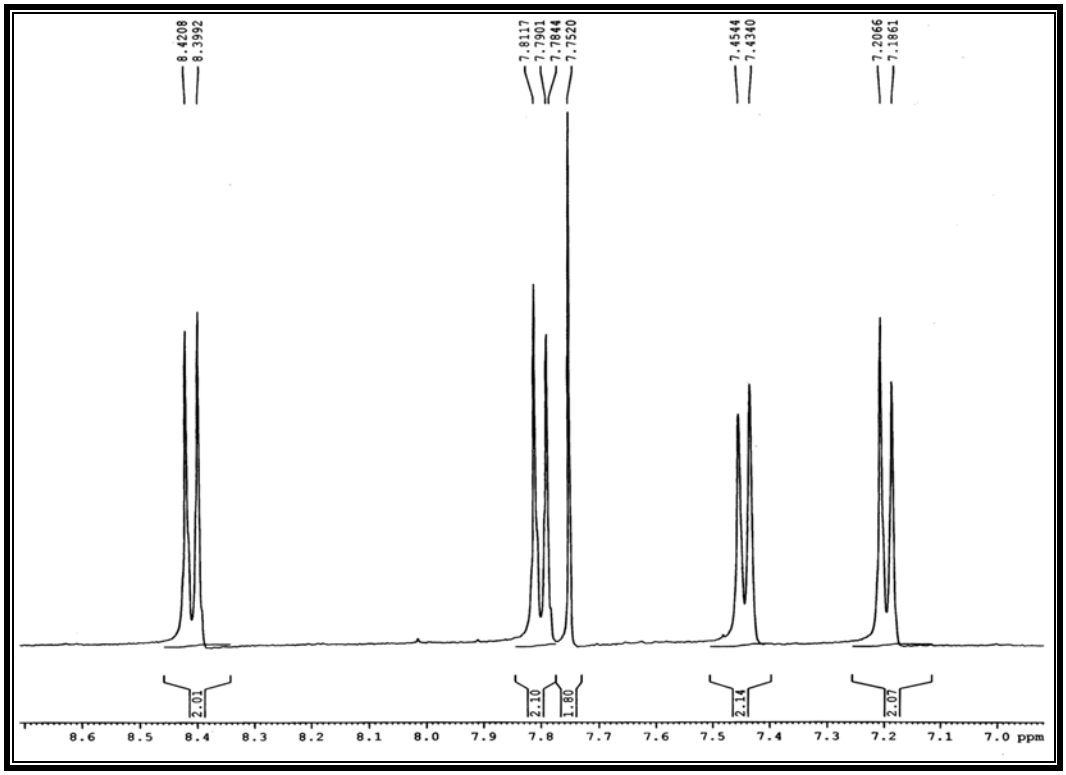
IR spectrum of BHD-124



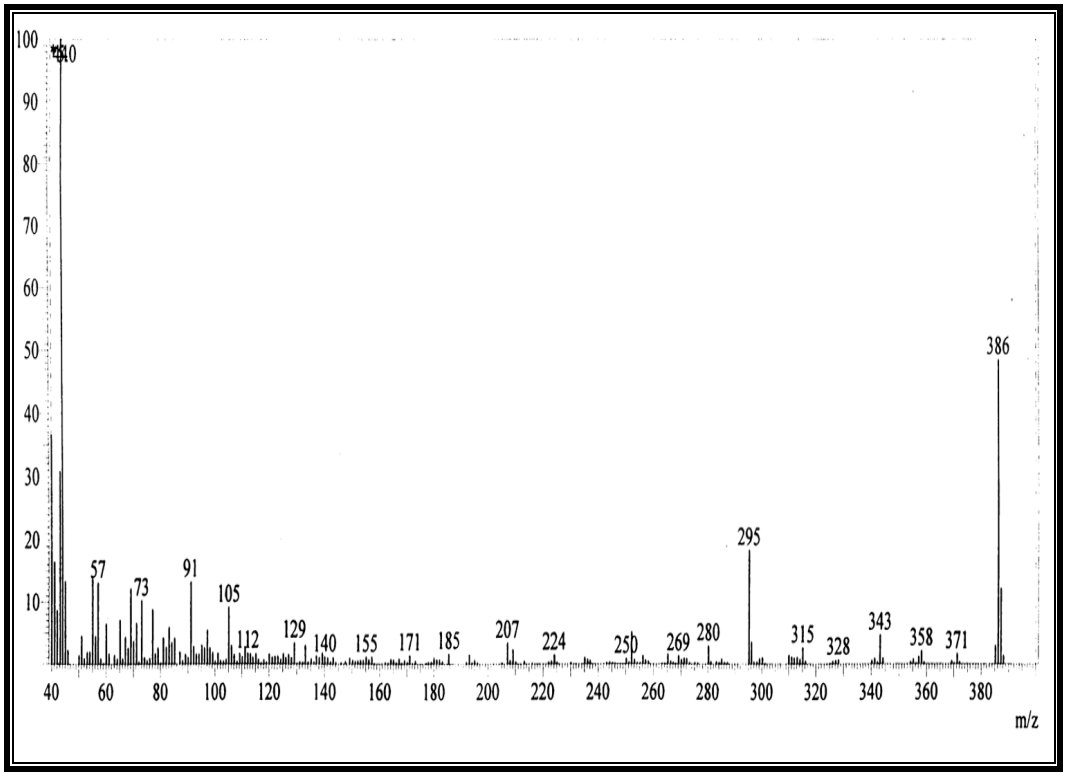
^{1}H NMR spectrum of BHD-124



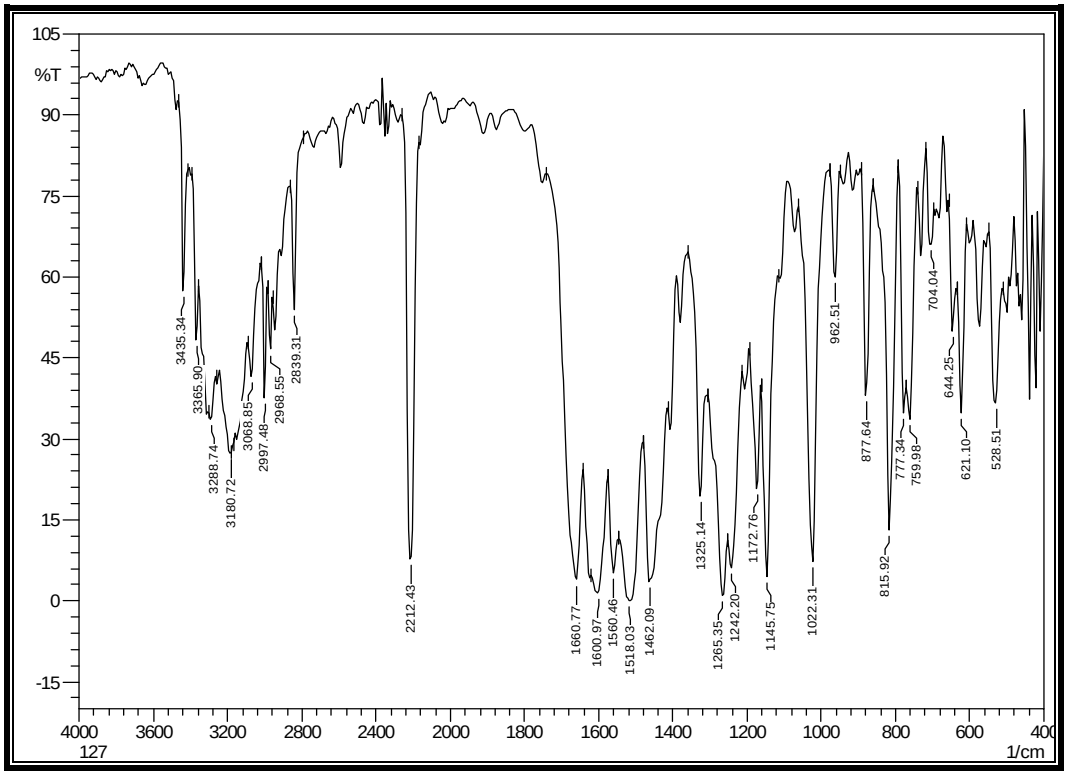
Expanded ^{1}H NMR spectrum of BHD-124



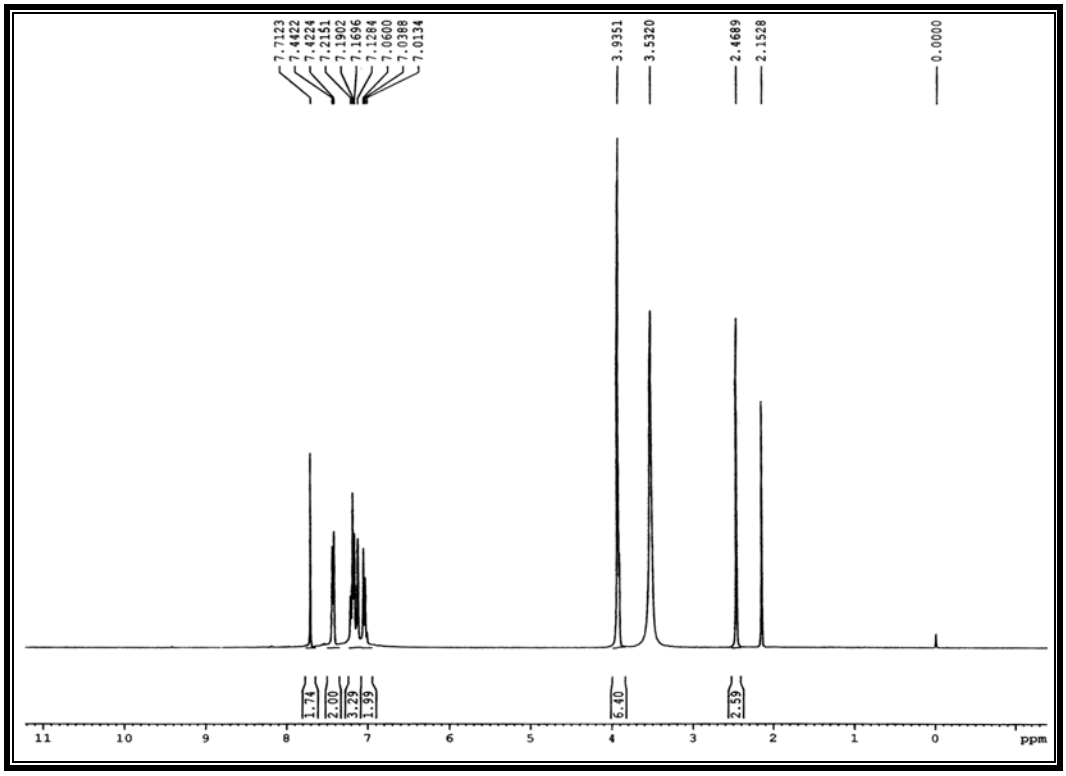
Mass spectrum of BHD-127



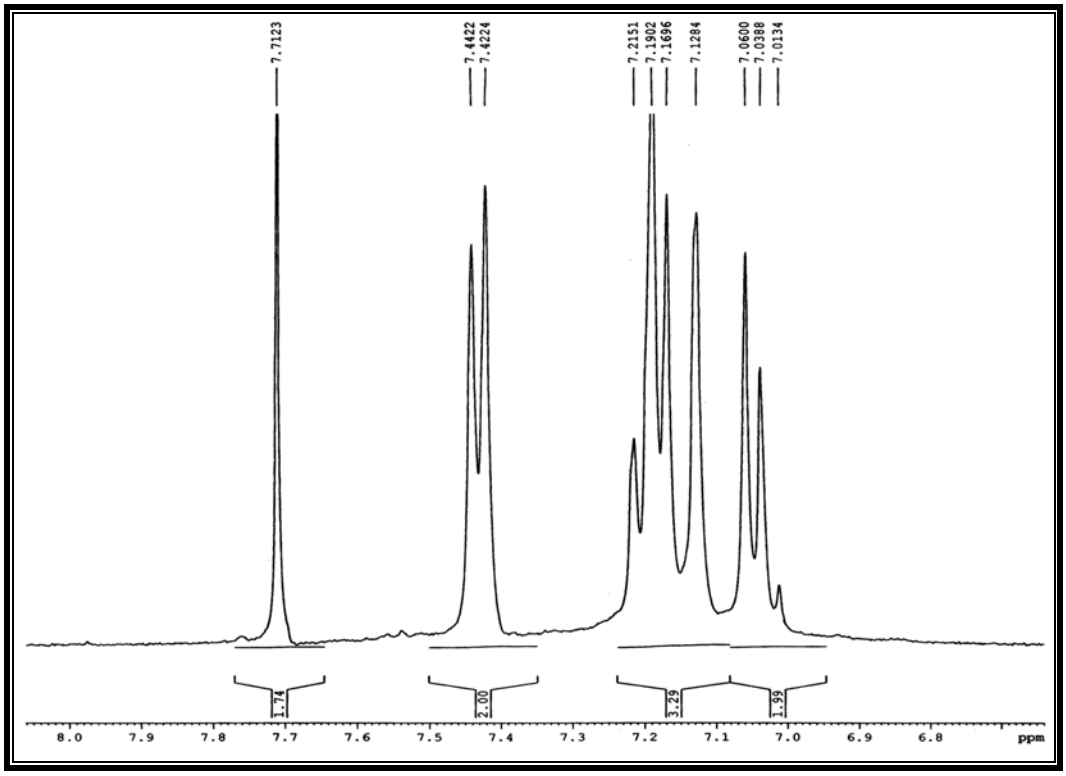
IR spectrum of BHD-127



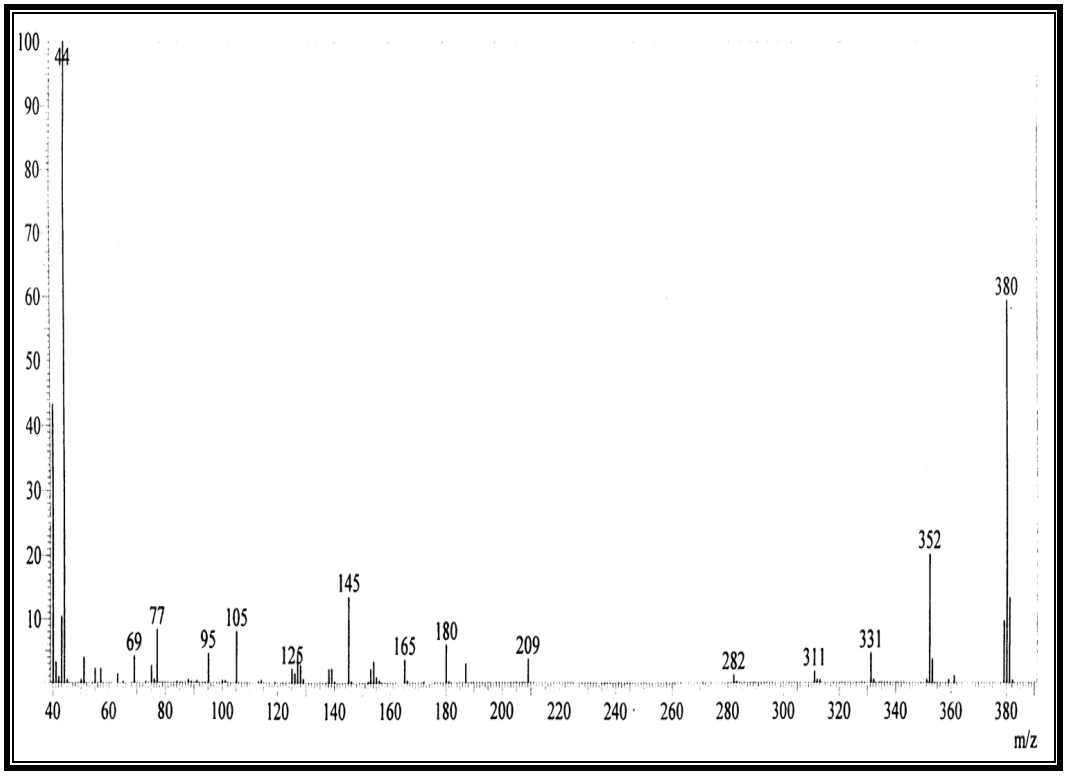
^{1}H NMR spectrum of BHD-127



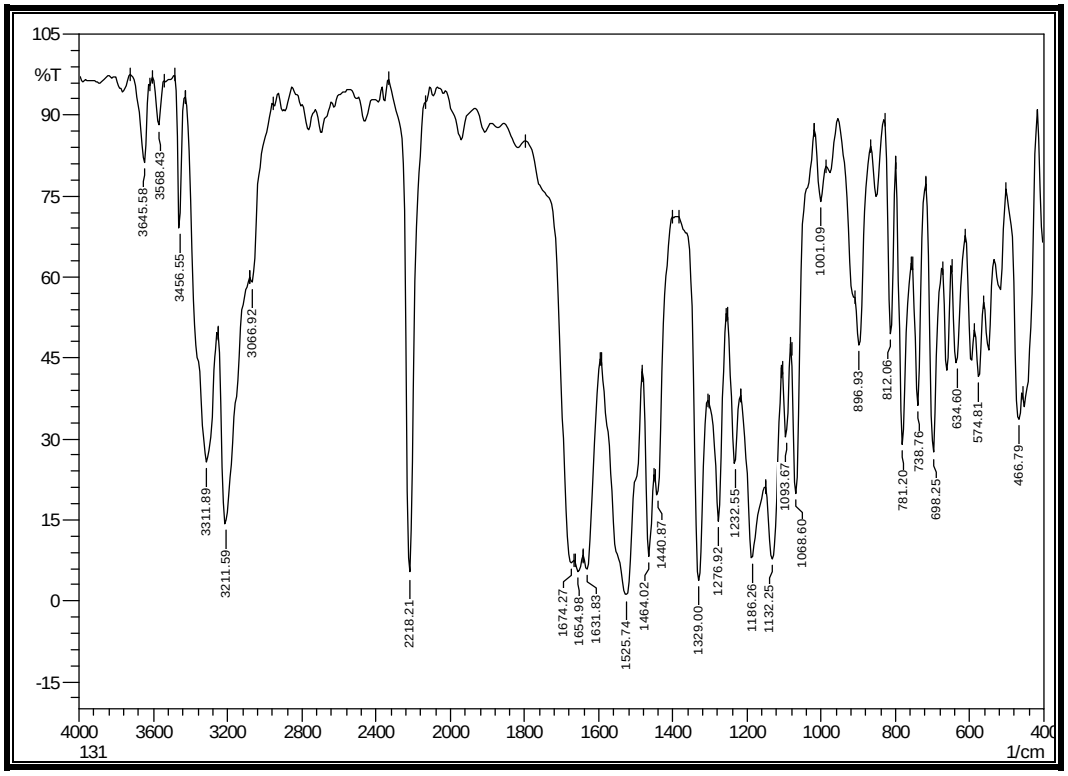
Expanded ^{1}H NMR spectrum of BHD-127



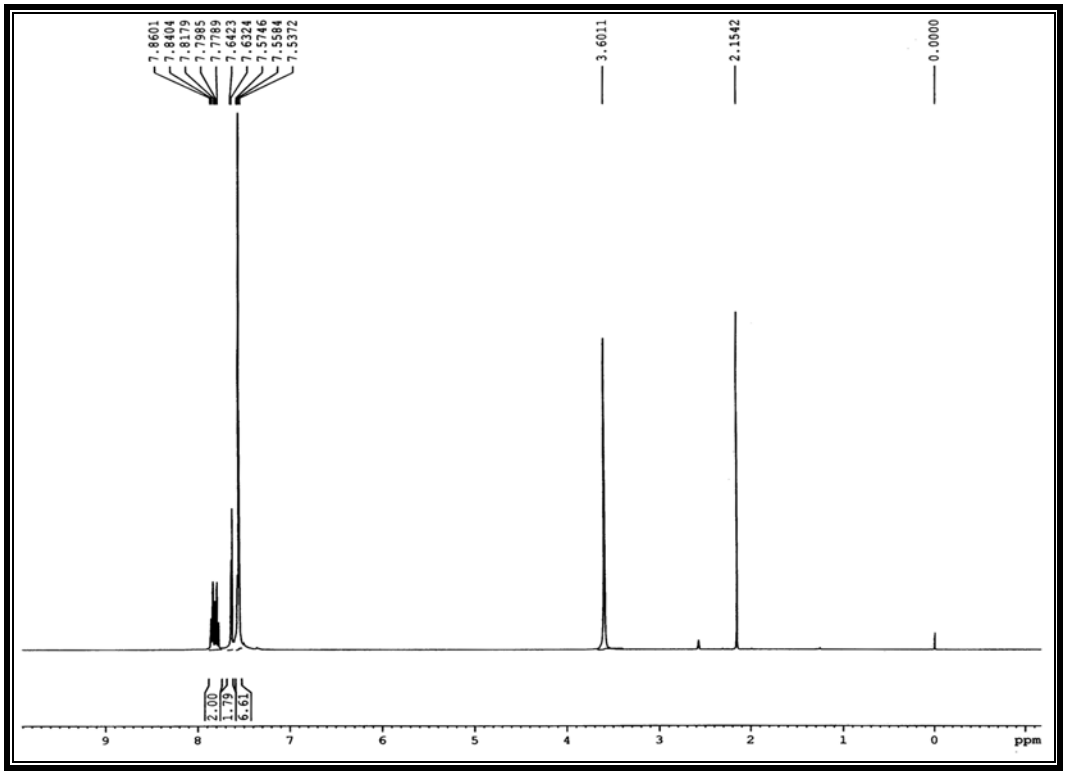
Mass spectrum of BHD-131



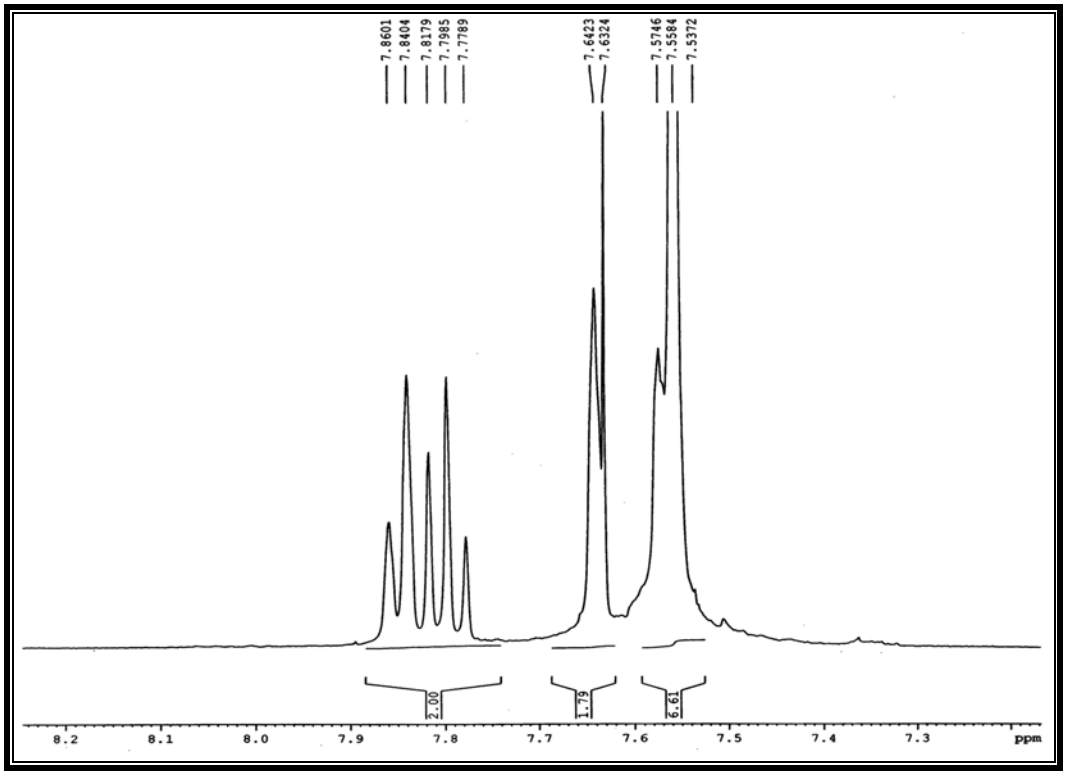
IR spectrum of BHD-131



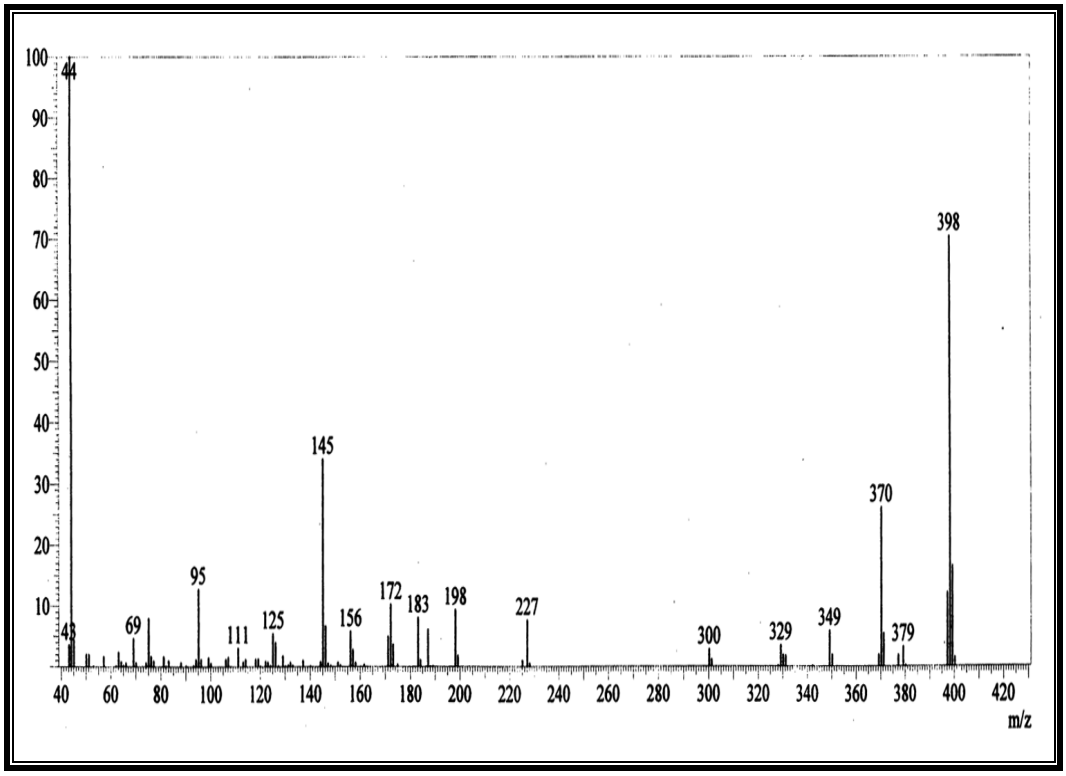
^{1}H NMR spectrum of BHD-131



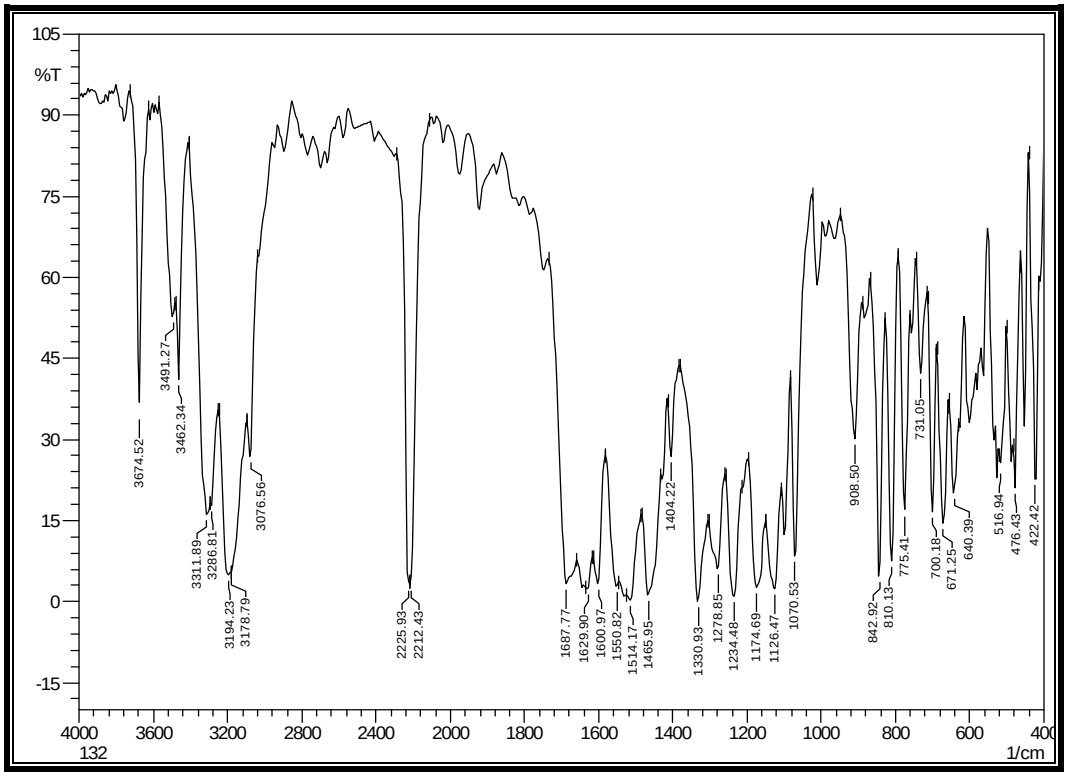
Expanded ^{1}H NMR spectrum of BHD-131



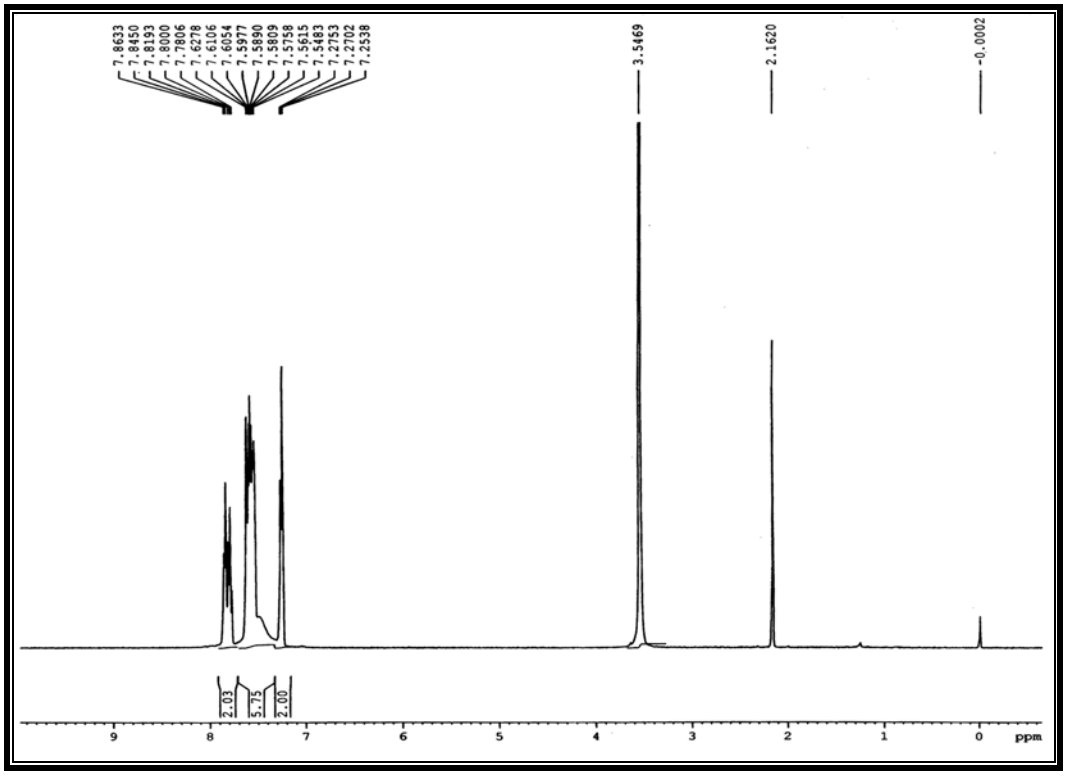
Mass spectrum of BHD-132



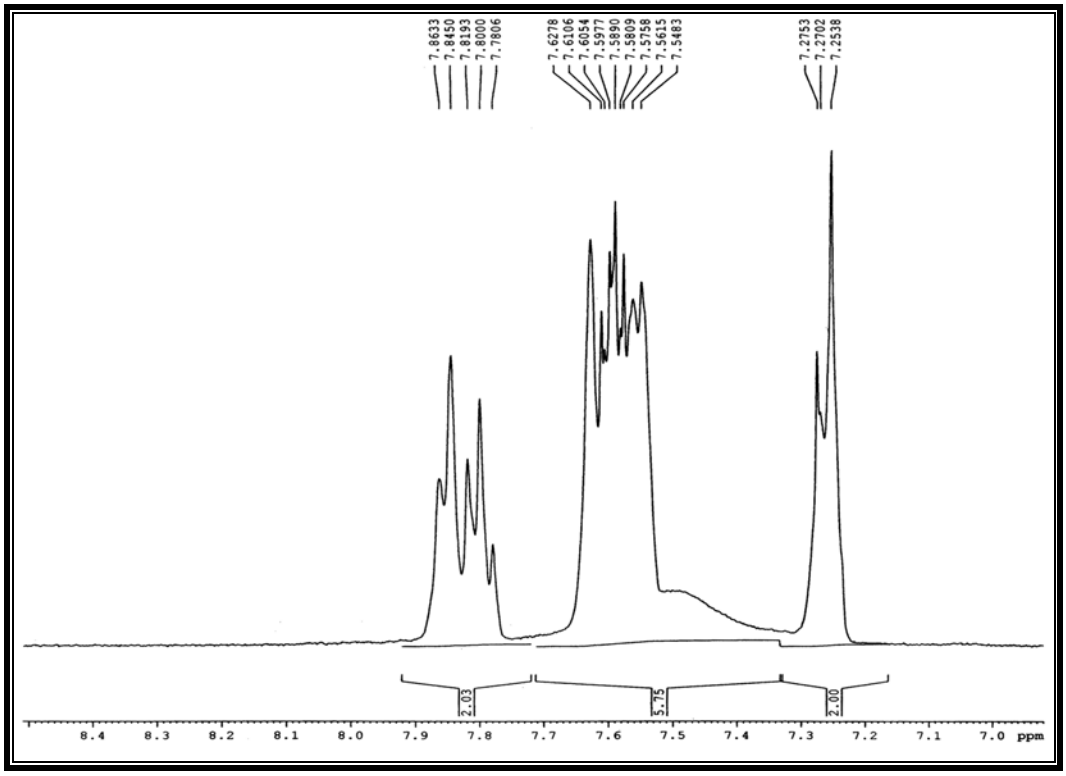
IR spectrum of BHD-132



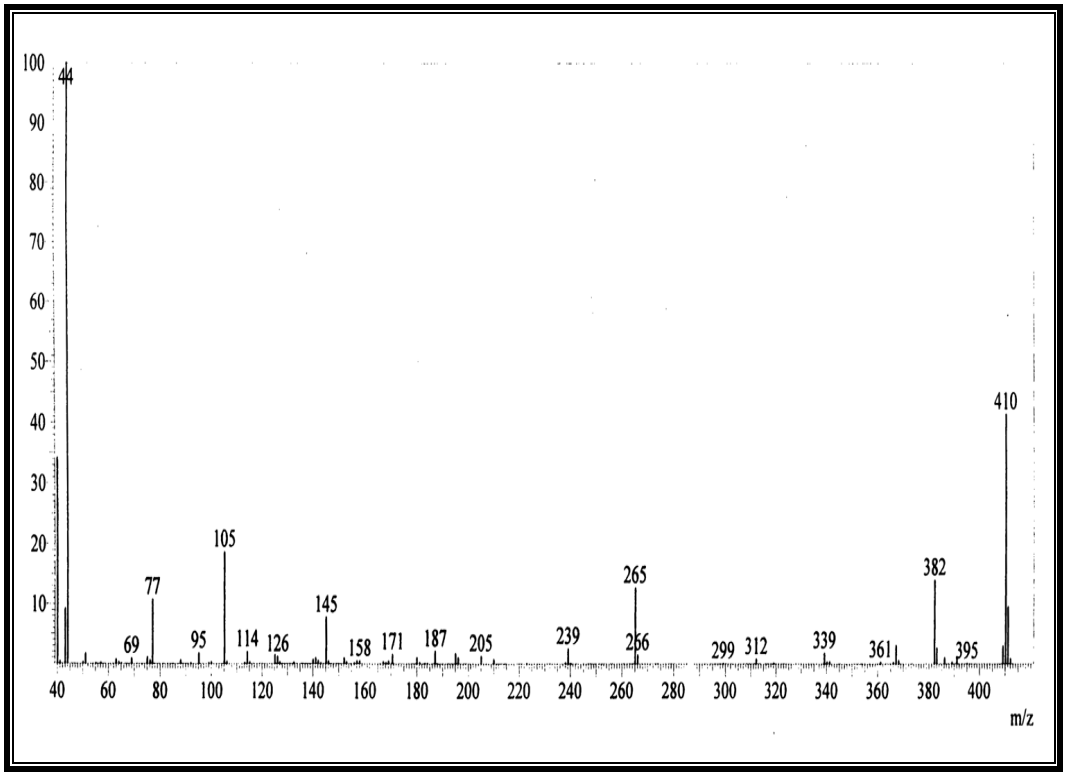
^{1}H NMR spectrum of BHD-132



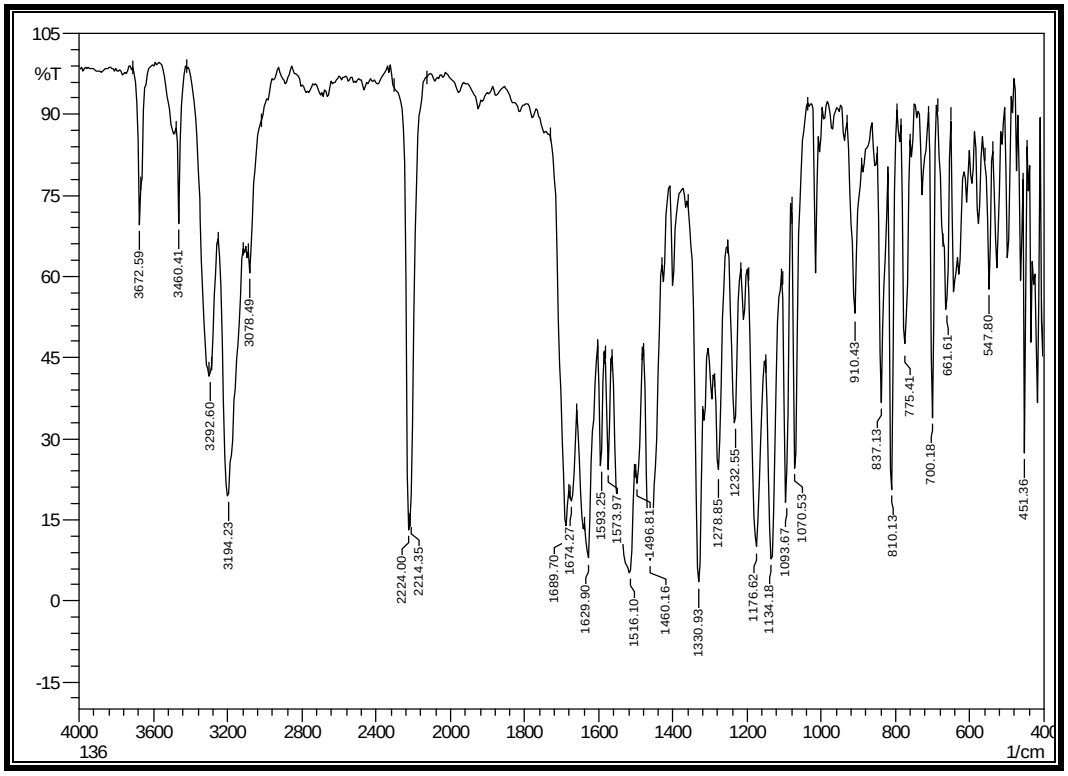
Expanded ^{1}H NMR spectrum of BHD-132



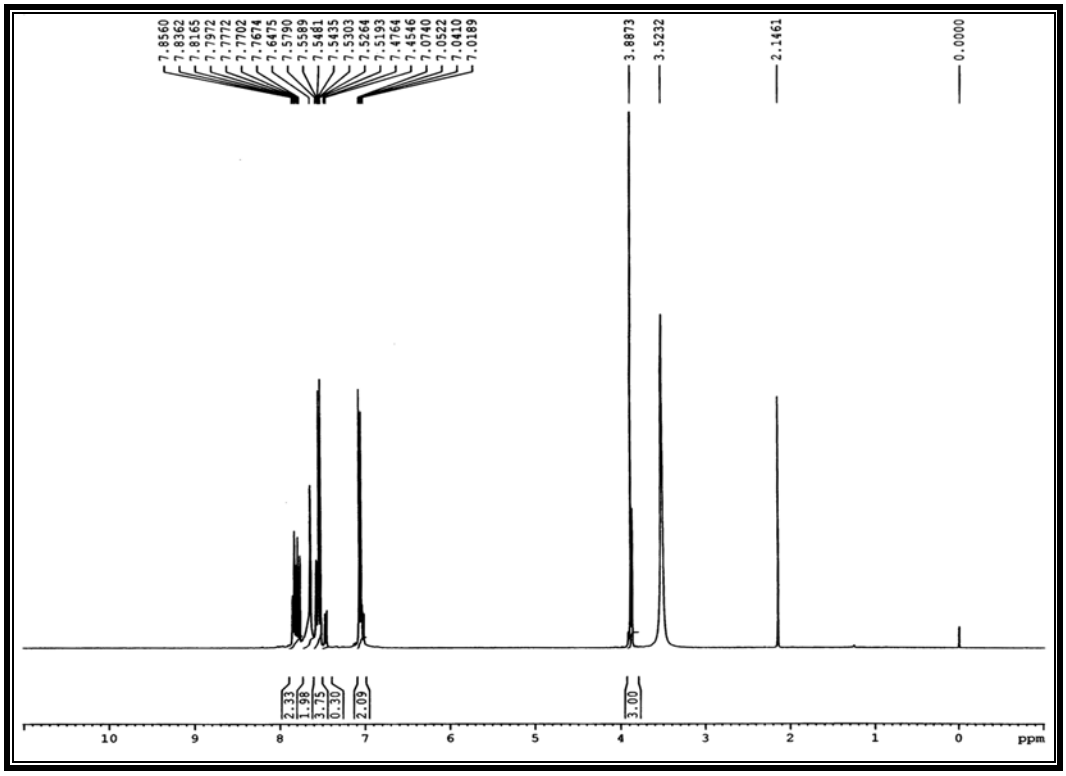
Mass spectrum of BHD-136



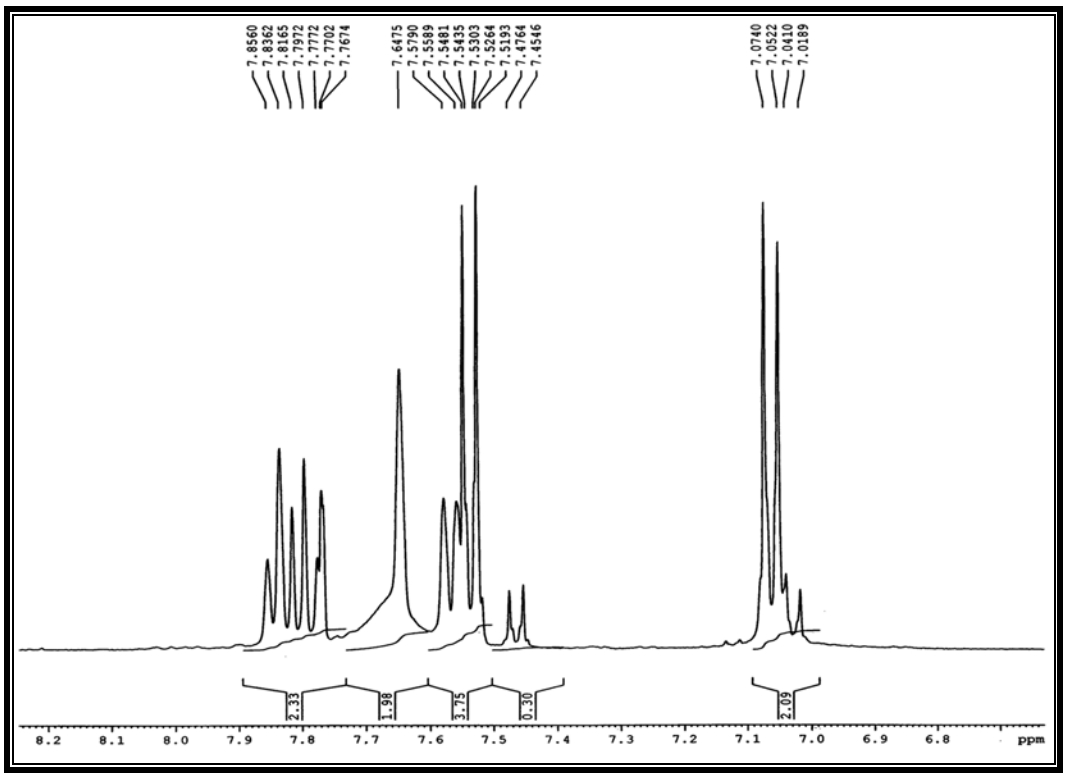
IR spectrum of BHD-136



^{1}H NMR spectrum of BHD-136



Expanded ^{1}H NMR spectrum of BHD-136



2.7 Biological evaluation

2.7.1 Antimicrobial evaluation

All the synthesized compounds (BHD-101 to BHD-140) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [102, 103] 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 greseofulvin 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 [102]. 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 200 μg mL^{-1}, 100 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1}, and 6.25 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 BHD-101 to 140

Code	Minimal 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.***
BHD-101	200	500	250	200	1000	500	500
BHD-102	200	500	200	200	500	>1000	>1000
BHD-103	100	250	62.5	100	>1000	500	>1000
BHD-104	250	250	100	250	1000	500	1000
BHD-105	250	62.5	50	100	500	250	500
BHD-106	500	200	62.5	200	250	1000	1000
BHD-107	200	500	250	250	500	1000	1000
BHD-108	500	500	200	200	1000	500	>1000
BHD-109	100	100	250	250	>1000	500	1000
BHD-110	150	100	62.5	100	500	250	500
BHD-111	200	500	250	250	>1000	>1000	>1000
BHD-112	500	250	200	100	500	1000	1000
BHD-113	250	500	250	500	>1000	1000	1000
BHD-114	250	250	100	250	500	1000	500
BHD-115	200	200	100	100	500	>1000	1000
BHD-116	250	500	500	200	>1000	500	>1000
BHD-117	200	100	250	62.5	500	500	>1000
BHD-118	500	500	200	250	500	1000	500
BHD-119	200	200	100	250	500	1000	>1000
BHD-120	200	250	100	250	>1000	>1000	1000
BHD-121	500	500	500	200	>1000	>1000	>1000
BHD-122	500	500	250	250	>1000	500	>1000
BHD-123	100	250	250	200	500	>1000	500
BHD-124	250	250	100	100	>1000	>1000	>1000
BHD-125	200	200	500	500	>1000	1000	>1000
BHD-126	500	500	250	500	>1000	>1000	>1000
BHD-127	200	200	100	200	1000	1000	250
BHD-128	250	500	200	250	500	1000	>1000
BHD-129	500	500	100	250	1000	1000	250
BHD-130	200	200	200	250	1000	1000	>1000
BHD-131	500	500	250	200	>1000	>1000	>1000
BHD-132	100	50	250	200	500	100	250
BHD-133	250	250	500	500	500	250	500
BHD-134	100	500	250	200	>100	>1000	>1000
BHD-135	500	500	250	500	250	>1000	>1000
BHD-136	250	200	150	100	500	500	250
BHD-137	200	500	250	500	250	500	>1000
BHD-138	200	100	62.5	250	1000	1000	500
BHD-139	500	250	500	500	>1000	>1000	>1000
BHD-140	250	250	500	250	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
Greseofulvin	-	-	-	-	500	100	100

2.7.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds BHD-101 to BHD-140 is currently under investigation and results are awaited.

2.8 References and notes

[1] Li, A. H.; Moro, S.; Forsyth, N.; Melman, N.; Ji, X. D.; Jacobsen, K. A. *J. Med. Chem.* **1999**, *42*, 706.

[2] Vacher, B.; Bonnand, B.; Funes, F.; Jubault, N.; Koek, W.; Assie, M. B.; Cosi, C.; Kleven, M. *J. Med. Chem.* **1999**, *42*, 1648.

[3] (a) Son, J. K.; Zhao, L. X.; Basnet, A.; Thapa, P.; Karki, R.; Na, Y.; Jahng, Y.; Jeong, T. C.; Jeong, B. S.; Lee, C. S.; Lee, E. S. *Eur. J. Med. Chem.* **2008**, *43*, 675. (b) Amr, A. G.; Abdulla, M. M.; *Bioorg. Med. Chem.* **2006**, *14*, 4341.

[4] Choi, W.; Houpis, I. N.; Charchil, H. R. O.; Molina, A.; Lynch, J. E.; Volante, R. P.; Reider, P. J.; King, A. O. *Tetrahedron* **1995**, *36*, 4571.

[5] Bhupathy, M.; Conlon, D. A.; Wells, K. M.; Wells, M.; Nolson, J. R.; Reider, P. J.; Rossen, K.; Sager, J.W.; Volante, R. P. *J. Heterocyclic Chem.* **1995**, *32*, 1283.

[6] Kappe, O. C.; Kappe, T. *Monatshefte fur Chemie* **1989**, *120*, 1095.

[7] (a) Saiki, A. Y. C.; Shen, L. L.; Chen, C. M.; Baranowski, J.; Lerner, C. G. *Antimicrob. Agents Chemother.* **1999**, *43*, 1574; (b) Li, Q.; Mitscher, L. A.; Shen, L. L. *Med. Res. Rev.* **2000**, 231.

[8] Brickner, S. *Chem. Ind.* **1997**, 131.

[9] (a) Goldman, M. E.; Nunberg, J. H.; O'Brien, J. A.; Quintero, J. C.; Schleif, W. A.; Freund, K. F.; Gaul, S. L.; Saari, W. S.; Wai, J. S.; Hoffman, J. M.; Anderson, P. S.; Hupe, D. J.; Emini, E. A.; Stern, A. M. *Proc. Natl. Acad. Sci.* **1991**, *88*, 6863; (b) Medina-Franco, J. L.; Martínez-Mayorga, K.; Juárez-Gordiano, C.; Castillo, R. *Chem. Med. Chem.* **2007**, *2*, 1141.

[10] (a) Li, Q.; Mitscher, L. A.; Shen, L. L. *Med. Res. Rev.* **2000**, *20*, 231; (b) Holzgrabe, U. *Pharm. Unserer Zeit.* **2001**, *30*, 446.

[11] Gupta, A. K.; Plott, T. *Int. J. Dermatol.* **2004**, *43*, 3.

[12] Collins, I.; Moyes, C.; Davey, W.; Rowley, M.; Bromidge, F.; Quirk, K.; Atack, J.; McKernan, R.; Thompson, S. A.; Wafford, K.; Dawson, G.; Pike, A.; Sohal, B.; Tsou, N.; Bull, R.; Castro, J. *J. Med. Chem.* **2002**, *45*, 1887.

[13] (a) Endoh, M.; Hori, M. *Exp. Opin. Pharmacother.* **2006**, *7*, 2179; (b) Robert, N.; Verrier, C.; Hoarau, C.; Celanire, S.; Marsais, F. *Arkivoc* **2008**, *7*, 92; (c) Presti, E. L.; Boggia, R.; Feltrin, A.; Menozzi, G.; Dorigo, P.; Mosti, L. *Il Farmaco* **1999**, *54*, 465.

[14] Sollogoub, M.; Fox, K. R.; Powers, V. E. C.; Brown, T. *Tetrahedron Lett.* **2002**, *43*, 3121; (b) Seidel, A.; Brunner, S.; Seidel, P.; Fritz, G. I.; Herbarth, O. *British J. Cancer* **2006**, *94*, 1726; (c) Sun, Z.; Ahmed, S.; McLaughlin, L. W. *J. Org. Chem.* **2006**, *71*, 2922; (d) Yang, Z.; Hutter, D.; Sheng, P.; Sismour, A. M.; Benner, S. A. *Nucl. Acids Res.* **2006**, *34*, 6095; (e) Rajeswaran, M.; Srikrishnan, T. *Nucleosides, Nucleotides and Nucleic Acids* **2008**, *27*, 1113.

[15] Parsons, S.; Winpenny, R. E. P. *Acc. Chem. Res.* **1997**, *30*, 89.

[16] Kawato, Y.; Terasawa, H. *Prog. Med. Chem.* **1997**, *34*, 69.

[17] Murray, T.; Zimmerman, S. *Tetrahedron Lett.* **1995**, *36*, 7627.

[18] Werbitzky, O.; Studer, P. *U.S. Patent* 6,022,974, Feb 9, **2000**.

[19] Harrison, T.; Moyes, C. R.; Nadin, A.; Owens, A. P.; Lewis, R. T. *PCT WO* 98/50384.

[20] Gary, P.; Soh, J. W.; Mao, Y.; Kim, M. G.; Pamukcu, R.; Li, H.; Thompson, W. J.; Weinstein, I. B. *Clin. Cancer Res.* **2000**, *6*, 4136.

[21] Cheng, J.; Grande, J. P. *Exp. Biol. Med.* **2007**, *232*, 38.

[22] Murata, T.; Shimizu, K.; Narita, M.; Manganiello, V.; Tagawa, T. *Anticancer Res.* **2002**, *22*, 3171.

[23] Murata, T.; Sugatani, T.; Shimizu, K.; Manganiello, V.; Tagawa, T. *Anticancer Drugs* **2001**, *12*, 79.

[24] Moon, E.; Lee, R.; Near, R.; Weintraub, L.; Wolda, S.; Lerner, A. *Clin. Cancer Res.* **2002**, *8*, 589.

[25] Shimizu, K.; Murata, T.; Okumura, K.; Manganiello, V. C.; Tagawa, T. T. *Anticancer Drugs* **2002**, *13*, 875.

[26] Cheney, I. W.; Yan, S.; Appleby, T.; Walker, H.; Vo, T.; Yao, N.; Hamatake, R.; Hong, Z.; Wu, J. Z. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1679.

[27] Wendt, M. D.; Sun, C.; Kunzer, A.; Sauer, D.; Sarris, K.; Hoff, E.; Yu, L.; Nettesheim, D. G.; Chen, J.; Jin, S.; Comess, K. M.; Fan, Y.; Anderson, S. N.; Isaac, B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3122.

[28] Aqui, N. A.; Vonderheide, R. H. *Cancer Biol. Ther.* **2008**, *7*, 1888.

[29] Ambrosini, G.; Adida, C.; Altieri, D. C. *Nat. Med.* **1997**, *3*, 917.

[30] (a) Hardie, D. G. *Endocrinology* **2003**, *144*, 5179; (b) Hardie, D. G.; Scott, J. W.; Pan, D. A.; Hudson, E. R. *FEBS Lett.* **2003**, *546*, 113; (c) Kahn, B. B.;

Alquier, T.; Carling, D.; Hardie, D. G. *Cell Metab.* **2005**, *1*, 15; (d) Hawley, S. A.; Boudeau, J.; Reid, J. L.; Mustard, K. J.; Udd, L.; Makela, T. P.; Alessi, D. R.; Hardie, D. G. *J. Biol.* **2003**, *2*, 28.

[31] (a) Carling, D.; Zammit, V. A.; Hardie, D. G. *FEBS Lett.* **1987**, *223*, 217; (b) McGarry, J. D.; Brown, N. F. *Eur. J. Biochem.* **1997**, *244*, 1; (c) Saha, A. K.; Schwarsin, A. J.; Roduit, R.; Masse, F.; Kaushik, V.; Tornheim, K.; Prentki, M.; Ruderman, N. B. *J. Biol. Chem.* **2000**, *275*, 24279; (d) Hardie, D. G.; Hawley, S. A. *Bioessays* **2001**, *23*, 1112.

[32] (a) Winder, W. W.; Hardie, D. G. *Am. J. Physiol.* **1999**, *277*, E1; (b) Moller, D. E.; Kaufman, K. D. *Annu. Rev. Med.* **2005**, *56*, 45; (c) Schimmack, G.; DeFronzo, R. A.; Musi, N. *Diab. Obes. Metab.* **2006**, *8*, 591.

[33] Zhao, G.; Iyengar, R. R.; Judd, A. S.; Cool, B.; Chiou, W.; Kifle, L.; Frevert, E.; Sham, H.; Kym, P. R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3254.

[34] Mosti, L.; Menozzi, G.; Schenone, P.; Dorigo, P.; Gaion, R. M.; Belluco, P. *Farmaco* **1992**, *47*, 427.

[35] Beheshtia, Y. S.; Khorshidi, M.; Heravi, M. M.; Baghernejad, B. *Bull. Chem. Soc. Ethiop.* **2010**, *24*, 433.

[36] Abadi, A. H.; Abouel-Ella, D. A.; Lehmann, J.; Tinsley, H. N.; Gary, B. D.; Piazza, G. A.; Abdel-Fattah, M. A. O. *Eur. J. Med. Chem.* **2010**, *45*, 90.

[37] Abdel-Latif, F. F.; Mekheimer, R.; Ahmed, E. K.; Abdel-Aleem, T. B. *Pharmazie* **1993**, *48*, 736.

[38] Elmoghayar, M. R. H.; Elagamey, A. G. A.; Nasr, M. Y. A.; Sallam, M. M. M. *J. Heterocycl. Chem.* **1984**, *21*, 1885.

[39] Hussein, A. H. M. *Heteroatom Chem.* **1997**, *8*, 1.

[40] Sakurai, A.; Midorikawa, H. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 430–432.

[41] Zoorob, H. *Z. Naturforsch.* **1976**, *31*, 1680.

[42] Abdalla, M.; Essawy, A.; Deeb, A. *Pakistan J. Sci. Ind. Res.* **1977**, *20*, 139.

[43] Karabasanagauda, T.; Adhikari, A. V.; Parameshwarappa,G. *J. Serb. Chem. Soc.* **2009**, *74*, 733.

[44] Yongmin, Z. Y. *J. Chem. Res.* **2002**, *6*, 288.

[45] Barat, C. *J. Indian Chem. Soc.* **1930**, *7*, 321.

[46] Al-Arab, M. M. *J. Heterocycl. Chem.* **1990**, *27*, 523.

[47] Yousif, M. Y.; Eisa, H. M.; Tayel, M. A.; Metwally, M. A. *Pakistan J. Sci. Ind. Res.* **1990**, *33*, 371.

[48] Chase, B. H.; Walker, J. *J. Chem. Soc.* **1953**, 3548.

[49] Kametani, T.; Ogasawara, K.; Kozuka, A.; Sato, K. *Yakugaku Zasshi* **1967**, *87*, 1189.

[50] Al-Najjar, A. Z.; Abdelkhalik, M. M.; Al-Enezi, A.; Elnagdi, M. H. *Molecules* **2009**, *14*, 68.

[51] Carles, L. Narkunan, K.; Penlou, S.; Rousset, L.; Bouchu, D.; Ciufolini, M. A. *J. Org. Chem.* **2002**, *67*, 4304.

[52] Jain, R.; Roschangar, F.; Ciufolini, M. A. *Tetrahedron Lett.* **1995**, *36*, 3307.

[53] Reddy, T. I.; Varma, R. S. *Tetrahedron Lett*. **1997**, *38*, 1721.

[54] Ghozlan, S. A. S.; Hassanien, A. Z. A. *Tetrahedron* **2002**, *58*, 9423.

[55] Yamagushi, Y.; Katsuyama, I.; Funabiki, K.; Matsui, M.; Shibata, K. *J. Heterocycl. Chem.* **1998**, *35*, 805.

[56] Mathews, A.; Anabha, E. R.; Sasikala, K. A.; Lathesh, K. C.; Krishnaraj, K. U.; Sreedevi, K. N.; Prasanth, M.; Devaky, K. S.; Asokan, C. V. *Tetrahedron* **2008**, *64*, 1671.

[57] Debenham, J. S.; Madsen-Duggan, C. B.; Walsh, T. F.; Wang, J.; Tong, X.; Doss, G. A.; Lao, J.; Fong, T. M.; Schaeffer, M. T.; Xiao, J. C.; Huang, C. R. C; Shen, C. P.; Feng, Y.; Marsh, D. J.; Stribling, D. S.; Shearman, L. P.; Strack, A. M.; MacIntyre, D. E.; Van der Ploeg, L. H. T.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 681.

[58] Bisht, S. S.; Jaiswal, N.; Sharma, A.; Fatima, S.; Sharma, R.; Rahuja, N.; Srivastava, A. K.; Bajpai, V.; Kumar, B.; Tripathi R. P. *Carbohydr. Res*. Accepted: 2 March **2011**.

[59] Bisht, S. S.; Fatima, S.; Tamrakar, A. K.; Rahuja, N.; Jaiswal, N.; Srivastava, A. K.; Tripathi, R. P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2699.

[60] Bisht, S. S.; Pandey, J.; Sharma, A.; Tripathi, R. P. *Carbohydr. Res*. **2008**, *343*, 1399.

[61] Misra, M.; Sharma, R; Kant, R.; Maulik, P. R.; Tripathi, R.P. *Tetrahedron* **2011**, *67*, 740.

[62] Rodrigues, F.; Canac, Y.; Lubineau, A. *Chem. Commun.* **2000**, 2049.

[63] Tyndall, D. V.; Nakib, T. A.; Meegan, M. J. *Tetrahedron Lett.* **1988**, *29*, 2703.

[64] Al-Arab, M. M. *J. Heterocycl. Chem.* **1989**, *26*, 1665.

[65] Elgemeie, G. E. H.; Zohdi, H. F.; Sherif, S. M. *Phosphorus, Sulfur, Silicon* **1990**, *54*, 215.

[66] Radwan, M. A. A.; Abbas, E. M. H. *Monatsh. Chem.* **2009**, *140*, 229.

[67] Sonar, S. S.; Shingate, B. B. *Organic Chemistry: An Indian Journal* **2007**, *3*, 1.

[68] Hamdy, N. A.; Gamal-Eldeen, A. M. *Eur. J. Med. Chem.* **2009**, *44*, 4547.

[69] Perez-Medina, L. A.; Mariella, R. P.; McElvain, S. M. *J. Am. Chem. Soc.* **1947**, *69*, 2574.

[70] Fanta, P. E.; Stein, R. A. *J. Am. Chem. Soc.* **1955**, *77*, 1045.

[71] Blackwood, R. K.; Hess, G. B.; Larrabee, C. E.; Pilgrim, F. J. *J. Am. Chem. Soc.* **1958**, *80*, 6244.

[72] Abd-el-Rahman, A. H.; Kandeel, E. M. *J. Heterocycl. Chem.* **1981**, *18*, 371.

[73] Elgemeie, G. H.; Alnaimi, I. S.; Alarab, H. F.; Heterocycles **1992**, *34*, 1721.

[74] Elgemeie, G. H.; Elfahham, H. A. *Bull. Chem. Soc. Jpn.* **1998**, *61*, 4431.

[75] Faty, R. A. M.; Youssef, A. M. S. *Curr. Org. Chem.* **2009**, *13*, 1577.

[76] Mijin, D. Z.; Uscumlic, G. S.; Valentic, N. V. *J. Serb. Chem. Soc.* **2001**, *66*, 507.

[77] Fan, X. S.; Wang, X.; Zhang, X. Y.; Feng, D.; Qu, Y. Y. *Chin. Chem. Lett.* **2009**, *20*, 1161.

[78] Okamoto, Y.; Zama, Y.; Itoh, T.; Aotsuka, T.i; Kurasawa, Y.; Takagi, K. *J. Chem. Res.* **1990**, 5, 136.

[79] Nohara, Akira; Ishiguro, Toshihiro; Sanno, Yasushi. *Tetrahedron Lett.* **1974**, *13*, 1183.

[80] Melikyan, G.; Piroyan, A. *Arkivoc* **2006,** *4*, 234.

[81] Basyouni, W. M. *Acta Chem. Slov.* **2003**, *50*, 223.

[82] Al-Najjar, A. A.; Amer, S. A.; Riad, M.; Elghamry, I.; Elnagdi, M. H. *J. Chem. Res. Synop.* **1996**, 296.

[83] Martin, N.; Seoane, C.; Soto, J. L. *Heterocycles* **1985**, *23*, 2013.

[84] Beckmann, S.; Schefczik, E. *Eur. Patent* **1994**, 628, 547.

[85] Doss, S. H.; Louca, N. A.; Elmegeed, G. A.; Mohareb, R. M. *Arch. Pharmacal Res.* **1999**, *22*, 496.

[86] Shams, H. Z.; Mohareb, R. M.; Helal, M. H.; Mahmoud, A. E. *Molecules* **2011**, *16*, 52.

[87] (a) Dyachenko, V. D.; Tkachev, R. P. *Russ. J. Org. Chem.* **2003**, *39*, 757; (b) Dyachenko, V. D.; Tkachev, R. P. *Russ. J. Org. Chem.* **2006**, *42*, 149.

[88] Tkachova, V. P.; Gorobets, N. Y.; Tkachov, R. P.; Dyachenko, O. D.; Rusanov, E. B.; Dyachenko, V. D. *ARKIVOC* **2010**, *11*, 254.

[89] El-Rady, E. A.; Khalil, M. A. *J. Chinese Chem. Soc.* **2004**, *51*, 779.

[90] Mijin, D.; Antonovic, D.; Misic-Vukovic, M.; *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **1994**, *33*, 309.

[91] Mijin, D.; Mišić-Vuković, M.; *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **1995**, *34*, 348.

[92] Mijin, D.; Mišić-Vuković, M.; *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **1998,** *37*, 988.

[93] Mijin, D.; Milić, B.; Mišić-Vuković, M.; *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **2006**, *45*, 61.

[94] Prlainovic, N. Z.; Bezbradica, D. I.; Knezevic-Jugovic, Z. D.; Kozlowska, R. T.; Mijin, D. Z. *J. Braz. Chem. Soc.* **2010**, 1.

[95] Al-Zaydi, K. M. *Arabian J. Chem.* **2009**, *2*, 89.

[96] Gorobets, N. Y.; Yousefi, B. H.; Belaj, F.; Kappe, C. O. *Tetrahedron* **2004**, *60*, 8633.

[97] (a) Loupy, A. *Microwaves in Organic Synthesis* Ed.; Wiley: Weinheim, **2002;** (b) Hayes, B. L. *Microwave Synthesis: Chemistry at the Speed of Light* CEM: Matthews, NC, **2002**; (c) Lidstom, P., Tierney, J. P. *Microwave-Assisted Organic Synthesis* Eds.; Blackwell: Oxford, **2004**; (d) For online resources, see: http://www.maos.net

[98] (a) Westman, J.; Lundin, R.; Stalberg, J.; Ostbye, M.; Franzen, A.; Hurynowicz, A. *Comb. Chem. High Throughput Screen.* **2002**, *5*, 565; (b) Giacommelli, G.; Porcheddu, A.; Salaris, M.; Taddei, M. *Eur. J. Org. Chem.* **2003**, 537; (c) Molteni, V.; Hamilton, M. M.; Mao, L.; Crane, C. M.; Termin, A. P.; Wilson, D. M. *Synthesis* **2002**, 1669.

[99] Zhang, X. H.; Zhong, W.; Li, X. Z.; Li, S. *Chinese Chem. Lett.* **2009**, *20*, 771.

[100] Mijin, D.; Uscumlic, G.; Perisic-Janjic, N.; Trkulja, I.; Radetic, M.; Jovanncic, P. *J. Serb. Chem. Soc.* **2006**, *71*, 435.

[101] Konstantinos, K. *Patent schrift* **1984**, 646, 418.

[102] National Committee for Clinical and Laboratory Standards, Method for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically Approved Standard, fourth ed. NCCLS, Villanova, Italy, **1997**, Document M 100-S7. S100-S157.

[103] (a) D.H. Isenberg, Essential Procedure for Clinical Microbiology, *American Society for Microbiology*, Washington, **1998**; (b) Zgoda, J. R.; Porter, J. R. *Pharm. Biol.* **2001**, *39*, 221.

Chapter 3

Multicomponent one-pot synthesis and biological evaluation of pyrano[2,3-*c*] pyrazoles

3.1 Introduction - MCRs

Despite recent advances in molecular biology and the progress in combinatorial synthetic methodology, the rate of introduction of new medicines has decreased markedly over the past two decades [1]. Structural diversity in a focused collection of potential therapeutics is believed to increase the positive hit rate. Most medicines in use are still small synthetic organic molecules that often contain a heterocyclic ring. However, the range of easily accessible and suitably functionalized heterocyclic building blocks for the synthesis of structurally diverse libraries is rather limited. Therefore, the development of new, rapid and clean synthetic routes towards focused libraries of such compounds is of great importance to both medicinal and synthetic chemists [2]. Undoubtedly, the most efficient strategies involve multicomponent reactions (MCRs), which have emerged as a powerful tool for the rapid introduction of molecular diversity. Consequently, the design and development of (new) MCRs for the generation of heterocycles receives growing interest [3-6].

Multicomponent reactions (MCRs) are special types of synthetically useful organic reactions in which three or more different starting materials react to a final product in the one-pot procedure [3]. Such reactions are atom-efficient processes by incorporating the essential parts of the starting materials into the final product. MCRs are powerful tools in the modern drug discovery process and allow the fast, automated, and high-throughput generation of organic compounds [7]. In the past years the pharmaceutical industry has focused more and more on diversity-oriented and biased combinatorial libraries [8, 9]. Furthermore, the discovery of novel MCRs can be considered as an interesting topic for academic research that also satisfies a practical interest of applied science [10-12].

3.2 Multicomponent Reactions - A Special Class of Tandem Reactions

In traditional organic synthesis individual bonds are formed in a stepwise procedure. This often involves isolation and purification of intermediates and alteration of reaction conditions for the next synthetic step. In the ideal case, however, a target molecule is prepared from readily available starting materials in one simple, safe, environmentally friendly and resource-efficient operation that proceeds quickly and in quantitative yield (Figure 1) [3].

In the past decade, many research groups have aimed for the realization of this concept of Ideal Synthesis by the development of multi-step, single operation processes for the construction of complex molecules in which several bonds are formed in a chain of events and without the necessity of isolating the intermediates. Reactions that meet these criteria, commonly referred to as tandem reactions [13], allow the economically and environmentally favorable synthesis of a wide range of organic molecules.

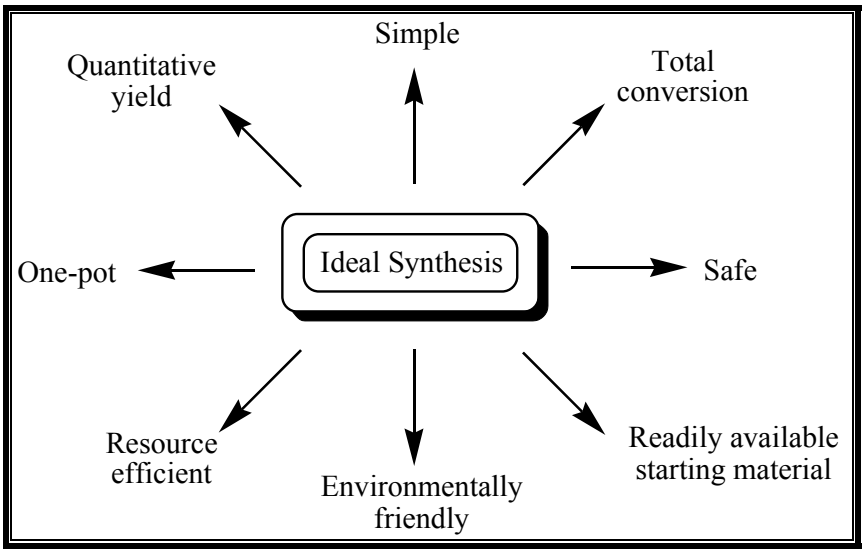


Figure 1. General aspects of the Ideal Synthesis

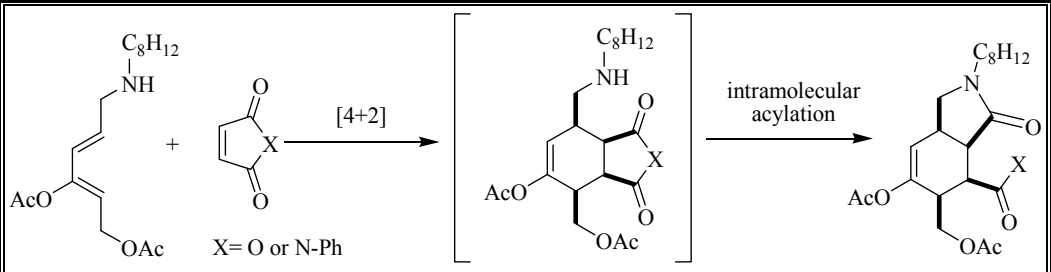
As suggested by Denmark [14], a distinction can be made between three categories of tandem reactions:

i) cascade or domino reactions

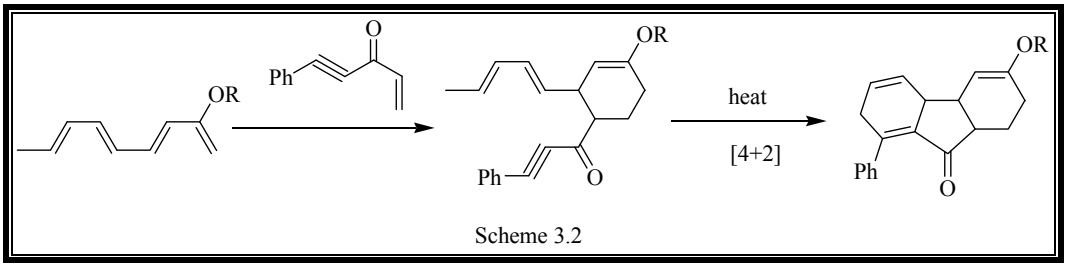
ii) consecutive reactions

iii) sequential reactions

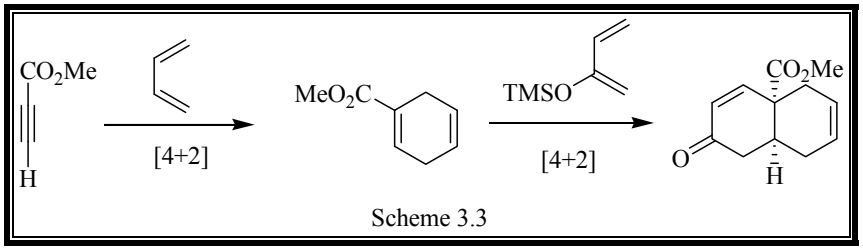
In a tandem cascade reaction, every reaction step causes the structural change required for the next reaction step. The overall reaction proceeds without modification of reaction conditions or the addition of extra reagents. The intermediates in cascade reactions cannot be isolated. An example of a cascade reaction is depicted in Scheme 3.1 [15].



Tandem consecutive reactions differ from cascade reactions in that the intermediate is an isolable entity. The intermediate contains the required functionality to undergo the second reaction step, but additional promotion in the form of energy (light or heat) is needed to overcome the activation barriers. In the consecutive reaction shown in Scheme 3.2, intermediate can be isolated, but the application of additional heating in the same reaction vessel promotes the second Diels-Alder cyclisation [16].

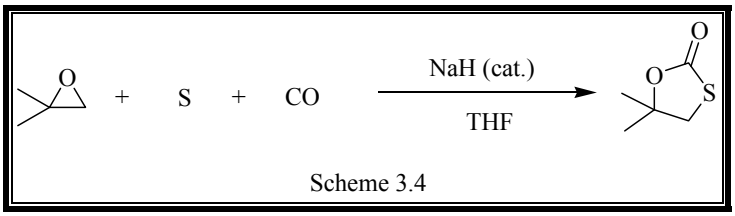


In tandem sequential reactions, the functionalities created in the first reaction step must enable the intermediate to engage in the following reaction step. However, these types of reactions require the addition of an extra component for the tandem process to proceed (Scheme 3.3) [17]. The intermediate may be isolable, but this is not necessary.



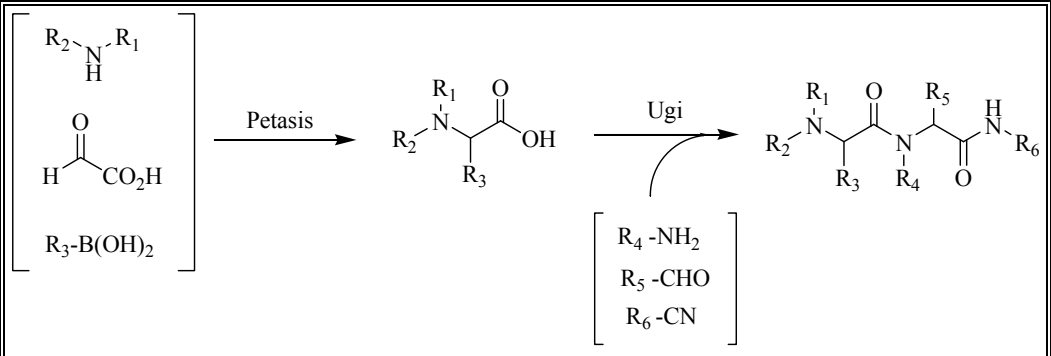
Scheme 3.3

A special class of tandem sequential reactions constitutes the multicomponent reaction (MCRs). MCRs are defined as one-pot reactions in which three or more components react to form a single product, incorporating essentially all atoms of the starting materials [11]. A MCR in which a small molecule, like water, is expelled is also referred to as a multicomponent condensation. MCRs are well appreciated because of their superior atom economy, simple procedures, the highly convergent character and the high and ever increasing number of accessible backbones. Different classification schemes of MCRs are possible, for example classifications according to variability, mechanisms or components involved. Although the three-component synthesis depicted in Scheme 3.4 gives access to 1,3-oxathiolan-2-one in good yield under mild conditions and in one operation [18], this is an example of a MCR of low variability, because only the substituents on the epoxide can be varied, while the other components are fixed. This MCR is therefore of low exploratory power (EN) [4].



Scheme 3.4

On the other hand, MCRs of high variability, like the tandem Petasis-Ugi reaction depicted in Scheme 3.5 [19], are of high exploratory power, because both high complexity and broad diversity are generated in this procedure. Given the numbers of commercially available starting materials, this MCR can virtually provide a library that spans a chemical space of $1000 \times 500 \times 1000 \times 1000 \times 1000 = 5 \times 10^{14}$ small molecules [20].



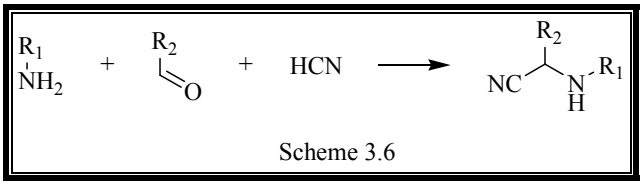
Scheme 3.5

Based on reaction mechanisms, MCRs are divided into three subclasses, as shown in Table 1 [3a].

Table 1. MCRs classified by reaction mechanism

MCR type	general reaction equation
I	A + B ⇌ C ⇌ D ... O ⇌ P
II	A + B ⇌ C ⇌ D ... O → P
III	A + B → C → D ... O → P

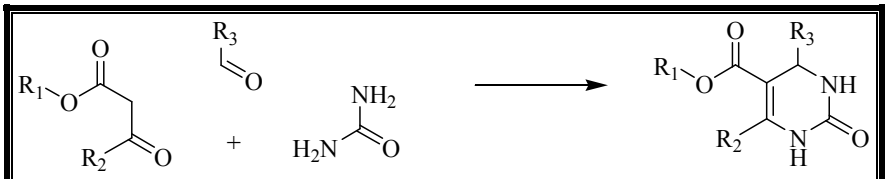
In type I MCRs, the starting materials, intermediates and products are in equilibrium with each other. The yield of the final product depends on the thermodynamics and the product is often isolated as a mixture with intermediates and starting materials. A well-known type I MCR is the Strecker reaction (Scheme 3.6).



Scheme 3.6

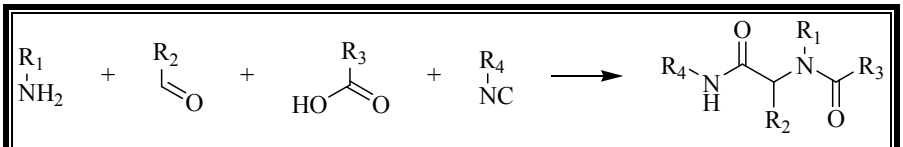
Type II MCRs are sequences of reversible reactions that are terminated by an irreversible reaction step, which drives the reaction to completion. This last step often involves a highly exothermic reaction like an aromatization, a ring closure or the oxidation of the C^{II} of isocyanides to C^{IV}.

Although they are not uncommon in biochemical pathways in living cells, type III MCRs, in which all reaction steps are irreversible, are very rare in preparative chemistry. Most successful MCRs are of type II, like the Biginelli reaction (Scheme 3.7). The irreversibility of the last reaction step does not exclude the formation of side products. However, very often possible side reactions are reversible as well and therefore, the irreversibly formed product P dominates. It should be noted that the classification of MCRs according to mechanism is not strict, but that transitions between the different types are fluid.



Scheme 3.7

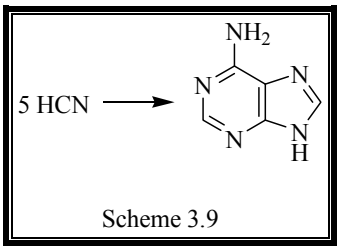
Finally, MCRs can be classified according to the functional groups involved in the reaction. Examples are imine based MCRs, isocyanide based MCRs (IMCRs) and carbine based MCRs. Many known MCRs belong to the first type because the first step involves the condensation of an amine and an aldehyde or ketone to form an imine. One of the most famous MCRs, the Ugi four-component reaction, belongs to both imine and isocyanide based MCRs (Scheme 3.8).



Scheme 3.8

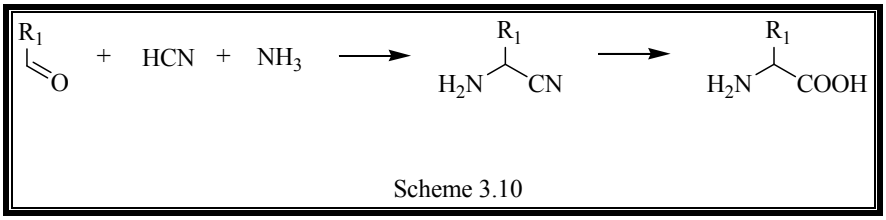
3.3 History of Multicomponent Chemistry

The concept of MCRs is not unknown in nature, it is important especially in evolution. It seems that adenine, one of the major constituents of DNA and RNA, was prebiotically formed by the condensation of five molecules of HCN, a plentiful component of prebiotic atmosphere, in a reaction catalyzed by NH_3 (Scheme 3.9) [21-25]. The other nucleic bases have been generated in similar reactions involving HCN and H_2O.



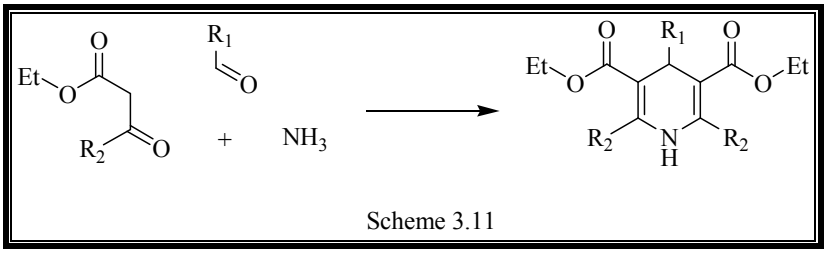
Scheme 3.9

The first modern contribution to the development of multicomponent chemistry was made in 1850 by Strecker. The crucial step in the well-known Strecker synthesis of α-amino acids is the formation of α-amino nitriles from aldehydes, HCN and NH_3 in one-pot [26]. Subsequent hydrolysis of these synthetically valuable intermediates results in the amino acids (Scheme 3.10).



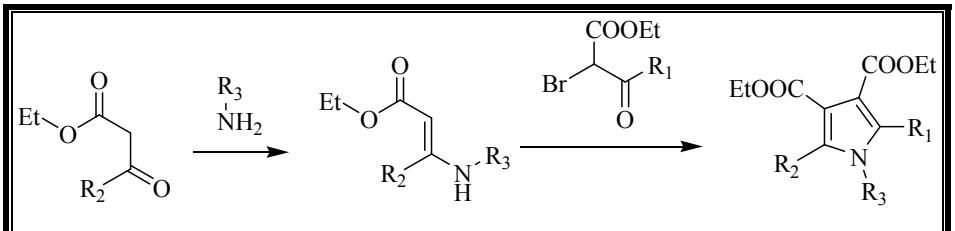
Scheme 3.10

Further progress of multicomponent chemistry can be attributed to the work of Hantzsch in 1882. He synthesized symmetrically substituted dihydropyridines from NH_3, aldehydes and two equivalents of *β*-ketoesters (Scheme 3.11) [27].



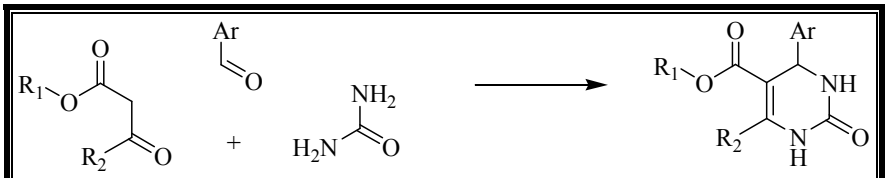
Scheme 3.11

Another contribution made by Hantzsch to MCRs was the synthesis of pyrroles by reacting primary amines 16, *β*-ketoesters and *α*-halogenated *β*-ketoesters (Scheme 3.12).[27]



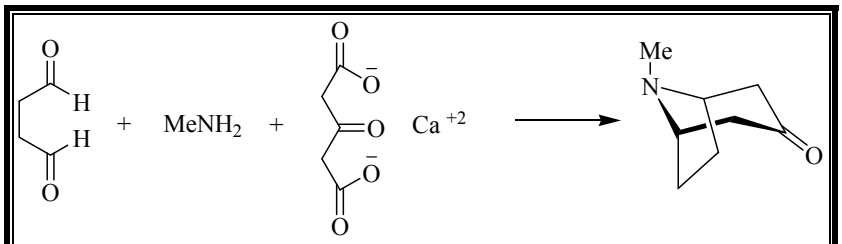
Scheme 3.12

The Biginelli reaction first described in 1893 represents multicomponent synthesis of substituted dihydropyrimidines by acid-catalyzed cyclocondensation of *β*-ketoesters, aromatic aldehydes and urea (Scheme 3.13) [28-31].



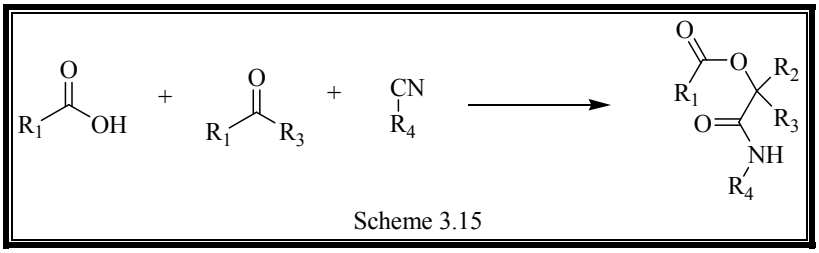
Scheme 3.13

The first important application of MCRs in natural product synthesis was the Robinson synthesis of the alkaloid tropinone from succinic dialdehyde, methylamine and calcium salt of acetonedicarboxylic acid, carried out in 1917 (Scheme 3.14) [33].



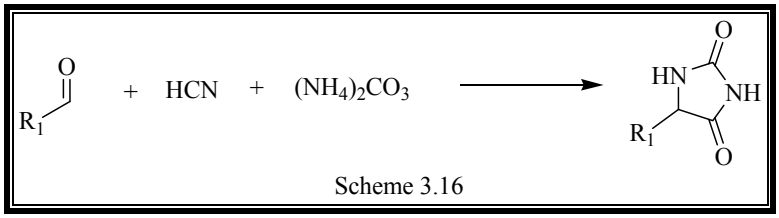
Scheme 3.14

The first MCR involving isocyanides was discovered in 1921 by Passerini [21], Carboxylic acids, carbonyl compounds and isocyanides afforded *α*-acyloxy carboxamides in a one-pot procedure (Scheme 3.15) [23, 33].



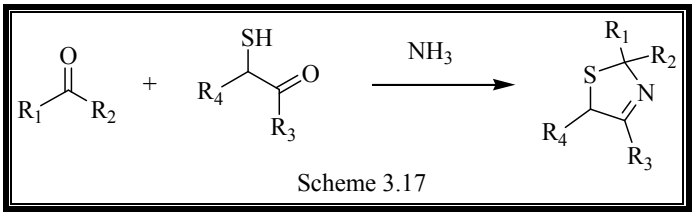
Scheme 3.15

In 1934, Bucherer and Bergs described a four-component reaction for synthesis of hydantoins. One-pot reaction of hydrogen cyanide, aldehydes, NH_3 and CO_2 afforded hydantoins [34], which can be easily transformed into *α*-amino acids by simple hydrolysis (Scheme 3.16) [35, 36].



Scheme 3.16

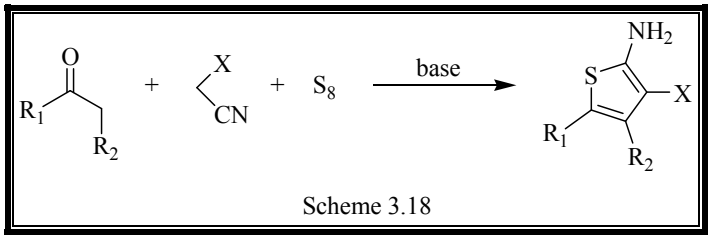
The next important example is the Asinger reaction reported in 1958. *α*-Halogenated carbonyl compounds and sodium hydrogen sulfide generated *in situ* thiols which reacted with carbonyl compounds and ammonia to afford thiazolines (Scheme 3.17) [37].



Scheme 3.17

One of the most utilized multicomponent reactions was discovered in 1959 by Ugi *et al.*[23] Synthesis of *α*-acylamino amides was achieved by reacting aldehydes, primary amines, carboxylic acids and isocyanides (Scheme 3.8). [23-25, 33]

In 1961 Gewald and co-workers described the synthesis of polysubstituted thiophenes with electron withdrawing substituents such as cyano, carboethoxy and carboxamido in the 3-positions and alkyl, aryl, cycloalkyl and hetaryl groups in the 4- and 5-positions. Three major modifications of this method are described in literature which gives access to various 2-aminothiophenes (Scheme 3.18) [38]. The most elegant and simplest version consists of a one-pot procedure which includes the condensation of aldehydes, ketones or 1,3-dicarbonyl compounds with activated nitriles and sulfur in the presence of amine at room temperature. Ethanol, methanol, dimethylformamide, dioxane, excess ketone such as methyl ethyl ketone or cyclohexanone are preferred solvents and the most often employed amines are diethylamine, morpholine or triethylamine.



Scheme 3.18

The year 1993 began a new era of the formation and investigation of the products and the libraries of the Ugi reaction (U-4CR) and higher MCRs of the isocyanides. Already in 1961, Ugi and Steinbrückner [24] described the libraries of U-4CR products, and this was mentioned again in 1971. For many decades nobody had been interested in libraries. In 1982, Furka [39] formed peptide libraries by the solid-phase method of Merrifield [40], and since then this was increasingly applied. Libraries of other compounds were subsequently also formed by multistep solid-phase procedures [41].

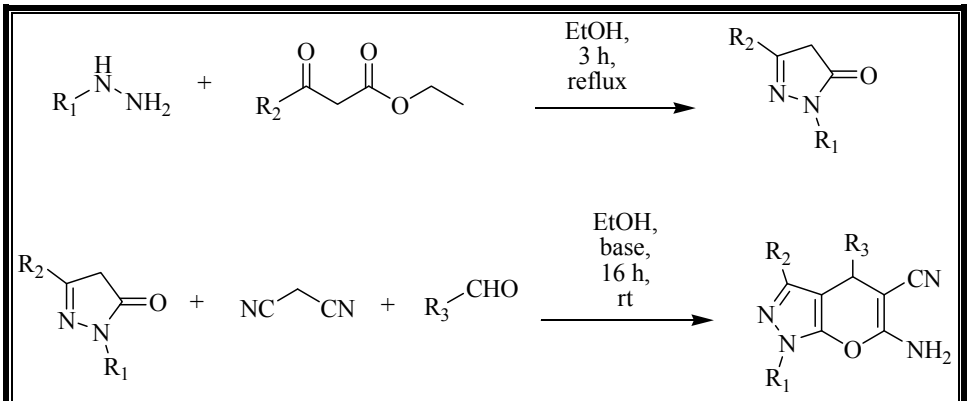
In 1995, Armstrong *et al.* [42] and in the Hofmann LaRoche AG Weber *et al.* [43] published the first industrial libraries of U-4CR products; the latter group had then found two Thrombine inhibitors, whereas the search for such products by the then conventional methods was not successful for a decade. A still-increasing number of industrial companies began to produce and to investigate the libraries of the U-4CR products and related compounds. Nowadays, the search for new pharmaceutical and plantprotecting compounds is accomplished by forming libraries of up to 20,000 and more different compounds in one day by a single person.

3.4 Reported synthetic strategies

3.4.1 Three-component synthesis of Dihydropyrano[2,3-*c*]pyrazoles

Laufer *et al.* [44] have synthesized 1,4-dihydropyrano[2,3-*c*]pyrazoles (Scheme 3.19) with various substituents at the 1-, 3-, and 4-position. Given the large number of commercially available aldehydes and the easy access to hydrazines and *β*-keto esters, this method should be applicable to synthesis of libraries with high diversity.

The corresponding *β*-keto esters were synthesized either according to Yuasa and Tsuruta [45] or by deprotonation of esters and subsequent reaction with ethyl acetate. This second procedure (deprotonation of esters), described in a patent application for the synthesis of ethyl 3-oxo-3-(pyridin-4-yl)propanoate [46], is more advantageous because the reaction can be performed using ethyl acetate as both the solvent and reagent without further purification. The reaction was performed at room temperature overnight, and nearly all products precipitated as discrete crystals.



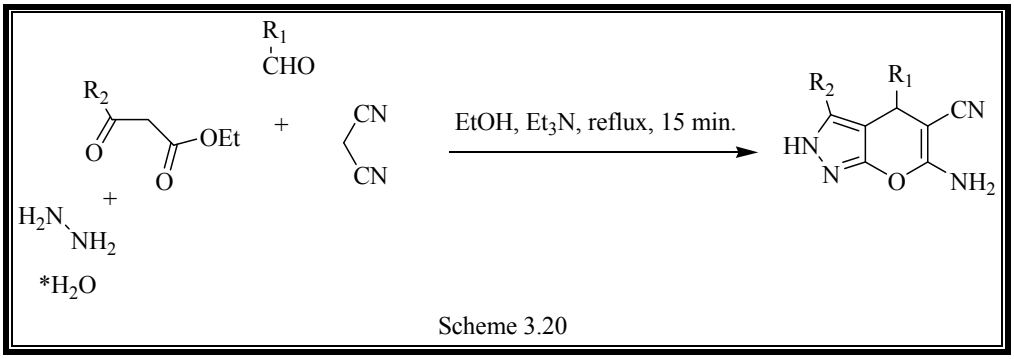
Scheme 3.19

3.4.2 Four-component pyrano[2,3-*c*]pyrazoles synthesis

Shestopalov *et al.* [47] demonstrated that a four-component reaction of aromatic aldehydes, malononitrile, *β*-ketoesters, and hydrazine hydrate successfully yields 6-aminopyrano[2,3-*c*]pyrazol-5-carbonitriles without the need of prior pyrazolin-5-ones isolation [48]. The multicomponent synthesis of pyranopyrazoles was carried out by

simultaneously refluxing all four starting materials in ethanol for 15 min. in the presence of Et_3N (Scheme 3.20).

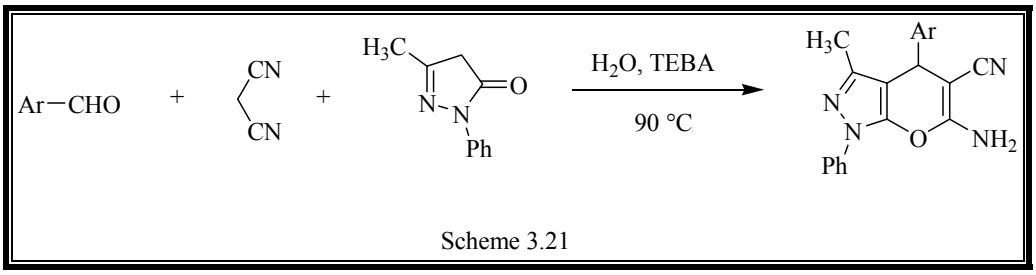
They showed that aromatic aldehydes with electronwithdrawing, electron-donating, withdrawing and donating groups, as well as napthaldehydes and hetero-aromatic aldehydes can be successfully reacted with *β*-ketoesters, malonodinitrile, and hydrazine hydrate to yield final pyrano[2,3-*c*]pyrazoles with high regio-selectivity.



Scheme 3.20

3.4.3 1,4-dihydropyrano[2,3-*c*]pyrazoles synthesis in aqueous media

Shi *et al*. [49] report one-pot synthesis of 1,4-dihydropyrano[2,3-*c*]pyrazole derivatives by three-component reaction in aqueous media. When aromatic aldehydes, malononitrile, 3-methyl-1-phenyl-2-pyrazolin-5-one, and triethylbenzylammonium chloride (TEBA) were stirred at 90 °C for 6-10 h in water, the products were obtained in good yields (Scheme 3.21).



Scheme 3.21

The three-component reaction of aromatic aldehydes, malononitrile, and 3-methyl-1-phenyl-2-pyrazolin-5-one to 6-amino-5-cyano-4-aryl-1,4-dihydropyrano[2,3-*c*]pyrazoles has been efficiently performed in aqueous media. The easy purification of products by simple crystallization, and the use of water as solvent combined with

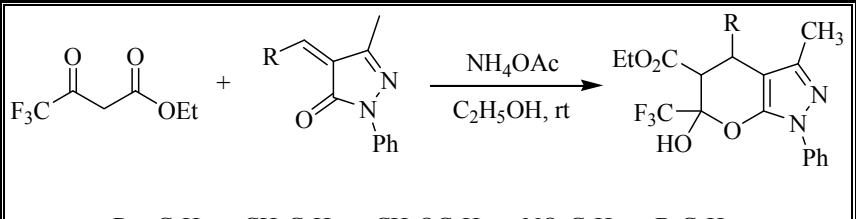
the exploitation of the multicomponent strategy open to this process suggest good prospects for its industrial applicability.

3.4.4 Base-catalyzed rout of Dihydropyrazolo[3,4-*b*]pyrans

3.4.4.1 By using Ammonium acetate

Li *et al.* [50] reported the preparation of 2*H*,4*H*-dihydropyrazolo[3,4-*b*]pyrans from the reaction of 4-Arylidene-3-methyl-1-phenyl-5-pyrazolones and *β*-ketoester using ammonium acetate as a catalyst (Scheme 3.22).

Ammonium acetate has been used widely as a base or a catalyst in Biginelli reactions [51, 52], Hantsch reactions [53] and other reactions [54, 55]. With this aim in view, they applied the ammonium acetate to this reaction. Treatment of 3-methyl-1-phenyl-4-phenylidene-5-pyrazolone with 1 equiv. of ammonium acetate followed by *β*-ketoester in ethanol at room temperature for 2 h gave the corresponding 1,4,5,6-tetrahydropyrazolo[3,4-*b*]pyrans.



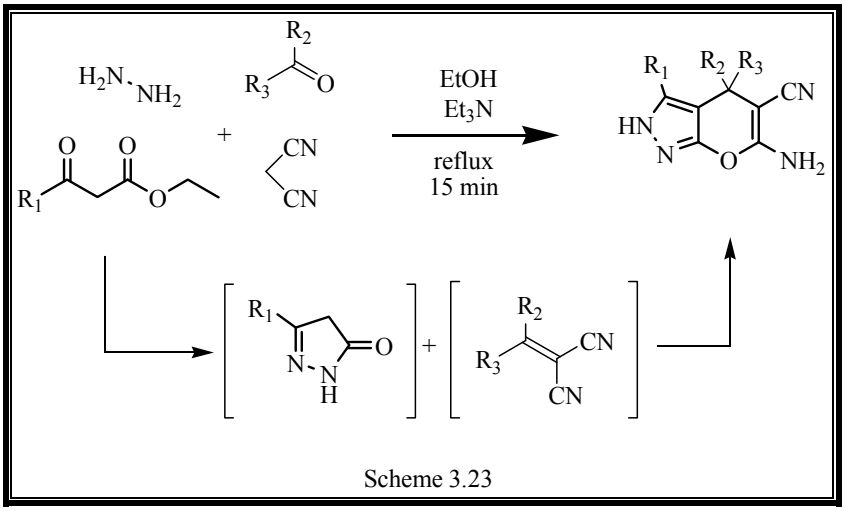
R = C_6H_5, *p*-$CH_3C_6H_4$, *p*-$CH_3OC_6H_4$, *p*-$NO_2C_6H_4$, *p*-BrC_6H_4, o-$CH_3C_6H_4$, o-FC_6H_4, *m*-ClC_6H_4, o-OHC_6H_4

Scheme 3.22

3.4.4.2 By using TEA

Mixing equimolecular amounts of ethyl acetoacetate with hydrazine hydrate, benzaldehyde and malononitrile has produced corresponding pyranopyrazoles (Scheme 3.23). This same product could be obtained in almost the same yield by reacting 3-amino-2-pyrazoline-5-one and arylidenemalononitriles in ethanol in the presence of chitosan or, as originally reported, in the presence of piperidine. Despite the recently claimed isolation of Michael adduct, this could not be repeated even when the reaction was conducted at room temperature for a short period. Only either unchanged starting materials or cyclic products were isolated. It is of value to report

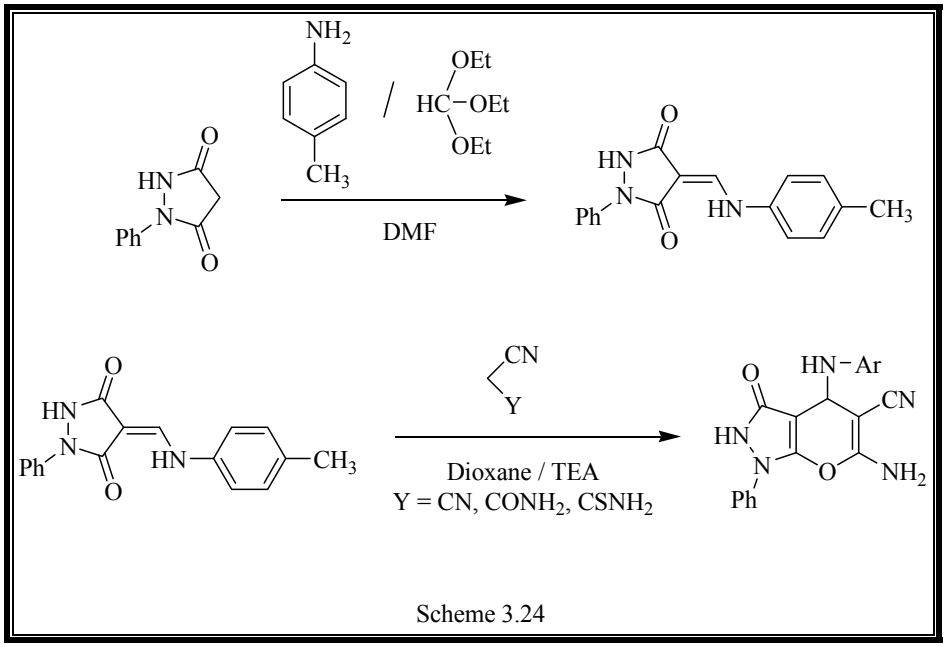
that after an induction period the reaction is exothermic and temperature control is somewhat difficult.



Scheme 3.23

The reaction of compound 4-(*p*-Methylphenylaminomethylidine)-1-phenyl-3,5-pyrazolidinedione with active nitriles and cyclic ketones, namely malononitrile, cyanoacetamide, cyanothioacetamide, cyanoacetic hydrazide, 1-phenyl-3,5-pyrazolidinedione, 3-methyl-1-phenyl-5-pyrazolone, cyclopentanone, cyclohexanone and cycloheptanone in the presence of a catalytic amount of triethylamine gave pyrano[2,3-*c*]pyrazole derivatives (Scheme 3.24) [56]. The reaction pathway of such compound was assumed to follow a preliminary formation of carbanion of the active methylene reagent, which was added to the double bond followed by a nucleophilic attack of the NH group at the CN, CO, and CS groups with the elimination of a water molecule in the case of cyanoacetamide and a H_2S molecule in the case of cyanothioacetamide [57].

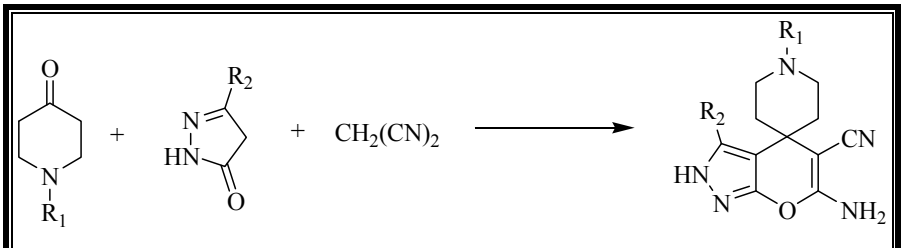
Here, 4-(*p*-Methylphenylaminomethylidine)-1-phenyl-3,5-pyrazolidinedione was prepared from the reaction of 1-phenylpyrazolidine-3,5-dione with ethyl orthoformate and *p*-toluidine in boiling dimethylformamide.



Scheme 3.24

3.4.5 Synthesis of spiro-dihydropyrazolo[3,4-*b*]pyrans

Evans *et al.* [58] describe a three-component condensation in which substituted piperidin-4-ones have been used in place of aromatic aldehydes to synthesize a new spiro heterocyclic system. They report that the base-catalyzed reaction of substituted piperidin-4-ones, pyrazol-5-ones, and malononitrile proceeds in ethanol at 20 °C with the formation of substituted 6-amino-5-cyanospiro-4-(piperidine-4')-2*H*,4*H*-dihydropyrazolopyrans (Scheme 3.25).

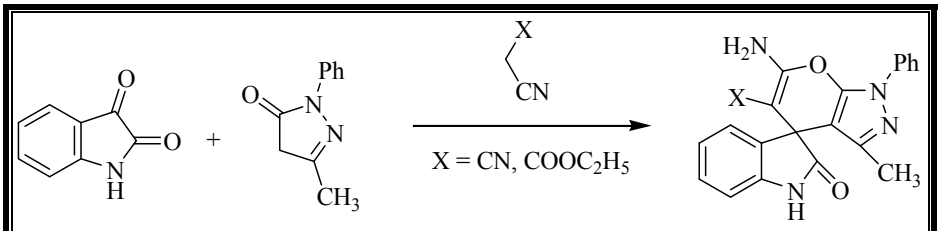


Scheme 3.25

Three-component condensation of 4-piperidinones, 5-pyrazolones, and malononitrile proceeds chemically and electrochemically and is a convenient one-step means of synthesis of substituted 6-amino-5-cyanospiro-4-(piperidine-4')-2*H*,4*H*-dihydropyrazolo[3,4-*b*]pyrans. The electrochemical reactions proceed under milder conditions and with yields 12-15% greater than those of the reactions catalyzed by chemical bases.

3.4.6 Dihydropyrazolopyrans from 1*H*-indole-2,3-dione

Aly H. Atta [59] reported the synthesis of a new series of compounds containing both the two moieties is likely to result in the formation of some interesting bioactive compounds. The one-pot reaction of 1*H*-indole-2,3-dione, 3-methyl-1-phenyl-2-pyrazolin-5-one, and active methylenes, namely malononitrile, ethyl cyanoacetate, pyrazolone, and acetyl acetone afforded the products. These products can be obtained via reaction of 3-[3-methyl-5-oxo-1-phenyl-1,5-dihydro-pyrazol-(4*Z*)-ylidene]-1,3-dihydro-indol-2-one with the corresponding active methylenes (Scheme 3.26).



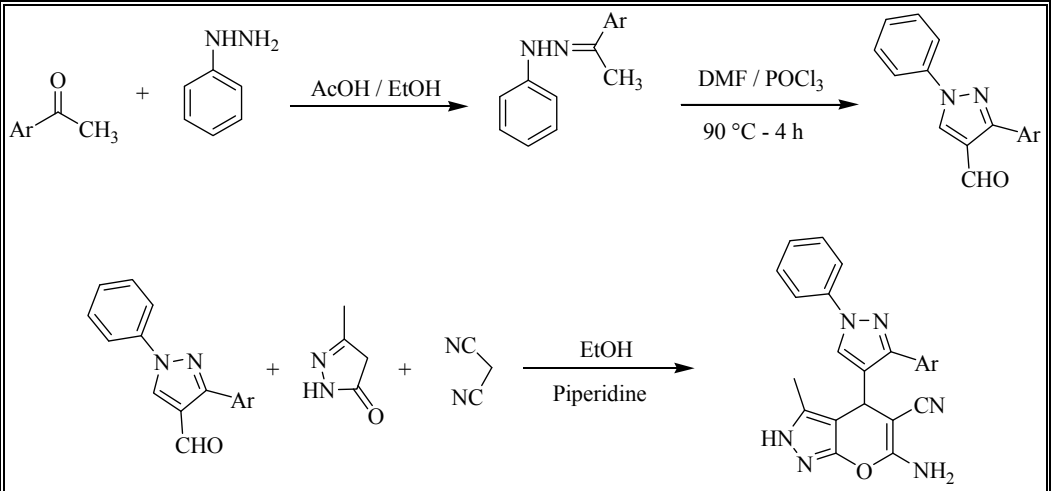
Scheme 3.26

3.4.7 Pyrazolopyrans from pyrazole-aldehydes

Thumar and Patel [60] reported a series of 4-pyrazolyl-4*H*-pyrazolopyran derivatives by one-pot three-component cyclocondensation reaction of 1-phenyl-3-(het)aryl-pyrazole-4-carbaldehyde, malononitrile and substituted pyrazolin-5-ones in the presence of piperidine as catalyst. The mixture refluxing under ethanol or acetonitrile gives pyran derivatives (Scheme 3.27).

The reaction occurs *via* an *in situ* initial formation of the heterylidenenitrile, containing the electron-poor C=C double bond, from the Knoevenagel condensation between pyrazole-4-carbaldehyde and malononitrile by loss of water molecules.

Finally, Michael addition into the initially formed unsaturated nitrile, i.e. nucleophilic attack of hydroxyl moiety to the cyano moiety affords cyclized pyran derivatives.

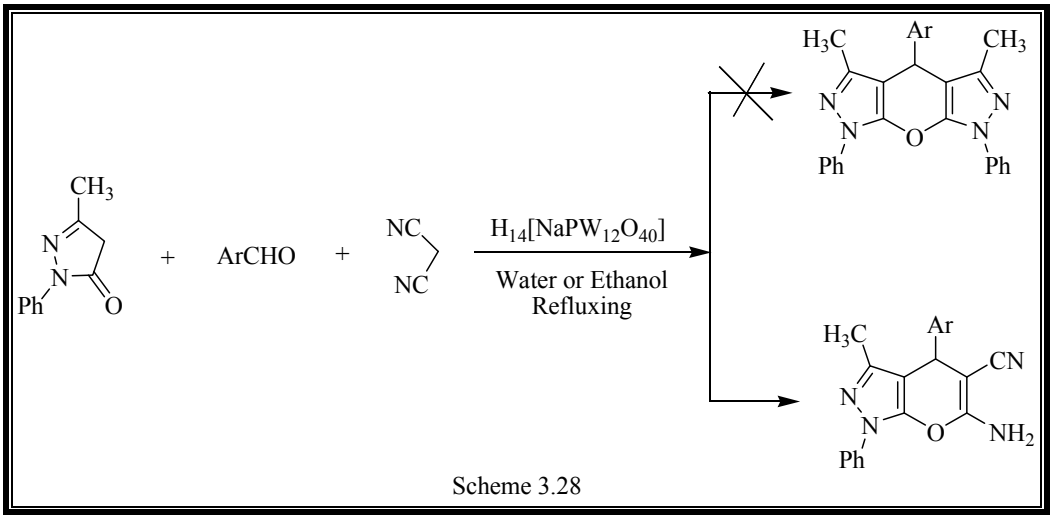


Scheme 3.27

3.4.8 Pyranopyrazoles by using heteropolyacid as a catalyst

Heravi *et al.* [61] reported facile method for the synthesis of 1,4-dihydropyrano[2,3-*c*] pyrazole derivatives *via* three-component one-pot condensation of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one, aldehydes and malononitrile in the presence of a catalytic amount of preyssler type heteropolyacid as a green and reusable catalyst in water or ethanol under refluxing conditions (Scheme 3.28).

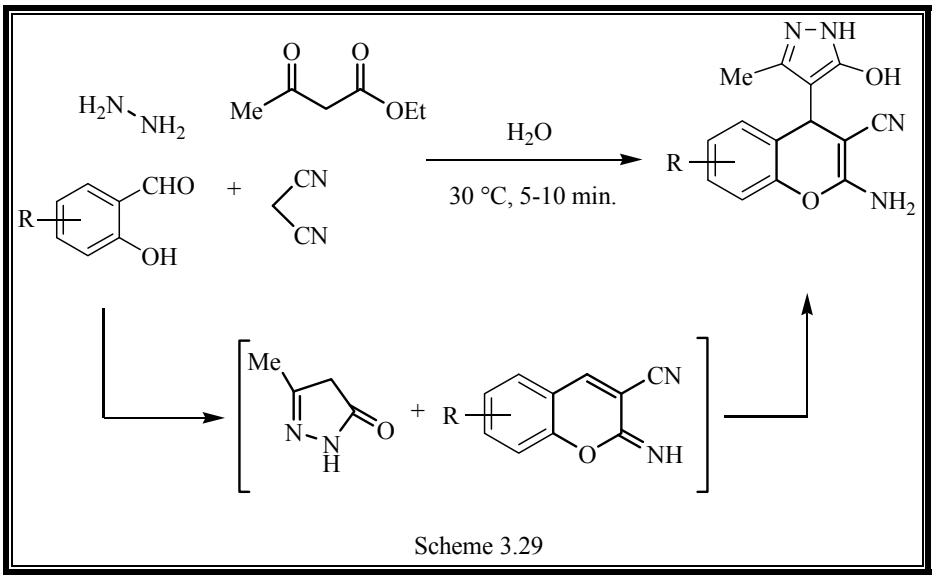
There has been considerable interest in the use of heteropolyacids as environmentally benign catalysts due to their unique properties such as high thermal stability, low cost, ease of preparation and recyclability. Numerous chemical reactions can occur in the presence of heteropolyacids [62]. Preyssler type heteropolyacid, $H_{14}[NaP_5W_{30}O_{110}]$, is remarkable owing to its exclusive physicochemical properties. These include strong Bronsted acidity, reversible transformations, solubility in polar and non-polar solvents, high hydrolytic and thermal stability, which are all essential in catalytic processes. Preyssler polyanion, as a large anion, can provide many ‘‘sites’’ on the oval-shaped molecule that are likely to render the catalyst effective [63]. This heteropolyanion with [64] acidic protons, is an efficient ‘‘supper acid’’ solid catalyst which can be used both in the homogeneous and heterogeneous phases [65].



Scheme 3.28

3.4.9 Synthesis of aminochromenes

A four component Knoevenagel-Michael addition-cyclization sequence has been studied for the synthesis of dihydropyranopyrazole derivatives from hydrazine hydrate, a malonitrile, a *β*-ketoester, and an aldehyde or a ketone. The reaction was described under catalyst- and solvent-free conditions [66] and using piperidine in ultrapure aqueous media [67], both at room temperature. But this methodology was intensively developed by Shestopalov and co-workers since they used a wide range of aldehydes, ketones, and *β*-ketoesters to form a series of these fused heterocyclic skeletons, even if substituted hydrazines were unreactive in this protocol [47].

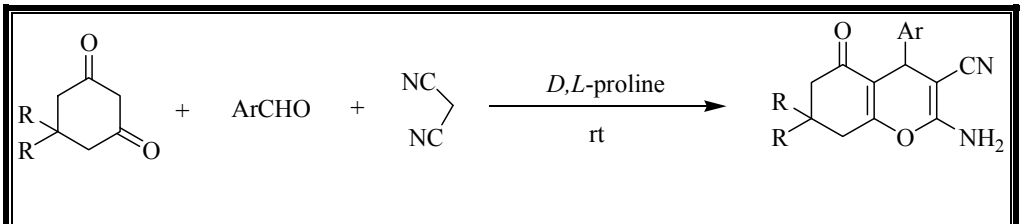


Scheme 3.29

More recently, an adaptation of this four-component transformation in water was proposed as a green combinatorial synthesis of novel aminochromene derivatives bearing an hydroxymethyl pyrazole functional group in the four-position, instead of a fused skeleton. In this unexpected transformation, 2-hydroxybenzaldhyde plays a crucial role by reacting selectively with malonitrile to form the chromene intermediate (Scheme 3.29) [68].

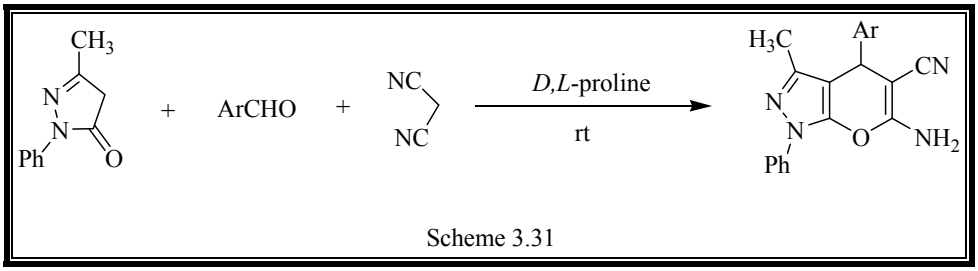
3.4.10 Solvent-free multicomponent synthesis of pyranopyrazoles

The conventional synthesis of 2-amino-3-cyano-4*H*-pyrans use organic solvent, but these solvents make the workup procedure complicated and lead to poor yields of products [69]. In recent years, 2-amino-3-cyano-4*H*-pyrans have also been synthesized under microwave [70], with ultrasound irradiation [71], or in aqueous media [49, 72, 73]. Some two-component [74] and three-component [72] condensations have been introduced for the synthesis of 2-amino-3-cyano-4*H*-pyrans. Each of these methods has its own merit, with at least one of the limitations of low yields, long reaction time, effluent pollution, harsh reaction conditions, and tedious workup procedues. All of these reasons spur us to study the possibility of synthesis of 2-amino-3-cycano-4-aryl-7,7-dimethyl-5,6,7,8-tetrahydrobenzo[*b*]pyrans and 6-amino-5-cyano-4-aryl-1,4-dihydro-pyrano[2,3-*c*]pyrazoles under solvent-free conditions.



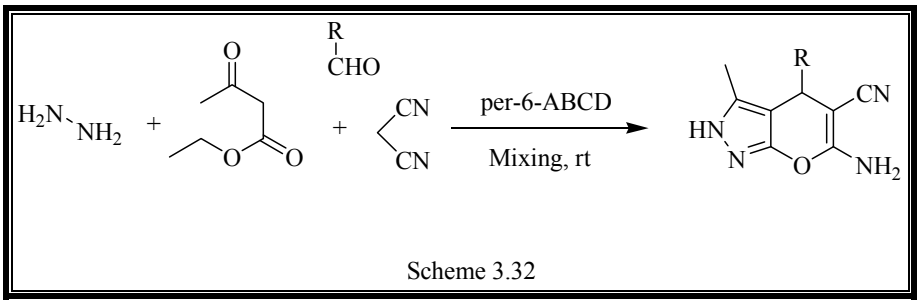
Scheme 3.30

Li *et al.* [75] report a highly efficient procedure for the synthesis of 2-amino-3-cycano-4-aryl-7,7-dimethyl-5,6,7,8-tetrahydrobenzo[*b*]pyrans and 6-amino-5-cyano -4-aryl-1,4-dihydropyrano[2,3-*c*]pyrazoles via a one-pot grinding method under solvent-free conditions using an inexpensive and commercially available *D,L*-proline as catalyst (Schemes 3.30 and 3.31).



Scheme 3.31

In a typical general experimental procedure, aromatic aldehydes, malononitrile, dimedone [1,3-cyclohexanedione or 3-methyl-1-phenyl-2-pyrazolin-5-one], and a catalytic amount of *D,L*-proline are added to a mortar. The mixture is ground by mortar and pestle at room temperature for a period. The solid product is obtained from an intermediate melt and then is laid up at room temperature for 30 min. The mixture is transferred to ice water and then is filtered off. The crude products are purified by recrystallization by ethanol to afford the products in good yields.



Scheme 3.32

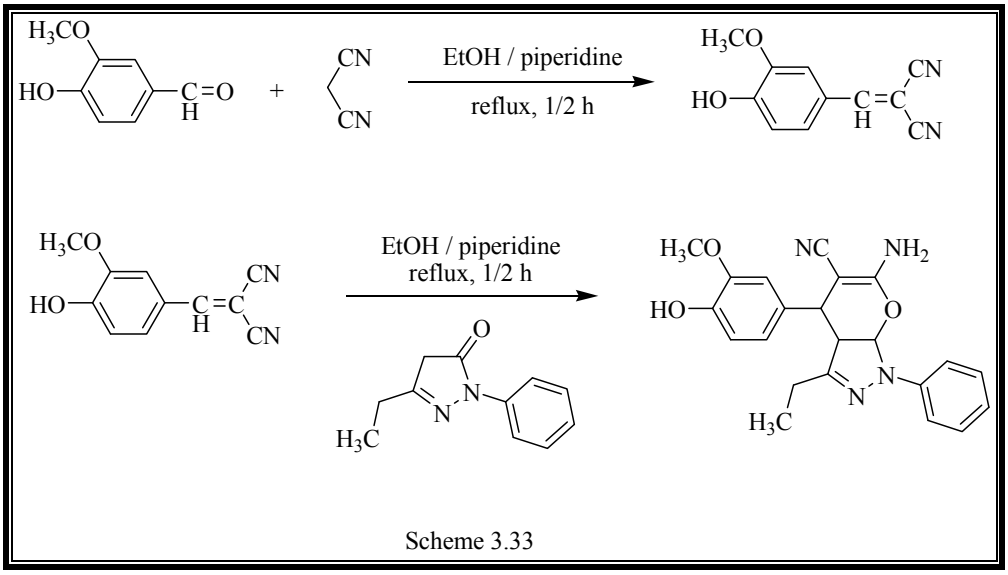
A simple, green and efficient protocol is developed with per-6-amino-b-cyclodextrin (per-6-ABCD) which acts simultaneously as a supramolecular host and as an efficient solid base catalyst for the solvent-free syntheses of various dihydropyrano[2,3-*c*]pyrazole derivatives involving a four-component reaction (Scheme 3.32).

Per-6-amino-b-cyclodextrin (per-6-ABCD) is used extensively as a supramolecular chiral host and as a base catalyst for Cu-catalyzed N-arylation [76] and for Michael addition of nitromethane to chalcones [77]. Kanagaraj and Pitchumani [78] have utilized per-6-ABCD as an excellent supramolecular host for the synthesis of pyranopyrazole derivatives, in an efficient and ecofriendly four-

component reaction protocol under solventfree conditions at room temperature. It is also interesting to note that the catalyst can be recovered and reused several times.

3.4.11 Syntheses of Polyfunctionalized Phenols Linked to Heterocycles

Boghdadie *et al.* [79] reported that, a solution of 4-(hydroxyl-3-methoxybenzylidine) malononitrile and 3-ethyl-1-phenyl-2-pyrazolin-5-one, in ethanol (50 ml) and two drops of piperidine was heated under reflux for 2 hours, cooled and poured onto water. The products were recrystallized from ethanol to give the corresponding compound 6-amino-3-ethyl-4-(4-hydroxy-3-methoxyphenyl)-1-phenyl-4*H*-pyrano[3,2-*d*]pyrazole-5-carbonitrile (scheme 3.33).



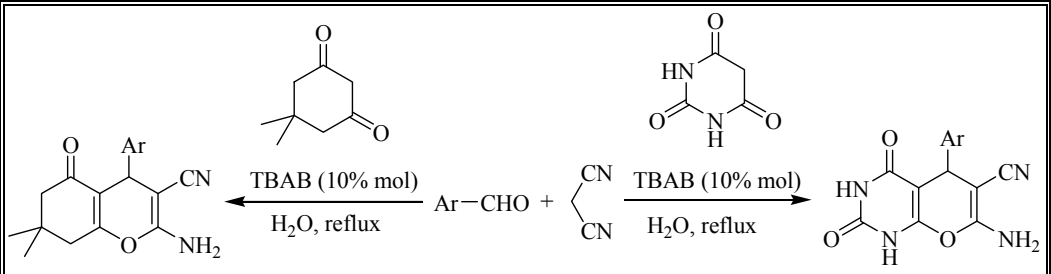
Scheme 3.33

3.4.12 Benzopyran Derivatives

3.4.12.1 4*H*-benzo[*b*]pyrans using TBAB as a catalyst

Fard *et al.* [80] reported a highly efficient procedure for the preparation of 4*H*-benzo[*b*]pyrans and pyrano[2,3-*d*]pyrimidinones via a domino Knoevenagelcyclo-condensation reaction using TBAB as a catalyst in water.

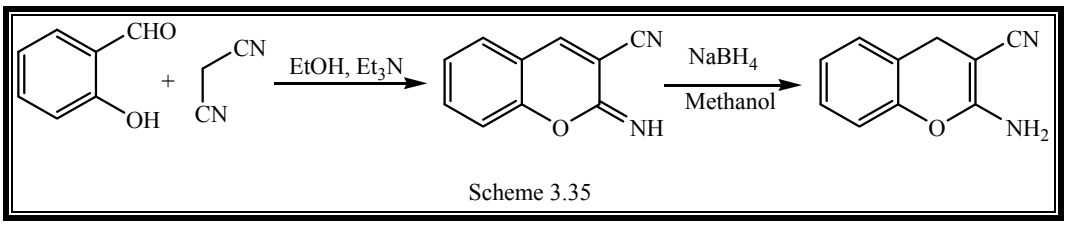
In a typical experimental procedure, a mixture of aromatic aldehyde, malononitrile, dimedone or barbituric acid in water under reflux condition, was stirred in the presence of a catalytic amount of TBAB (10 mol%) to afford the 4*H*-benzo[*b*]pyrans and pyrano[2,3- *d*]pyrimidinones (Scheme 3.34).



Scheme 3.34

3.4.12.2 2-Imino-2*H*-chromene-3-carbonitrile using $NaBH_4$ as a catalyst

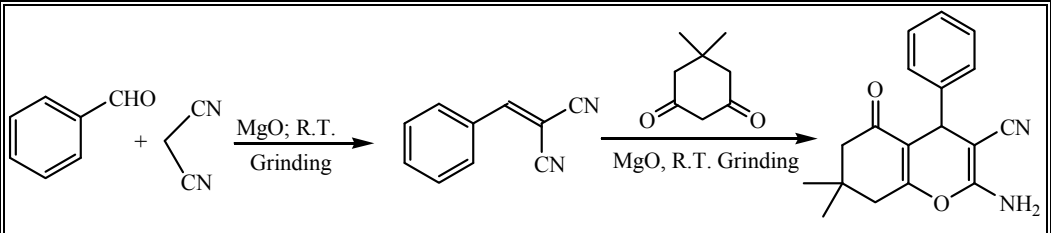
Rai *et al.* [81] have reported a synthesis (Scheme 3.35) in ethanol using triethylamine to first get 2-Imino-2*H*-chromene-3-carbonitrile which they reduced using sodium borohydride in methanol to give the essential 2-Amion-3-Cyanochromane derivative. The reaction mixture here has been conventionally refluxed for 3 h.



Scheme 3.35

3.4.12.3 2-Amino-3-cyanochromene using MgO as a catalyst

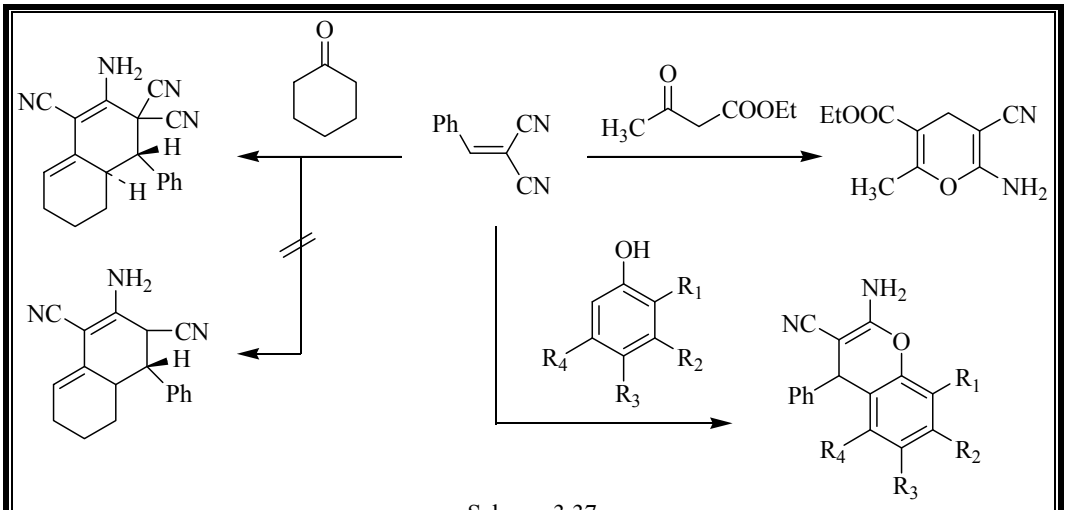
Kumar *et al.* [82] have reported an environmentally benign synthetic process using Magnesium oxide as the catalyst and by process of grinding (Scheme 3.36). This is the classical reaction where in a benzaldehyde or ketone has first been reacted with a malanonitrile which has got an active hydrogen site, yielding the benzylidene malanonitrile which when reacted to a 1,3-Diketo compound herein a meldurms acid afforded the 2-Amino-3-cyanochromene derivative the only difference than the classical methodology is that the reaction has been carried at room temperature and it is grinded which means there are absolutely no solvent which makes it a green process and which is also faster and gives a higher yield.



Scheme 3.36

3.4.12.4 Benzopyrans using chitosan as a catalyst

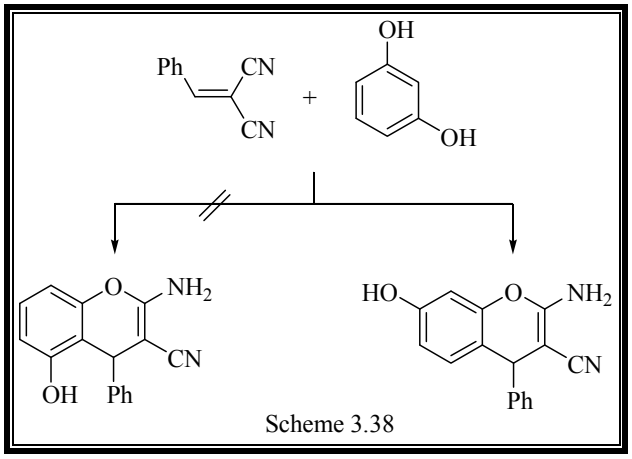
Similarly, Al-Matar *et al.* [83] have synthesized many compounds of this class using chitosan as the catalyst (Scheme 3.37).



Scheme 3.37

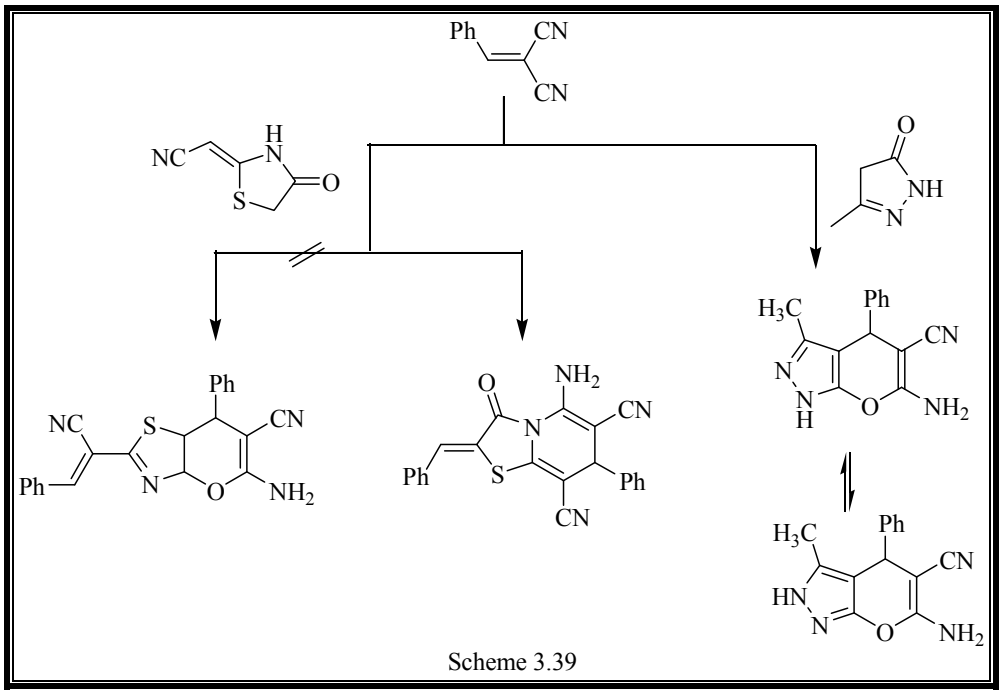
3.4.12.5 Benzopyrans using piperidine as a catalyst

More so ever Al-Matar *et al.* [83] have also studied the formation of the exact product i.e. 2-Amino-3-cyano-7-hydroxy-4*H*-chromene instead of 5-hydroxy derivative when resorcinol is reacted with malanonitrile using piperidine and ethanol. They have come out with this result using the Nuclear Overhauser Effect calculation from the proton NMR spectrum. The reaction scheme is shown in Scheme 3.38.



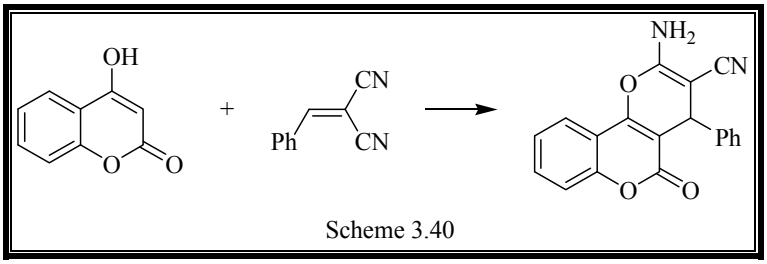
Scheme 3.38

They have also prepared many such compounds using the same methodology but different starting materials as shown in Scheme 3.39.



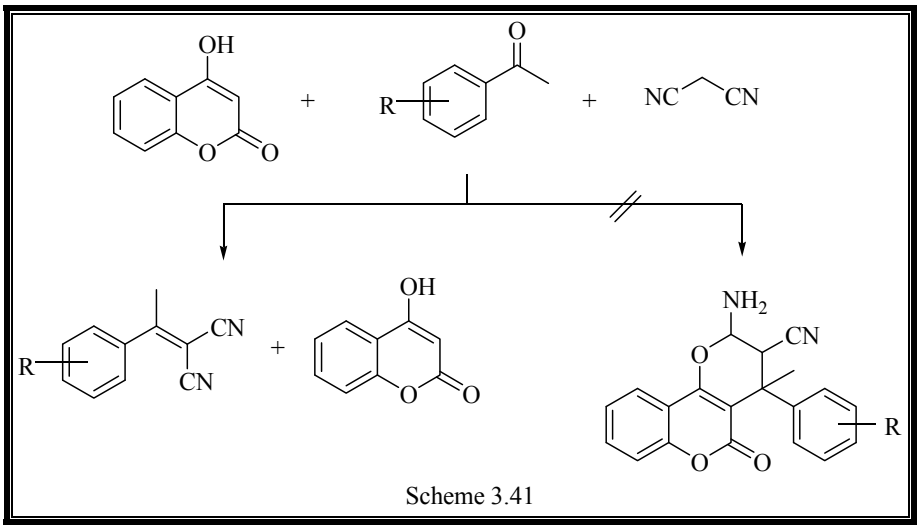
Scheme 3.39

Naliyapara *et al.* [84] have extended this work using 4-Hydroxy coumarin as a starting product (Scheme 3.40).



Scheme 3.40

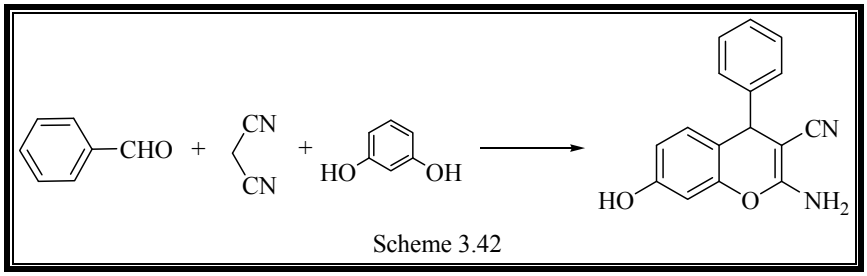
They have synthesized the spiro-compounds using the cyclic ketones to produce the desired results but failed to obtain the chromenes when the aryl ketones were used in the reaction. The reaction schemes followed are shown in Scheme 3.41.



Scheme 3.41

3.4.12.6 Benzopyrans using potassium carbonate as a catalyst

Kidwai *et al*. [85] has prepared the same class of the compounds using water as a solvent and potassium carbonate as the required base catalyst (Scheme 3.42).

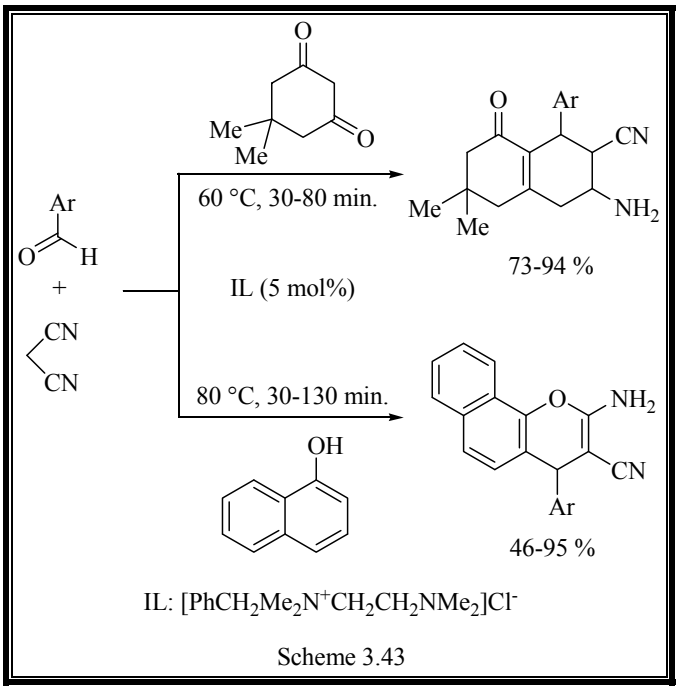


Scheme 3.42

Such compounds were prepared using different starting materials as diverse kinds of aldehydes viz. Phenyl, Quinolyl, Indolyl and alkyl were reacted with malanonitrile in presence of saturated potassium carbonate solution and then microwave irradiation was induced upon the reaction mixture which afforded the 2-Amino-3-cyano-4-substituted phenyl-7-hydroxy-4*H*-chromene dervatives.

3.4.12.7 Ionic liquids as catalyst for the synthesisi of benzopyrans

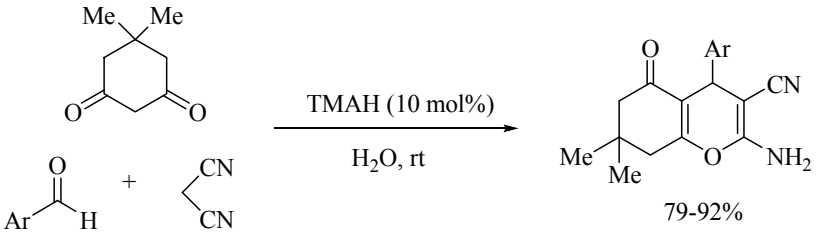
The synthesis of 4*H*-benzo[*b*]pyran derivatives has also been proposed by means of a basic ionic liquid-catalyzed three-component approach involving malononitrile, aromatic aldehydes and dimedone. The conventional method, requiring the use of refluxing DMF or acetic acid, lead to low yields and renders the isolation step troublesome. Other procedures have been described but all of them suffer at least from one limitation. Alternatively, it has been found that a small amount of *N,N*-dimethylaminoethylbenzyl-dimethylammonium chloride catalyzed a rapid and high yielding solvent-free transformation at 60 °C with a wide variation of the aldehyde partner (Scheme 3.43) [86].



Scheme 3.43

While, Peng and Song conducted this MCR in a mixture of catalytically active ionic liquid and water [87], and Lingaiah and co-workers reported the use of a

heterogeneous strong basic Mg/La mixed oxide catalyst in methanol [88]. Compared to the utilization of more classical solvents and organic bases, these strategies combine advantages in efficiency such as shorter reaction times and higher yields, with ecological advantages in terms of recovery and reusability of the catalyst.



Scheme 3.44

This approach has been extended to cyclic 1,3-dicarbonyls for the synthesis of tetrahydrobenzopyrane derivatives, also known as tetrahydrochromenes, which have attracted much attention due to their wide range of biological properties. Thus, a mixture of an aromatic aldehyde, dimedone, and malonitrile in aqueous media catalyzed either by (*S*)-proline [89] or tetramethylammonium hydroxide (TMAH) [90] gave the bicyclic heterocycle in excellent yields (Scheme 3.44).

3.5 Aim of current work

Pyran and fused pyran derivatives have attached a great deal of interest due to their association with various kinds of biological properties. They have been reported for their antimicrobial [91-94], antiviral [95, 96], anticonvulsant [97], cytotoxic [98] and antigenotoxic [99] activities. The incorporation of another heterocyclic moiety in pyrans either in the form of a substituent or as a fused component changes its properties and converts it into an altogether new and important heterocyclic derivative.

Pyrazole have attracted particular interest over the last few decades due to use of such ring system as the core nucleus in various drugs. They are well-known for their activities such as antidiabitic [110], antipyretic [101], anti-inflammatory [102], anti-hypertansive [103], antitumour [104], peptide deformylase inhibitor [105], and antidepressant agents [106]. Considering the importance of pyran and pyrazole derivatives, it was thought worthwile to synthesize new compounds incorporating both these moieties.

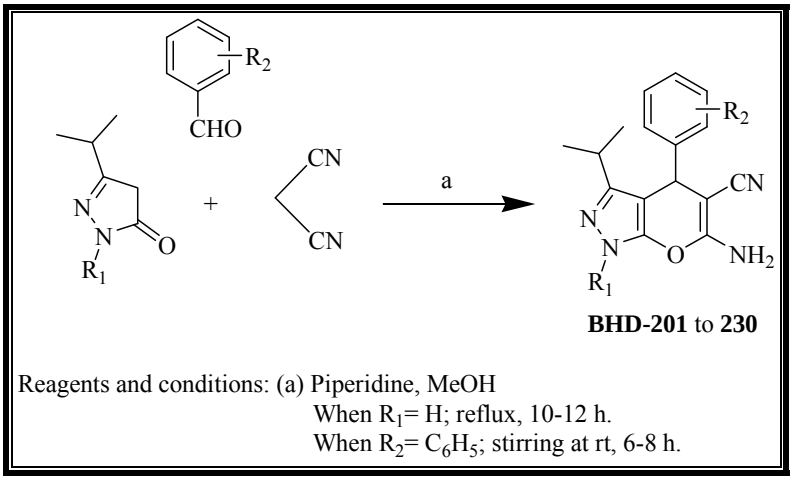
It is pertinent to mention that a large number of pyrazole fused and pyrazole substituted pyran derivatives are reported as biologically important compounds and their chemistry have received considerable attention of chemists in recent days [107-111]. Thus, pyranopyrazoles exhibit useful biological properties such as antimicrobial [112], insecticidal [113], and anti-inflammatory [114]. Furthermore Dihydropyrano [2,3-*c*]pyrazoles showed molluscicidal activity [115, 116] and was identified as a screening hit for Chk1 kinase inhibitor [117].

Over the last years, the chemistry of dihydropyrano[2,3-*c*]pyrazoles has received great interest. The first approach to synthesize these substances was undertaken by Otto [118], in which he initiated the reaction sequence by the base-catalyzed cyclization of 4-aryliden-5-pyrazolone. In a further report, this same group showed that weak bases can also be used for a Michael-type cyclization [119]. Extending the work of Otto, Klokol and colleagues performed the direct conversion of 3-methyl-3-pyrazolin-5-one with malononitrile in the presence of a weak base [120]. Recent methods for the synthesis of 1,4-dihydropyrano[2,3-*c*]pyrazoles include synthesis in aqueous media [49, 72b], under microwave irradiation [70d], and under solvent-free conditions [75, 121].

Thus, in view of the diverse therapeutic activity of pyrano[2,3-*c*]pyrazoles, we report one-pot synthesis of pyrano[2,3-*c*]pyrazole derivatives (**BHD-201** to **230**) by

three-component reaction, a scaffold from which a diverse range of other biologically important New Chemical Entities (NCE's) could be generated. A series of novel 1,4-dihydropyrano[2,3-*c*]pyrazole derivatives (**BHD-201** to **230**) has been synthesized by one-pot three-component cyclocondensation reaction of aromatic aldehydes, malononitrile and substituted pyrazolin-5-ones in the presence of piperidine as catalyst. The mixture refluxing under methanol gives 1,4-dihydropyrano[2,3-*c*]pyrazole derivatives. The products were characterized by FT-IR, mass, ^{1}H NMR spectroscopy and elemental analyses. The newly synthesized compounds were subjected to various biological activities *viz.,* antimicrobial, antimycobacterial, anti-cancer and antiviral.

3.6 Reaction Scheme



BHD-201 to **230**

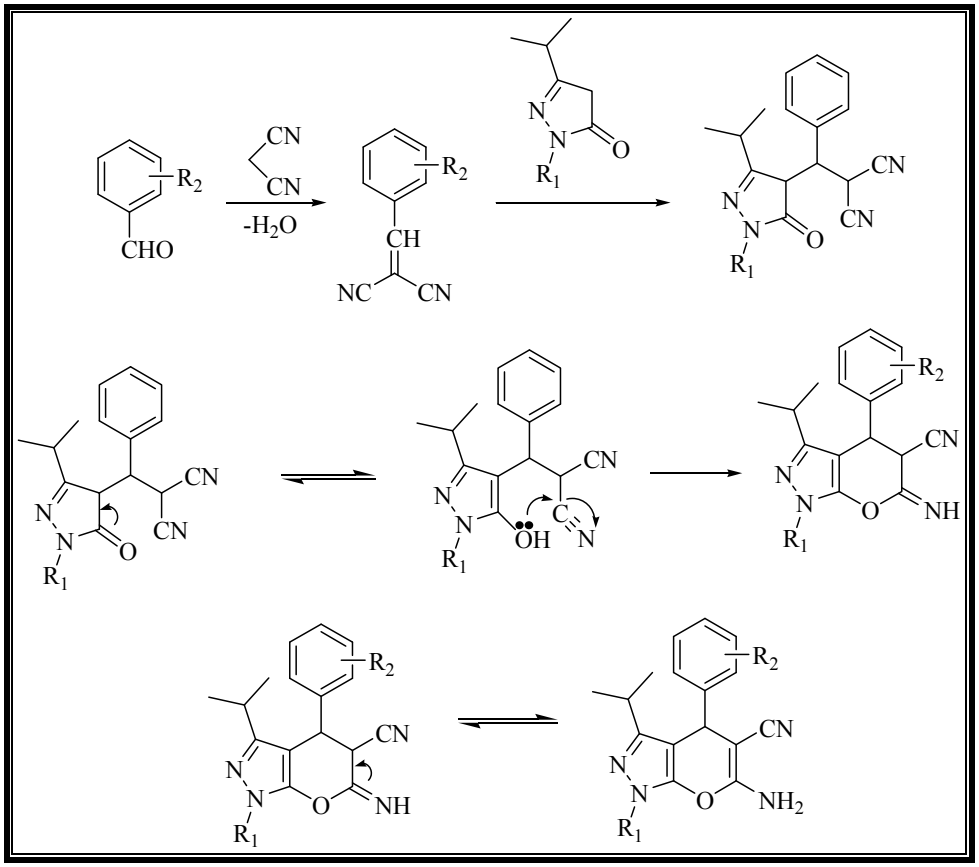
Reagents and conditions: (a) Piperidine, MeOH
When R_1= H; reflux, 10-12 h.
When R_2= C_6H_5; stirring at rt, 6-8 h.

Code	R_1	R_2	M.F.	M.W.	M.P. ºC	Yield %	R_{f1}	R_{f2}
BHD-201	H	H	$C_{16}H_{16}N_4O$	280	201-203	70	0.55	0.70
BHD-202	H	4-F	$C_{16}H_{15}FN_4O$	298	169-171	78	0.51	0.65
BHD-203	H	4-Cl	$C_{16}H_{15}ClN_4O$	314	188-190	81	0.61	0.78
BHD-204	H	4-Br	$C_{16}H_{15}BrN_4O$	358	147-149	72	0.57	0.72
BHD-205	H	4-NO_2	$C_{16}H_{15}N_5O_3$	325	221-223	69	0.48	0.67
BHD-206	H	4-CH_3	$C_{17}H_{18}N_4O$	294	173-175	86	0.60	0.74
BHD-207	H	4-OH	$C_{16}H_{16}N_4O_2$	296	207-209	76	0.52	0.68
BHD-208	H	4-OCH_3	$C_{17}H_{18}N_4O_2$	310	179-181	70	0.62	0.79
BHD-209	H	3,4-OCH_3	$C_{18}H_{20}N_4O_3$	340	142-144	68	0.50	0.68
BHD-210	H	3-Cl	$C_{16}H_{15}ClN_4O$	314	183-185	77	0.56	0.76
BHD-211	H	3-Br	$C_{16}H_{15}BrN_4O$	358	206-208	73	0.49	0.69
BHD-212	H	3-NO_2	$C_{16}H_{15}N_5O_3$	325	227-229	82	0.47	0.68
BHD-213	H	3-OH	$C_{16}H_{16}N_4O_2$	296	234-236	62	0.52	0.73
BHD-214	H	2-Cl	$C_{16}H_{15}ClN_4O$	314	168-170	70	0.50	0.70
BHD-215	H	2-NO_2	$C_{16}H_{15}N_5O_3$	325	213-215	66	0.58	0.74
BHD-216	C_6H_5	H	$C_{22}H_{20}N_4O$	356	157-159	75	0.61	0.81
BHD-217	C_6H_5	4-F	$C_{22}H_{19}FN_4O$	374	162-164	79	0.56	0.67
BHD-218	C_6H_5	4-Cl	$C_{22}H_{19}ClN_4O$	390	142-144	83	0.49	0.65
BHD-219	C_6H_5	4-Br	$C_{22}H_{19}BrN_4O$	434	200-202	67	0.53	0.72
BHD-220	C_6H_5	4-NO_2	$C_{22}H_{19}N_5O_3$	401	187-189	79	0.59	0.78
BHD-221	C_6H_5	4-CH_3	$C_{23}H_{22}N_4O$	370	135-137	85	0.60	0.79
BHD-222	C_6H_5	4-OH	$C_{22}H_{20}N_4O_2$	372	217-219	71	0.61	0.82
BHD-223	C_6H_5	4-OCH_3	$C_{23}H_{22}N_4O_2$	386	196-198	77	0.51	0.69
BHD-224	C_6H_5	3,4-OCH_3	$C_{24}H_{24}N_4O_3$	416	208-210	73	0.64	0.80
BHD-225	C_6H_5	3-Cl	$C_{22}H_{19}ClN_4O$	390	233-235	69	0.52	0.66
BHD-226	C_6H_5	3-Br	$C_{22}H_{19}BrN_4O$	434	156-158	70	0.56	0.70
BHD-227	C_6H_5	3-NO_2	$C_{22}H_{19}N_5O_3$	401	239-241	80	0.50	0.68
BHD-228	C_6H_5	3-OH	$C_{22}H_{20}N_4O_2$	372	214-216	75	0.55	0.71
BHD-229	C_6H_5	2-Cl	$C_{22}H_{19}ClN_4O$	390	174-176	68	0.49	0.63
BHD-230	C_6H_5	2-NO_2	$C_{22}H_{19}N_5O_3$	401	219-221	63	0.59	0.73

TLC Solvent system R_{f1}: Hexane: Ethyl acetate - 6:4;

TLC Solvent system R_{f2}: Chloroform: Methanol - 9:1

3.6.1 Plausible Reaction Mechanism



The mechanism reaction occurs *via* an *in situ* initial formation of the arylidene malonoitrile, containing the electron-poor C=C double bond, from the Knoevenagel condensation between aromatic aldehydes and malononitrile by loss of water molecules. Finally, Michael addition of pyrazolone to the initially formed unsaturated nitrile, i.e. nucleophilic attack of hydroxyl moiety to the cyano moiety affords cyclized pyran derivatives.

3.7 Experimental

3.7.1 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 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.

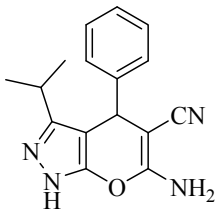
3.7.2 Synthesis of 3-isopropyl-1H-pyrazol-5(4H)-one/3-isopropyl-1-phenyl-1H-pyrazol-5(4H)-one

Synthesis of 3-isopropyl-1*H*-pyrazol-5(4*H*)-one/3-isopropyl-1-phenyl-1*H*-pyrazol-5 (4*H*)-one was prepared by known literature method [122].

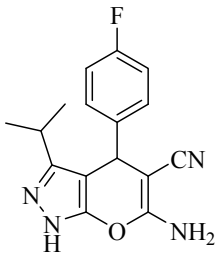
3.7.3 General procedure for the 6-amino-1,4-dihydro-3-isopropyl-4-(aryl)pyrano [2,3-c]pyrazole-5-carbonitrile (BHD 201-215)

A mixture of the malononitrile (0.01 mol), 3-isopropyl-1*H*-pyrazol-5(4*H*)-one (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) in 8-10 mL of MeOH with catalytic amount of piperidine were refluxed for 10-12 h. After completion of the reaction, the reaction mixture was filtered to give the solid products **BHD-201** to **215**, which were recrystallized from ethanol.

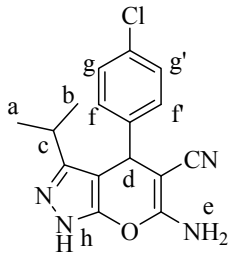
3.7.3.1 6-amino-1,4-dihydro-3-isopropyl-4-phenylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD-201) Yield: 70%; mp 201-203 ºC; MS: *m/z* 280; Anal. Calcd. for $C_{16}H_{16}N_4O$: C, 68.55; H, 5.75; N, 19.99. Found: C, 68.55; H, 5.75; N, 19.99%.



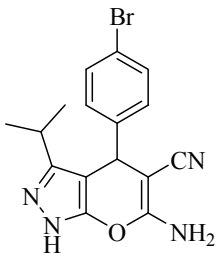
3.7.3.2 6-amino-4-(4-fluorophenyl)-1,4-dihydro-3-isopropylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD-202) Yield: 78%; mp 169-171 ºC; MS: *m/z* 298; Anal. Calcd. for $C_{16}H_{15}FN_4O$: C, 64.42; H, 5.07; N, 18.78. Found: C, 64.42; H, 5.07; N, 18.78%.



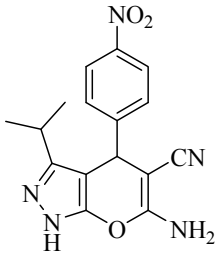
3.7.3.3 6-amino-4-(4-chlorophenyl)-1,4-dihydro-3-isopropylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD-203) Yield: 81%; mp 188-190 ºC; IR (cm^{-1}): 3485 (N-H stretching of free primary amine), 3290 (N-H stretching of pyrazolo ring), 3109 (C-H stretching of aromatic ring), 2198 (C≡N stretching of the nitrile group), 1641 (C=N stretching of pyrazolo ring), 1595 (N-H deformation pyrazolo ring), 1182 (N-N deformation of pyrazolo ring), 1028 (C-H in plane bending of aromatic ring), 813 (C-H out of plane bending for 1,4-disubstituted aromatic ring), 748 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 0.85-0.87 (d, 3H, H_a, J = 6.8 Hz), 1.01-1.03 (d, 3H, H_b, J = 6.9 Hz), 2.50-2.56 (m, 1H, H_c), 4.58 (s, 1H, H_d), 6.47 (s, 2H, H_e), 7.14-7.16 (d, 2H, $H_{ff'}$), 7.27-7.30 (d, 2H, $H_{gg'}$), 12.00 (s, 1H, H_h); MS: *m/z* 314; Anal. Calcd. for $C_{16}H_{15}ClN_4O$: C, 61.05; H, 4.80; N, 17.80. Found: C, 61.05; H, 4.80; N, 17.80%.



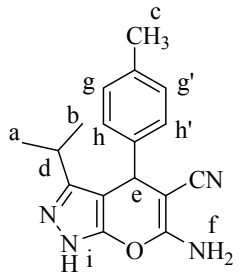
3.7.3.4 6-amino-4-(4-bromophenyl)-1,4-dihydro-3-isopropylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD-204) Yield: 72%; mp 147-149 ºC; MS: *m/z* 358; Anal. Calcd. for $C_{16}H_{15}BrN_4O$: C, 53.50; H, 4.21; N, 15.60. Found: C, 53.50; H, 4.21; N, 15.60%.



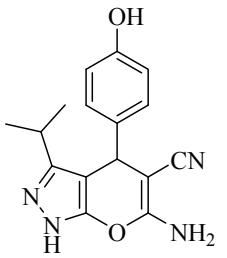
3.7.3.5 6-amino-1,4-dihydro-3-isopropyl-4-(4-nitrophenyl)pyrano[2,3-c]pyrazole-5-carbonitrile (BHD-205) Yield: 69%; mp 221-223 ºC; MS: *m/z* 325; Anal. Calcd. for $C_{16}H_{15}N_5O_3$: C, 59.07; H, 4.65; N, 21.53. Found: C, 59.07; H, 4.65; N, 21.53%.



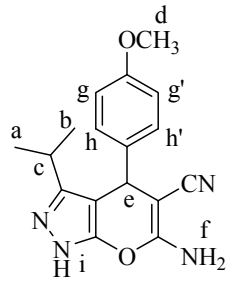
3.7.3.6 6-amino-1,4-dihydro-3-isopropyl-4-p-tolylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD-206) Yield: 86%; mp 173-175 ºC; IR (cm^{-1}): 3479 (N-H stretching of free primary amine), 3271 (N-H stretching of pyrazolo ring), 3111 (C-H stretching of aromatic ring), 3047 (C-H symmetrical stretching of CH_3 group), 2966 (C-H asymmetrical stretching of CH_3 group), 2193 (C≡N stretching of the nitrile group), 1639 (C=N stretching of pyrazolo ring), 1602 (N-H deformation pyrazolo ring), 1367 (C-N stretching of pyrazolo ring), 1188 (N-N deformation of pyrazolo ring), 1026 (C-H in plane bending of aromatic ring), 813 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 0.86-0.88 (d, 3H, H_a, J = 6.8 Hz), 1.01-1.03 (d, 3H, H_b, J = 6.9 Hz), 2.30 (s, 3H, H_c), 2.51-2.58 (m, 1H, H_d), 4.54 (m, 1H, H_e), 5.91 (s, 2H, H_f), 7.00-7.10 (m, 4H, $H_{g\text{-}h'}$), 11.80 (s, 1H, H_i); MS: *m/z* 294; Anal. Calcd. for $C_{17}H_{18}N_4O$: C, 69.37; H, 6.16; N, 19.03. Found: C, 69.37; H, 6.16; N, 19.03%.



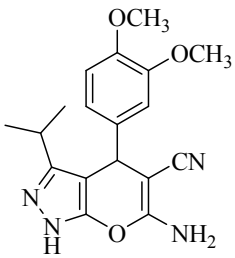
3.7.3.7 6-amino-1,4-dihydro-4-(4-hydroxyphenyl)-3-isopropylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD-207) Yield: 76%; mp 207-209 ºC; MS: *m/z* 296; Anal. Calcd. for $C_{16}H_{16}N_4O_2$: C, 64.85; H, 5.44; N, 18.91. Found: C, 64.85; H, 5.44; N, 18.91%.



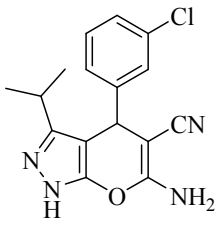
3.7.3.8 6-amino-1,4-dihydro-3-isopropyl-4-(4-methoxyphenyl)pyrano[2,3-c]pyrazole-5-carbonitrile (BHD-208) Yield: 70%; mp 179-181 ºC; IR (cm^{-1}): 3398 (N-H stretching of free primary amine), 3321 (N-H stretching of pyrazolo ring), 3101 (C-H stretching of aromatic ring), 3020 (C-H symmetrical stretching of CH_3 group), 2966 (C-H asymmetrical stretching of CH_3 group), 2193 (C≡N stretching of the nitrile group), 1654 (C=N stretching of pyrazolo ring), 1606 (N-H deformation pyrazolo ring), 1253 (C-O-C asymmetrical stretching of ether linkage), 1172 (N-N deformation of pyrazolo ring), 1107 (C-O-C symmetrical stretching of ether linkage), 1026 (C-H in plane bending of aromatic ring), 819 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 0.84-0.86 (d, 3H, H_a, J = 6.8 Hz), 1.00-1.02 (d, 3H, H_b, J = 6.9 Hz), 2.51-2.58 (m, 1H, H_c), 3.75 (s, 3H, H_d), 4.52 (s, 1H, H_e), 6.36 (s, 2H, H_f), 6.80-6.83 (d, 2H, $H_{gg'}$), 7.06-7.08 (d, 2H, $H_{hh'}$), 11.96 (s, 1H, H_i); MS: *m/z* 310; Anal. Calcd. for $C_{17}H_{18}N_4O_2$: C, 65.79; H, 5.85; N, 18.05. Found: C, 65.79; H, 5.85; N, 18.05%.



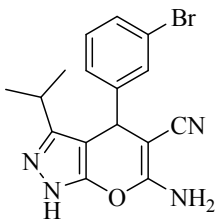
3.7.3.9 6-amino-1,4-dihydro-3-isopropyl-4-(3,4-dimethoxyphenyl)pyrano[2,3-c]pyrazole-5-carbonitrile (BHD-209) Yield: 68%; mp 142-144 ºC; MS: *m/z* 340; Anal. Calcd. for $C_{18}H_{20}N_4O_3$: C, 63.52; H, 5.92; N, 16.46. Found: C, 63.52; H, 5.92; N, 16.46%.



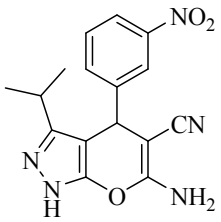
3.7.3.10 6-amino-4-(3-chlorophenyl)-1,4-dihydro-3-isopropylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD-210) Yield: 77%; mp 183-185 ºC; MS: *m/z* 314; Anal. Calcd. for $C_{16}H_{15}ClN_4O$: C, 61.05; H, 4.80; N, 17.80. Found: C, 61.05; H, 4.80; N, 17.80%.



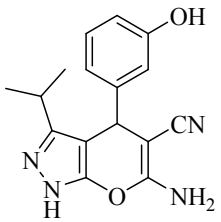
3.7.3.11 6-amino-4-(3-bromophenyl)-1,4-dihydro-3-isopropylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD-211) Yield: 73%; mp 206-208 ºC; MS: *m/z* 358; Anal. Calcd. for $C_{16}H_{15}BrN_4O$: C, 53.50; H, 4.21; N, 15.60. Found: C, 53.50; H, 4.21; N, 15.60%.



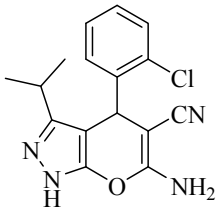
3.7.3.12 6-amino-1,4-dihydro-3-isopropyl-4-(3-nitrophenyl)pyrano[2,3-c]pyrazole-5-carbonitrile (BHD-212) Yield: 82%; mp 227-229 ºC; MS: *m/z* 325; Anal. Calcd. for $C_{16}H_{15}N_5O_3$: C, 59.07; H, 4.65; N, 21.53. Found: C, 59.07; H, 4.65; N, 21.53%.



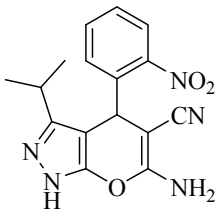
3.7.3.13 6-amino-1,4-dihydro-4-(3-hydroxyphenyl)-3-isopropylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD-213) Yield: 62%; mp 234-236 ºC; MS: *m/z* 296; Anal. Calcd. for $C_{16}H_{16}N_4O_2$: C, 64.85; H, 5.44; N, 18.91. Found: C, 64.85; H, 5.44; N, 18.91%.



3.7.3.14 6-amino-4-(2-chlorophenyl)-1,4-dihydro-3-isopropylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD-214) Yield: 70%; mp 168-170 ºC; MS: *m/z* 314; Anal. Calcd. for $C_{16}H_{15}ClN_4O$: C, 61.05; H, 4.80; N, 17.80. Found: C, 61.05; H, 4.80; N, 17.80%.



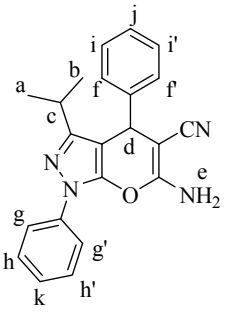
3.7.3.15 6-amino-1,4-dihydro-3-isopropyl-4-(2-nitrophenyl)pyrano[2,3-c]pyrazole-5-carbonitrile (BHD-215) Yield: 66%; mp 213-215 ºC; MS: *m/z* 325; Anal. Calcd. for $C_{16}H_{15}N_5O_3$: C, 59.07; H, 4.65; N, 21.53. Found: C, 59.07; H, 4.65; N, 21.53%.



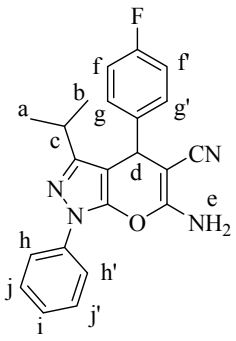
3.7.4 General procedure for the 6-amino-1,4-dihydro-3-isopropyl-4-(aryl)-1-phenylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD 216-230)

A mixture of the malononitrile (0.01 mol), 3-isopropyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) in 8-10 mL of MeOH with catalytic amount of piperidine were stirred at rt for 6-8 h. After completion of the reaction, the reaction mixture was filtered to give the solid products **BHD-216** to **230**, which were recrystallized from ethanol.

3.7.4.1 6-amino-1,4-dihydro-3-isopropyl-1,4-diphenylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD-216) Yield: 75%; mp 157-159 ºC; IR (cm^{-1}): 3471 (N-H stretching of free primary amine), 3321 (N-H stretching of pyrazolo ring), 3063 (C-H stretching of aromatic ring), 2196 (C≡N stretching of the nitrile group), 1658 (C=N stretching of pyrazolo ring), 1581 (N-H deformation pyrazolo ring), 1338 (C-N stretching of pyrazolo ring), 1180 (N-N deformation of pyrazolo ring), 1026 (C-H in plane bending of aromatic ring), 846 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 0.81-0.83 (d, 3H, H_a, J = 6.8 Hz), 1.00-1.02 (d, 3H, H_b, J = 6.9 Hz), 2.51-2.57 (m, 1H, H_c), 4.56 (s, 1H, H_d), 6.29 (s, 2H, H_e), 7.14-7.31 (m, 10H, $H_{ff\text{-}k}$); MS: *m/z* 356; Anal. Calcd. for $C_{22}H_{20}N_4O$: C, 74.14; H, 5.66; N, 15.72. Found: C, 74.14; H, 5.66; N, 15.72%.

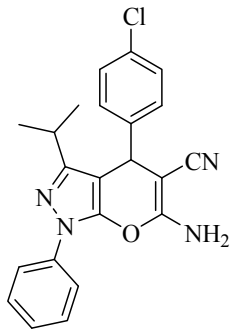


3.7.4.2 6-amino-4-(4-fluorophenyl)-1,4-dihydro-3-isopropyl-1-phenylpyrano[2,3-c]



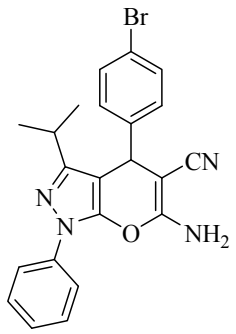
pyrazole-5-carbonitrile (BHD-217) Yield: 79%; mp 162-164 ºC; IR (cm^{-1}): 3458 (N-H stretching of free primary amine), 3325 (N-H stretching of pyrazolo ring), 3063 (C-H stretching of aromatic ring), 2200 (C≡N stretching of the nitrile group), 1664 (C=N stretching of pyrazolo ring), 1600 (N-H deformation pyrazolo ring), 1342 (C-N stretching of pyrazolo ring), 1155 (N-N deformation of pyrazolo ring), 1024 (C-H in plane bending of aromatic ring), 813 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ^{1}H NMR (DMSO-d_6) δ ppm: 0.89-0.91 (d, 3H, H_a, J = 6.8 Hz), 1.04-1.06 (d, 3H, H_b, J = 6.9 Hz), 2.43-2.48 (m, 1H, H_c), 4.64 (s, 1H, H_d), 6.62 (s, 2H, H_e), 6.90-7.76 (m, 10H, $H_{ff'-jj'}$); MS: *m/z* 374; Anal. Calcd. for $C_{22}H_{19}FN_4O$: C, 70.57; H, 5.11; N, 14.96. Found: C, 70.57; H, 5.11; N, 14.96%.

3.7.4.3 6-amino-4-(4-chlorophenyl)-1,4-dihydro-3-isopropyl-1-phenylpyrano[2,3-c]



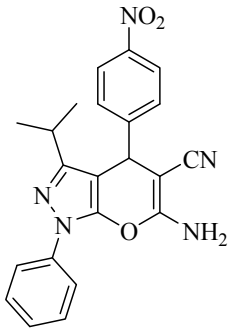
pyrazole-5-carbonitrile (BHD-218) Yield: 83%; mp 142-144 ºC; MS: *m/z* 390; Anal. Calcd. for $C_{22}H_{19}ClN_4O$: C, 67.60; H, 4.90; N, 14.33. Found: C, 67.60; H, 4.90; N, 14.33%.

3.7.4.4 6-amino-4-(4-bromophenyl)-1,4-dihydro-3-isopropyl-1-phenylpyrano[2,3-c]

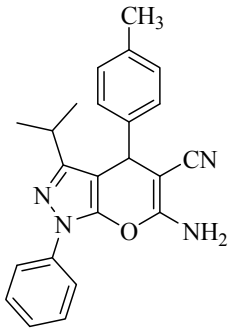


pyrazole-5-carbonitrile (BHD-219) Yield: 67%; mp 200-202 ºC; MS: *m/z* 434; Anal. Calcd. for $C_{22}H_{19}BrN_4O$: C, 70.57; H, 5.11; N, 14.96. Found: C, 70.57; H, 5.11; N, 14.96%.

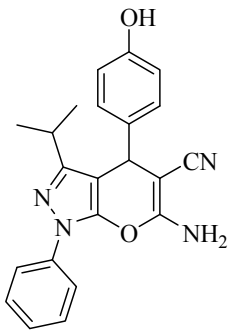
3.7.4.5 6-amino-1,4-dihydro-3-isopropyl-4-(4-nitrophenyl)-1-phenylpyrano[2,3-c] pyrazole-5-carbonitrile (BHD-220) Yield: 79%; mp 187-189 ºC; MS: *m/z* 401; Anal. Calcd. for $C_{22}H_{19}N_5O_3$: C, 65.83; H, 4.77; N, 17.45. Found: C, 65.83; H, 4.77; N, 17.45%.



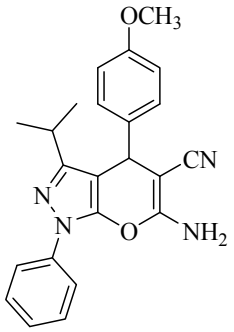
3.7.4.6 6-amino-1,4-dihydro-3-isopropyl-1-phenyl-4-p-tolylpyrano[2,3-c]pyrazole-5-carbonitrile (BHD-221) Yield: 85%; mp 135-137 ºC; MS: *m/z* 370; Anal. Calcd. for $C_{23}H_{22}N_4O$: C, 74.57; H, 5.99; N, 15.12. Found: C, 74.57; H, 5.99; N, 15.12%.



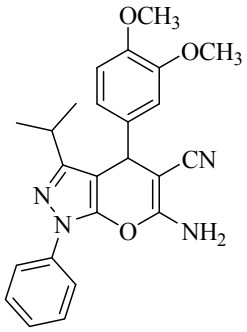
3.7.4.7 6-amino-1,4-dihydro-4-(4-hydroxyphenyl)-3-isopropyl-1-phenylpyrano[2,3-c] pyrazole-5-carbonitrile (BHD-222) Yield: 71%; mp 217-219 ºC; MS: *m/z* 372; Anal. Calcd. for $C_{22}H_{20}N_4O_2$: C, 70.95; H, 5.41; N, 15.04. Found: C, 70.95; H, 5.41; N, 15.04%.



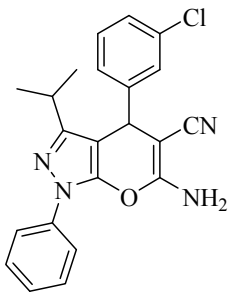
3.7.4.8 6-amino-1,4-dihydro-3-isopropyl-4-(4-methoxyphenyl)-1-phenylpyrano[2,3-c] pyrazole-5-carbonitrile (BHD-223) Yield: 77%; mp 196-198 ºC; MS: *m/z* 386; Anal. Calcd. for $C_{23}H_{22}N_4O_2$: C, 71.48; H, 5.74; N, 14.50. Found: C, 71.48; H, 5.74; N, 14.50%.



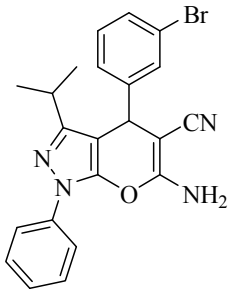
3.7.4.9 6-amino-1,4-dihydro-3-isopropyl-4-(3,4-dimethoxyphenyl)-1-phenylpyrano [2,3-c]pyrazole-5-carbonitrile (BHD-224) Yield: 73%; mp 208-210 ºC; MS: *m/z* 416; Anal. Calcd. for $C_{24}H_{24}N_4O_3$: C, 69.21; H, 5.81; N, 13.45. Found: C, 69.21; H, 5.81; N, 13.45 %.



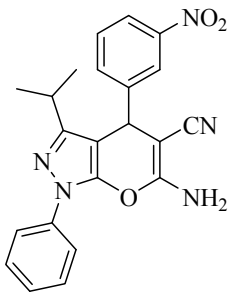
3.7.4.10 6-amino-4-(3-chlorophenyl)-1,4-dihydro-3-isopropyl-1-phenylpyrano[2,3-c] pyrazole-5-carbonitrile (BHD-225) Yield: 69%; mp 233-235 ºC; MS: *m/z* 390; Anal. Calcd. for $C_{22}H_{19}ClN_4O$: C, 67.60; H, 4.90; N, 14.33. Found: C, 67.60; H, 4.90; N, 14.33%.



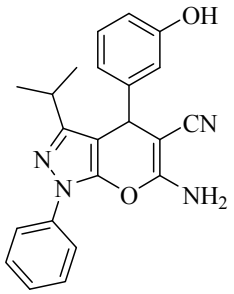
3.7.4.11 6-amino-4-(3-bromophenyl)-1,4-dihydro-3-isopropyl-1-phenylpyrano[2,3-c] pyrazole-5-carbonitrile (BHD-226) Yield: 70%; mp 156-158 ºC; MS: *m/z* 434; Anal. Calcd. for $C_{22}H_{19}BrN_4O$: C, 70.57; H, 5.11; N, 14.96. Found: C, 70.57; H, 5.11; N, 14.96%.



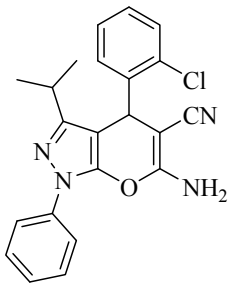
3.7.4.12 6-amino-1,4-dihydro-3-isopropyl-4-(3-nitrophenyl)-1-phenylpyrano[2,3-c] pyrazole-5-carbonitrile (BHD-227) Yield: 80%; mp 239-241 ºC; MS: *m/z* 401; Anal. Calcd. for $C_{22}H_{19}N_5O_3$: C, 65.83; H, 4.77; N, 17.45. Found: C, 65.83; H, 4.77; N, 17.45%.



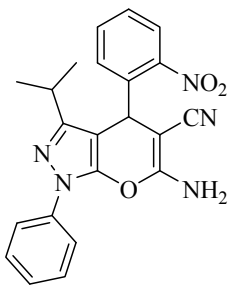
3.7.4.13 6-amino-1,4-dihydro-4-(3-hydroxyphenyl)-3-isopropyl-1-phenylpyrano[2,3-c] pyrazole-5-carbonitrile (BHD-228) Yield: 75%; mp 214-216 ºC; MS: *m/z* 372; Anal. Calcd. for $C_{22}H_{20}N_4O_2$: C, 70.95; H, 5.41; N, 15.04. Found: C, 70.95; H, 5.41; N, 15.04%.



3.7.4.14 6-amino-4-(2-chlorophenyl)-1,4-dihydro-3-isopropyl-1-phenylpyrano[2,3-c] pyrazole-5-carbonitrile (BHD-229) Yield: 68%; mp 174-176 ºC; MS: *m/z* 390; Anal. Calcd. for $C_{22}H_{19}ClN_4O$: C, 67.60; H, 4.90; N, 14.33. Found: C, 67.60; H, 4.90; N, 14.33%.



3.7.4.15 6-amino-1,4-dihydro-3-isopropyl-4-(2-nitrophenyl)-1-phenylpyrano[2,3-c] pyrazole-5-carbonitrile (BHD-230) Yield: 63%; mp 219-221 ºC; MS: *m/z* 401; Anal. Calcd. for $C_{22}H_{19}N_5O_3$: C, 65.83; H, 4.77; N, 17.45. Found: C, 65.83; H, 4.77; N, 17.45%.

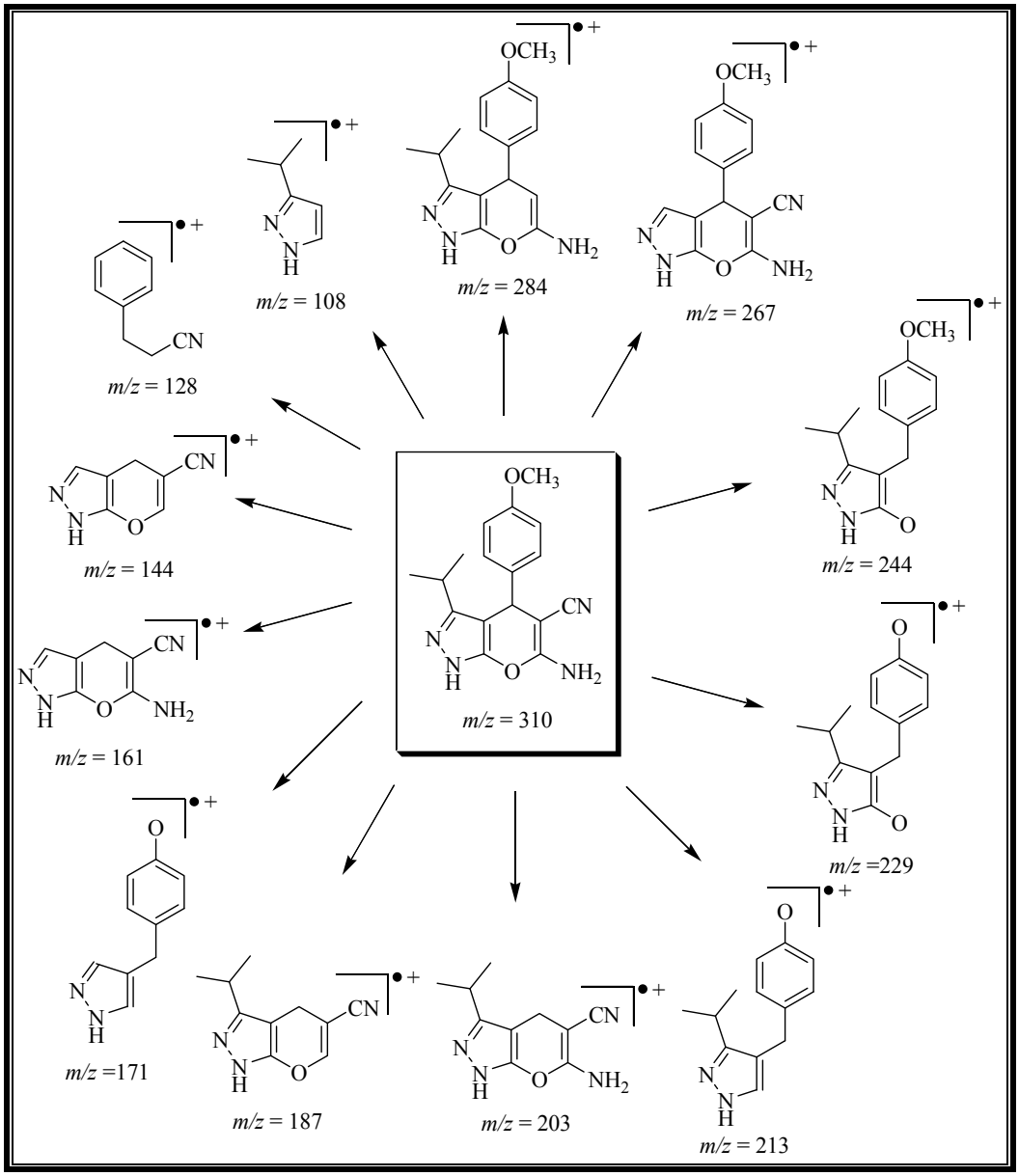


3.8 Spectral discussion

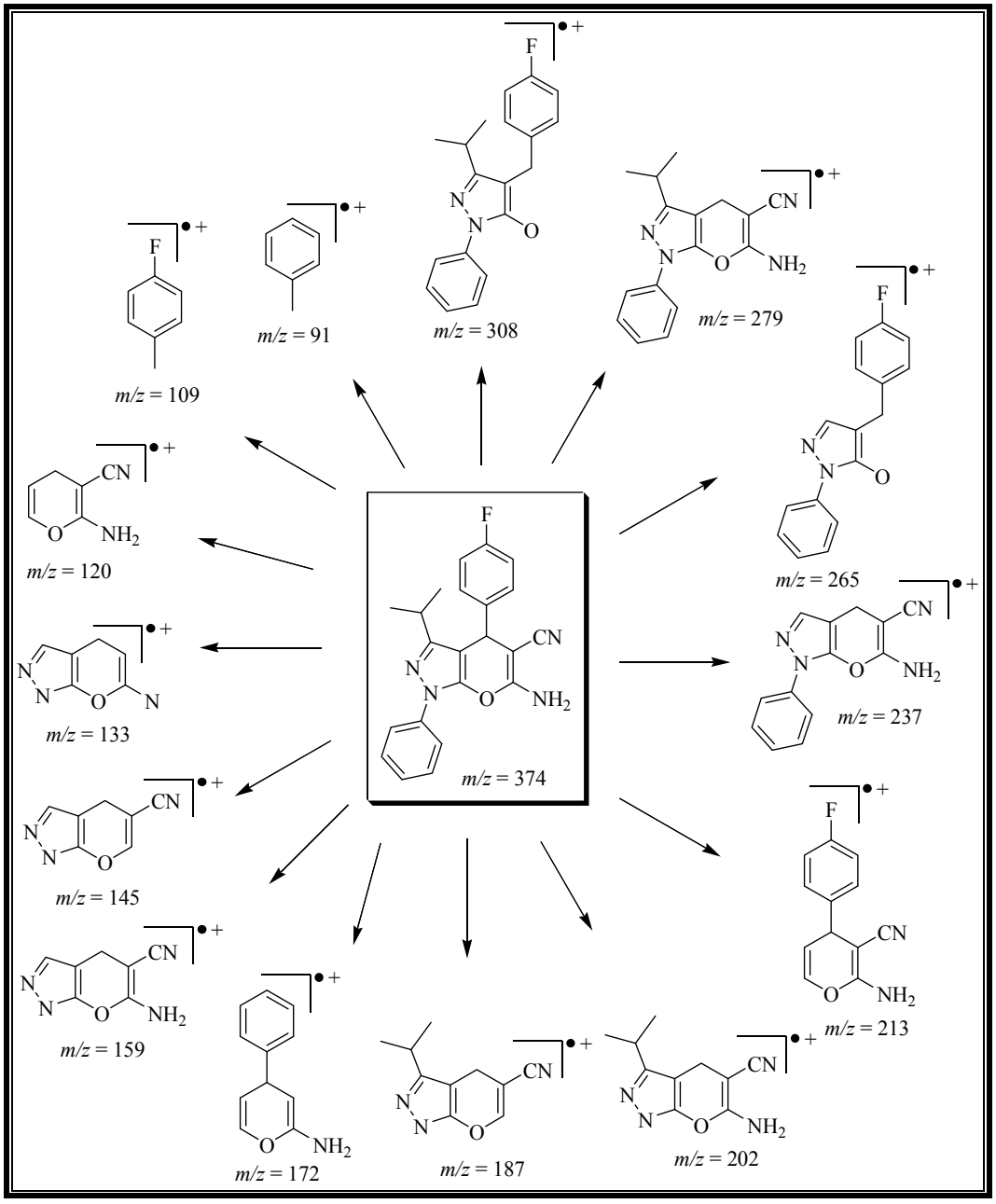
3.8.1 Mass spectral study

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.

3.8.1.1 Mass fragmentation pattern for BHD-208



3.8.1.2 Mass fragmentation pattern for BHD-217



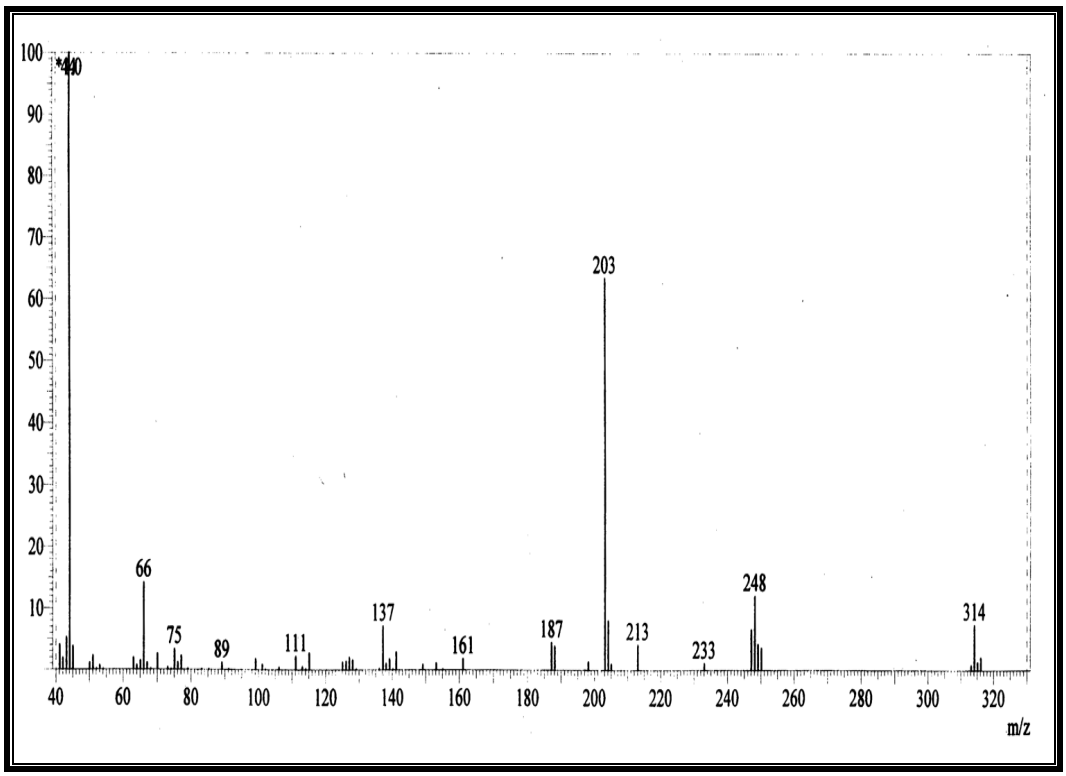
3.8.2 IR spectral study

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 pyrano[2,3-*c*]pyrazoles (BHD-201 to 230), confirmatory bands for primary amine (-NH_2) and nitrile (C≡N) stretching band was observed at 3400-3500 cm^{-1} and 2190-2220 cm^{-1} respectively. Another characteristic band for N-H deformation was observed at 1580-1620 cm^{-1}, which suggested the formation of pyranopyrazoles ring system.

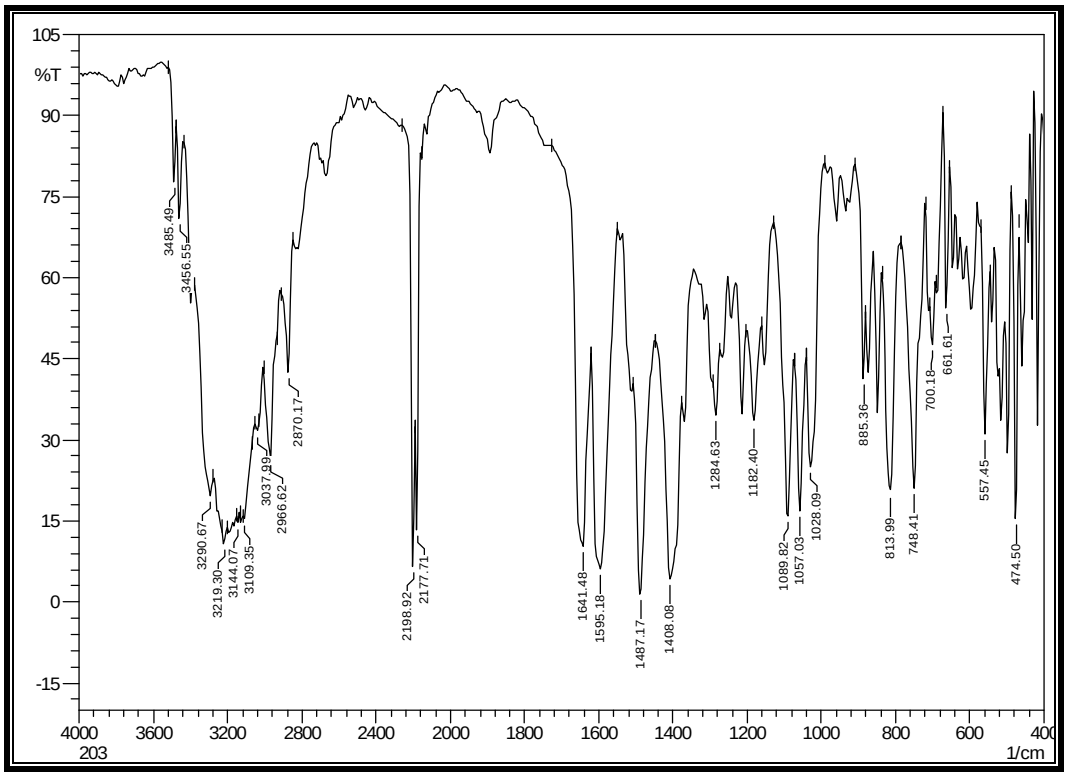
3.8.3 ^{1}H NMR spectral study

^{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. ^{1}H NMR spectra confirmed the structures of pyrano[2,3-*c*]pyrazoles (BHD-201 to 230) on the basis of following signals: singlet for primary amino group proton was observed at 5.91-6.62 δ ppm and a singlet for the methine proton of pyran ring at 4.52-4.64 δ ppm. The aromatic ring protons and *J* value were found to be in accordance with substitution pattern on phenyl ring.

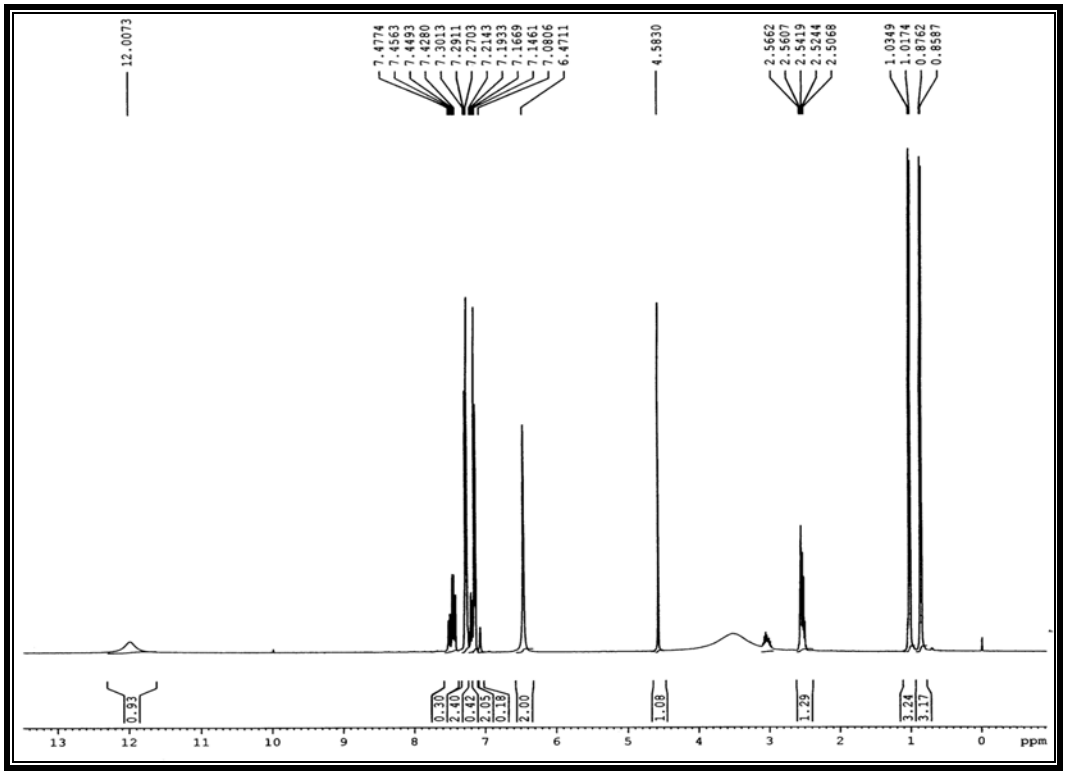
Mass spectrum of BHD-203



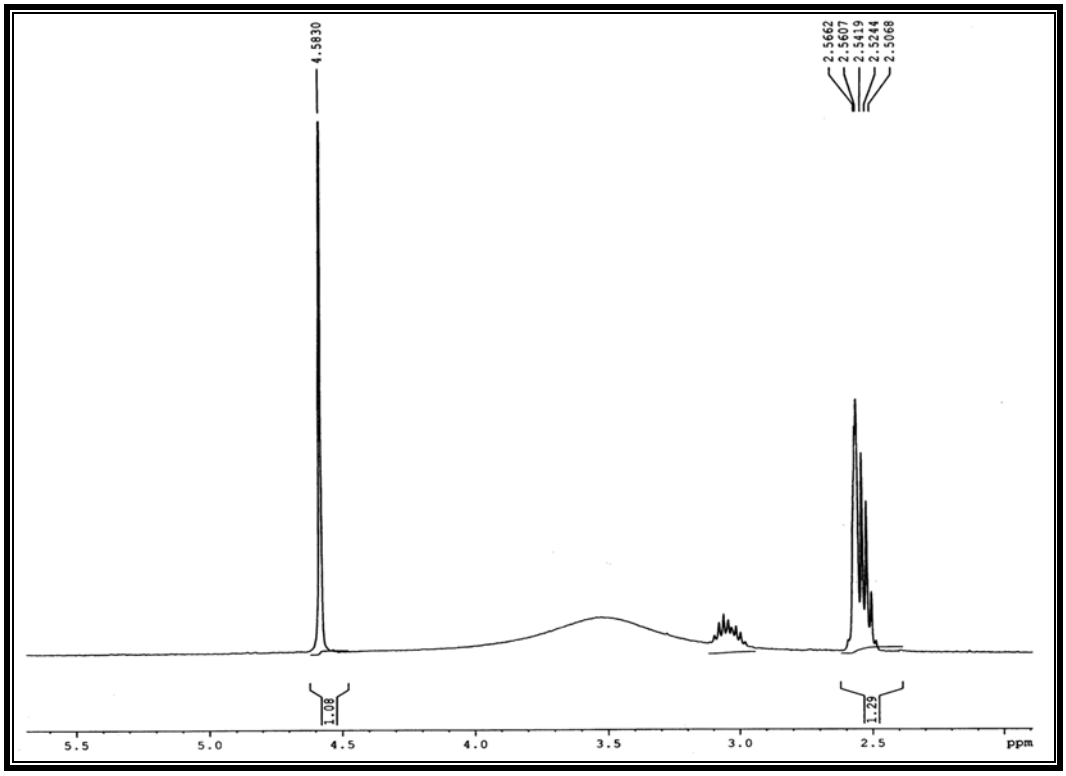
IR spectrum of BHD-203



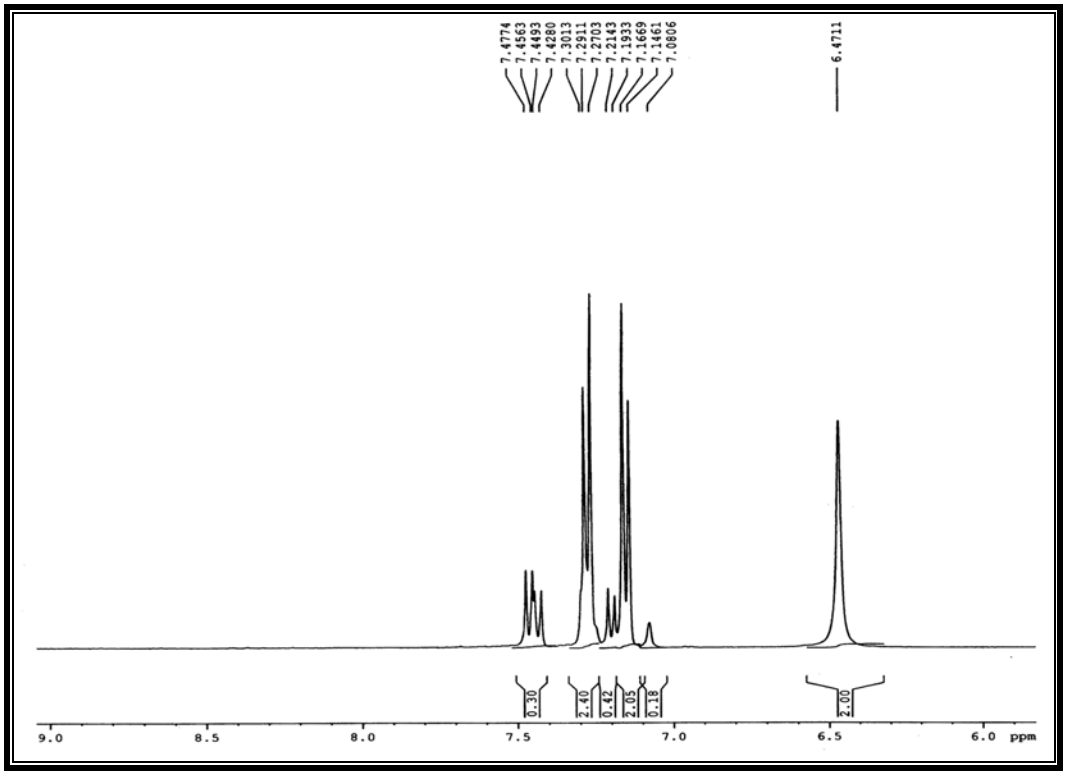
^{1}H NMR spectrum of BHD-203



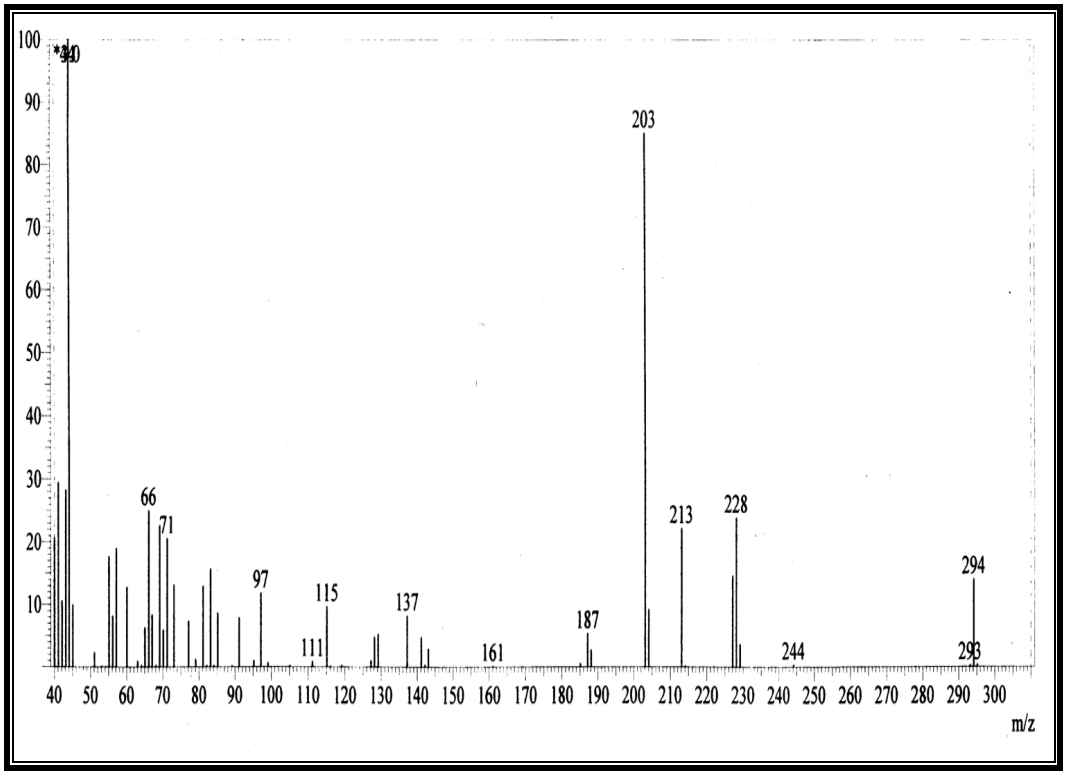
Expanded ^{1}H NMR spectrum of BHD-203



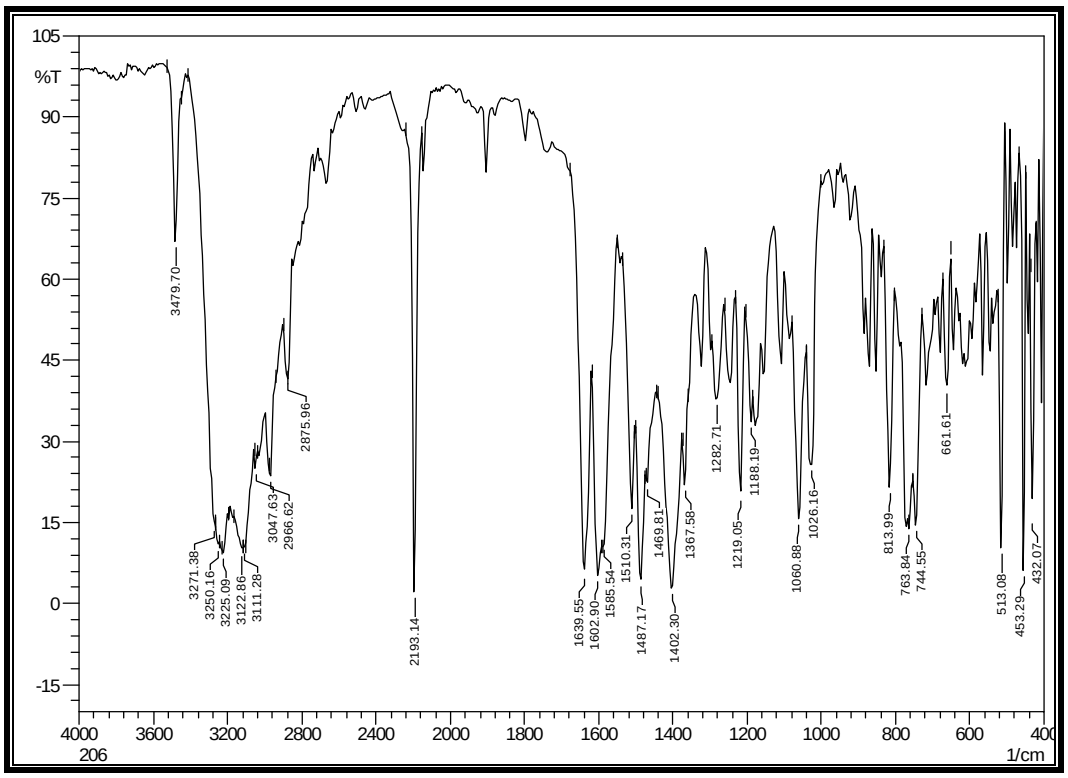
Expanded ^{1}H NMR spectrum of BHD-203



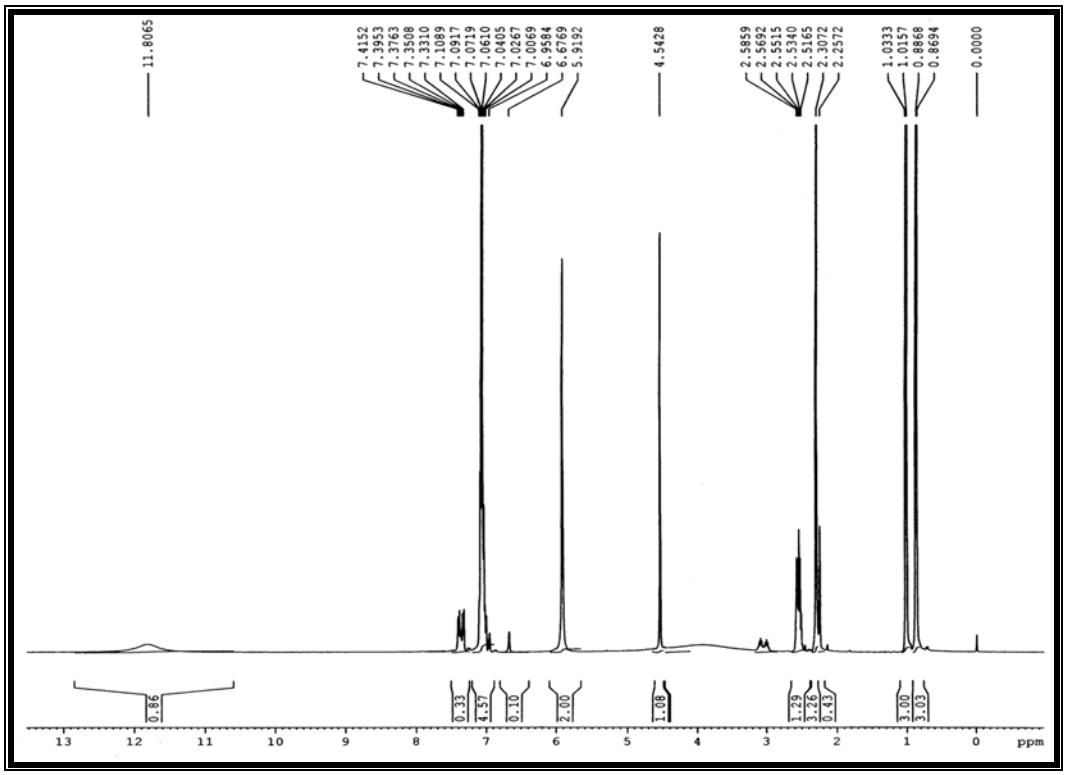
Mass spectrum of BHD-206



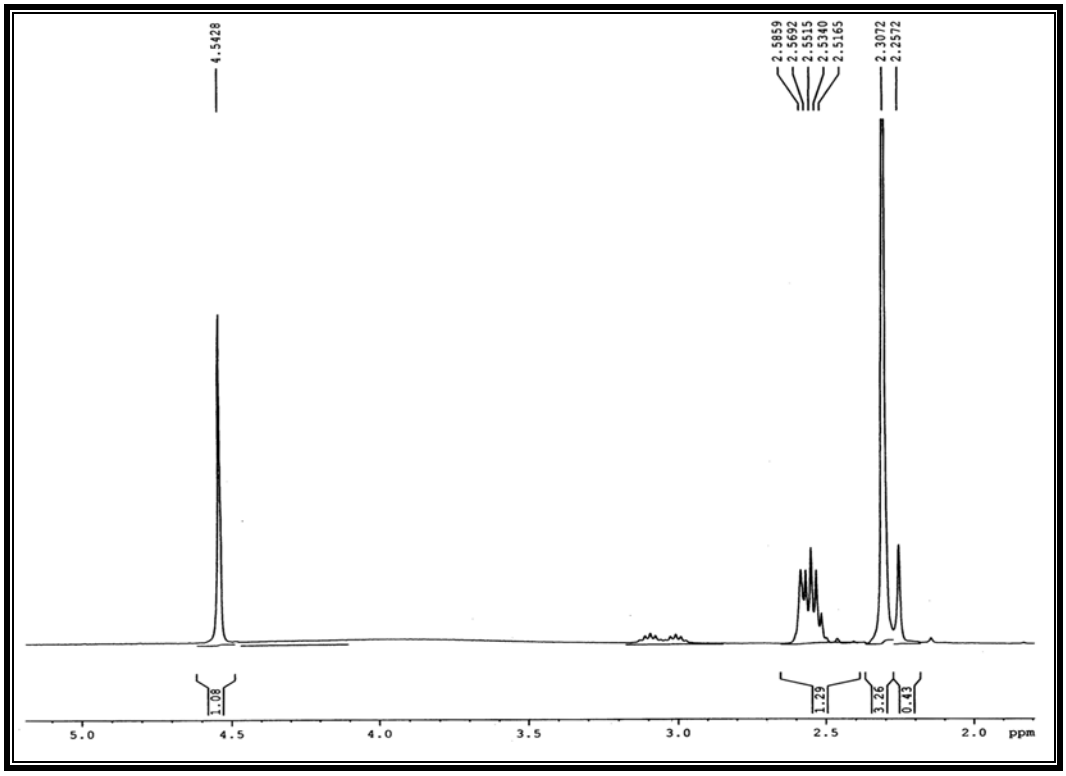
IR spectrum of BHD-206



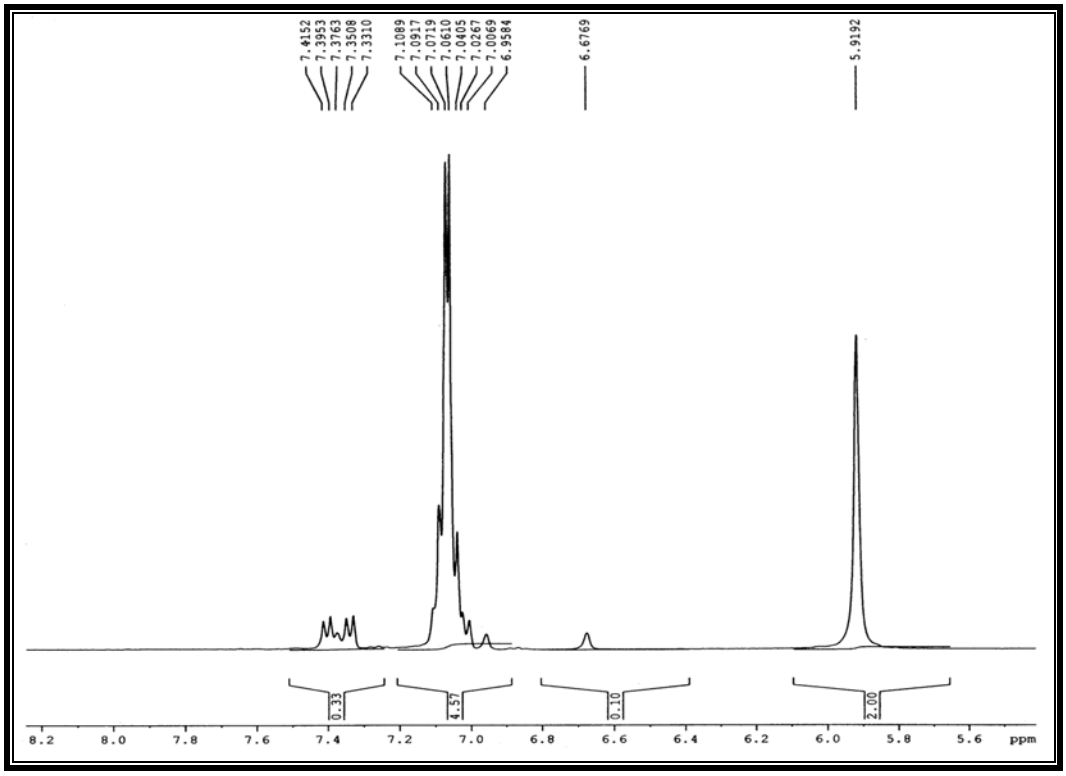
^{1}H NMR spectrum of BHD-206



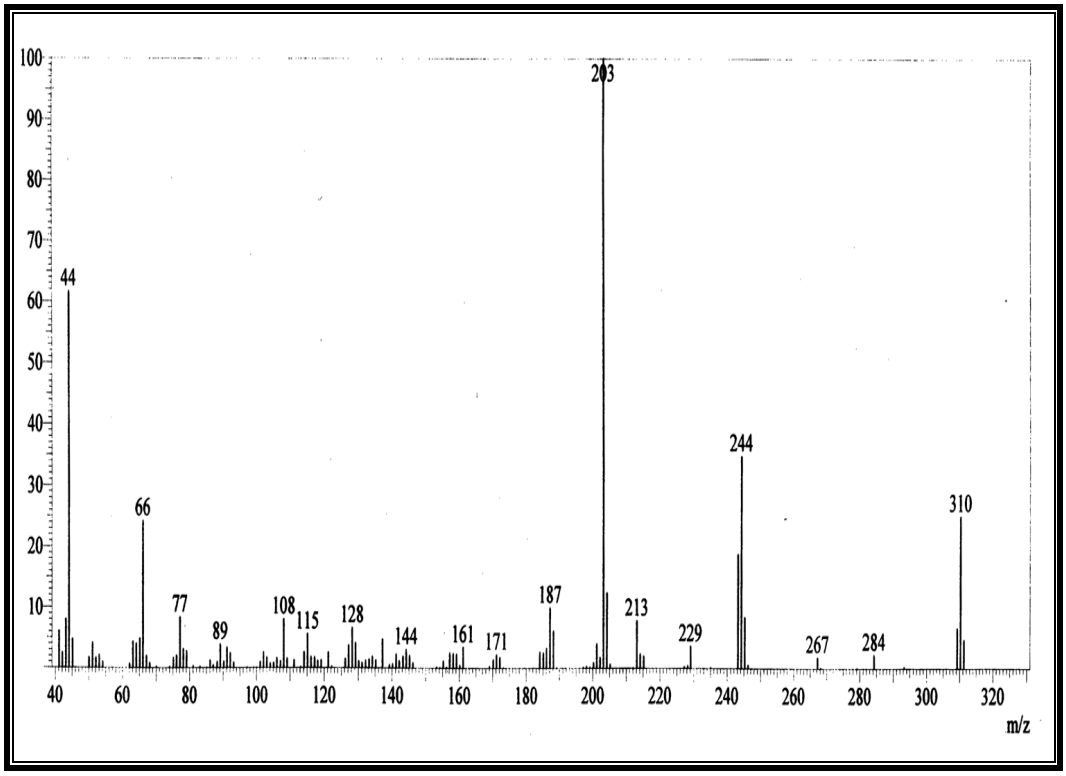
Expanded ^{1}H NMR spectrum of BHD-206



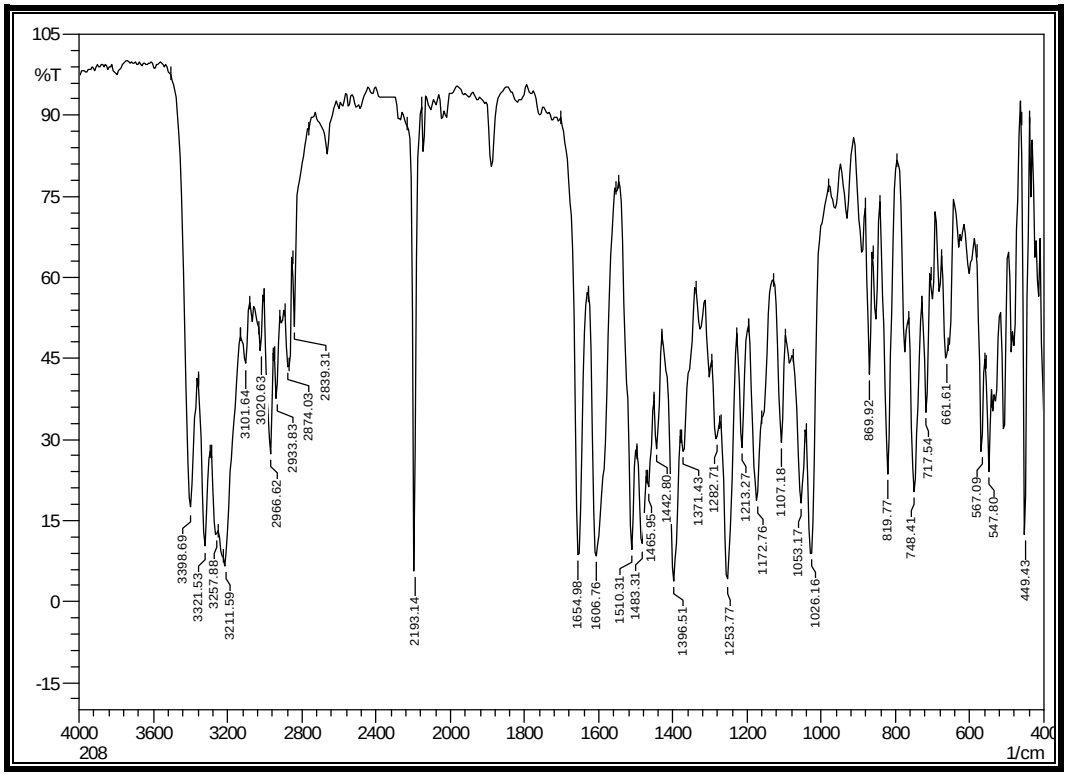
Expanded ^{1}H NMR spectrum of BHD-206



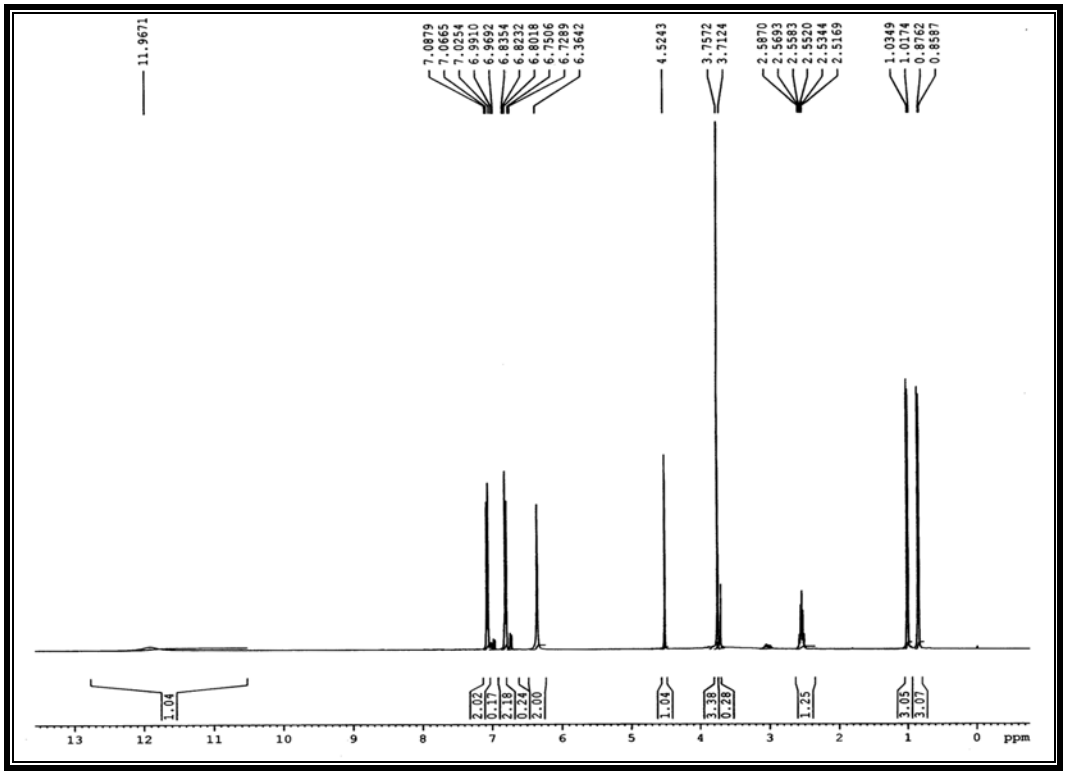
Mass spectrum of BHD-208



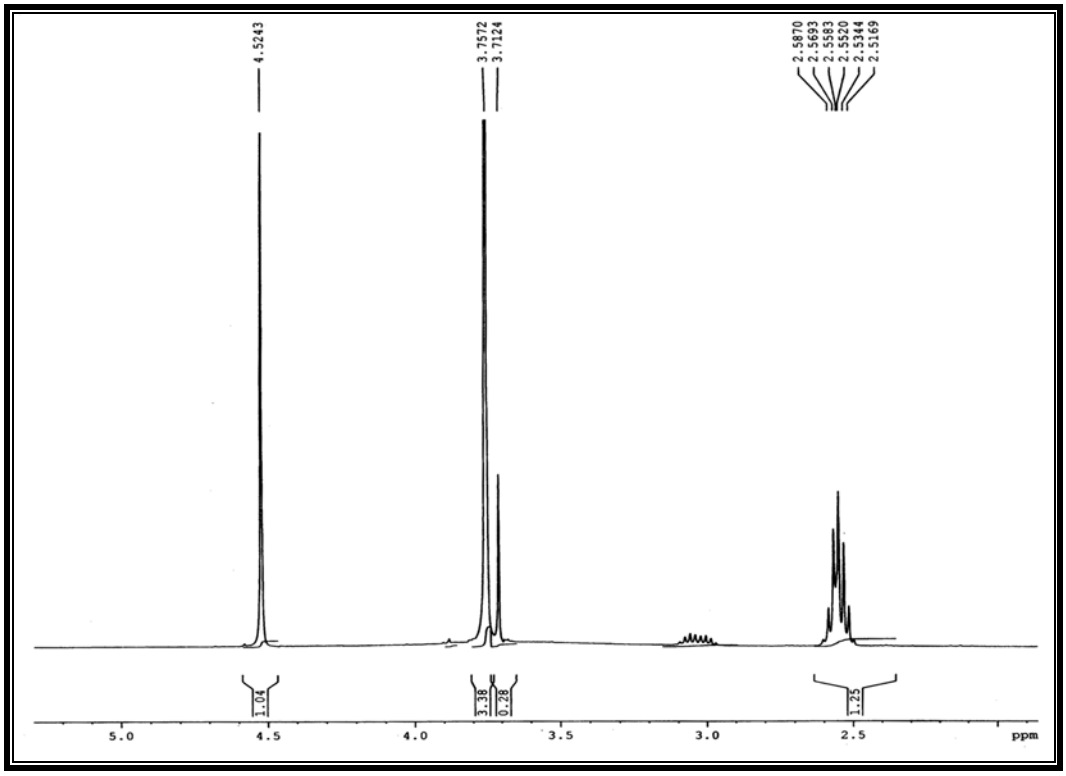
IR spectrum of BHD-208



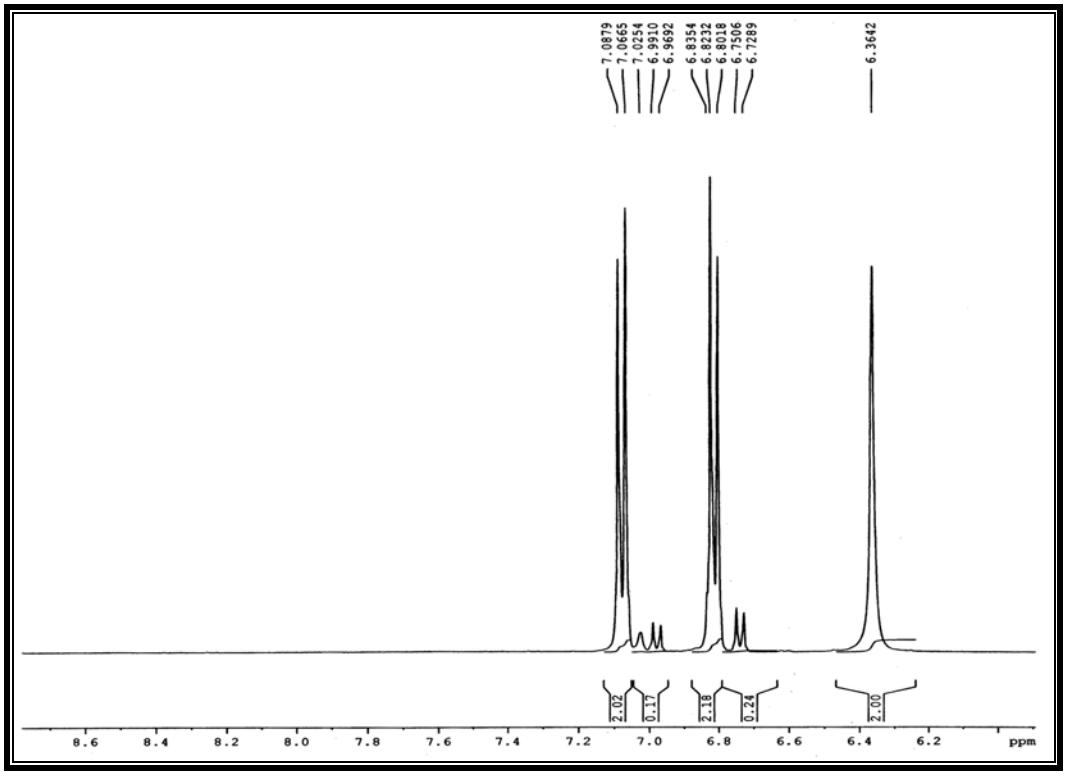
^{1}H NMR spectrum of BHD-208



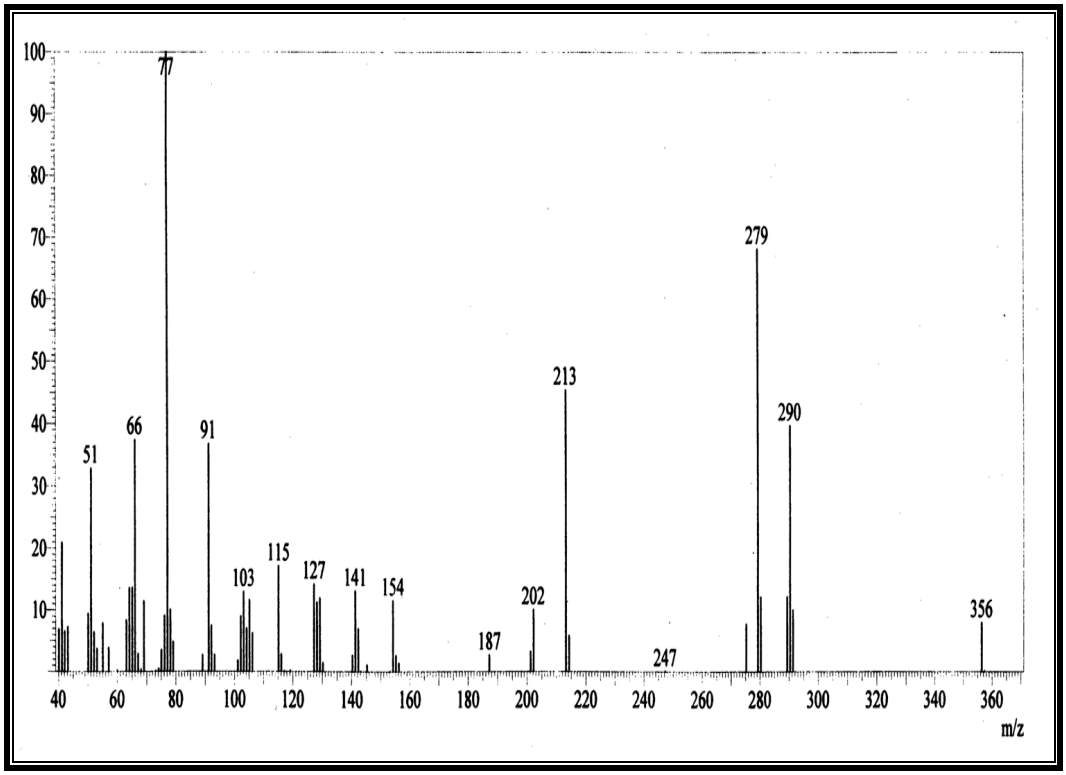
Expanded ^{1}H NMR spectrum of BHD-208



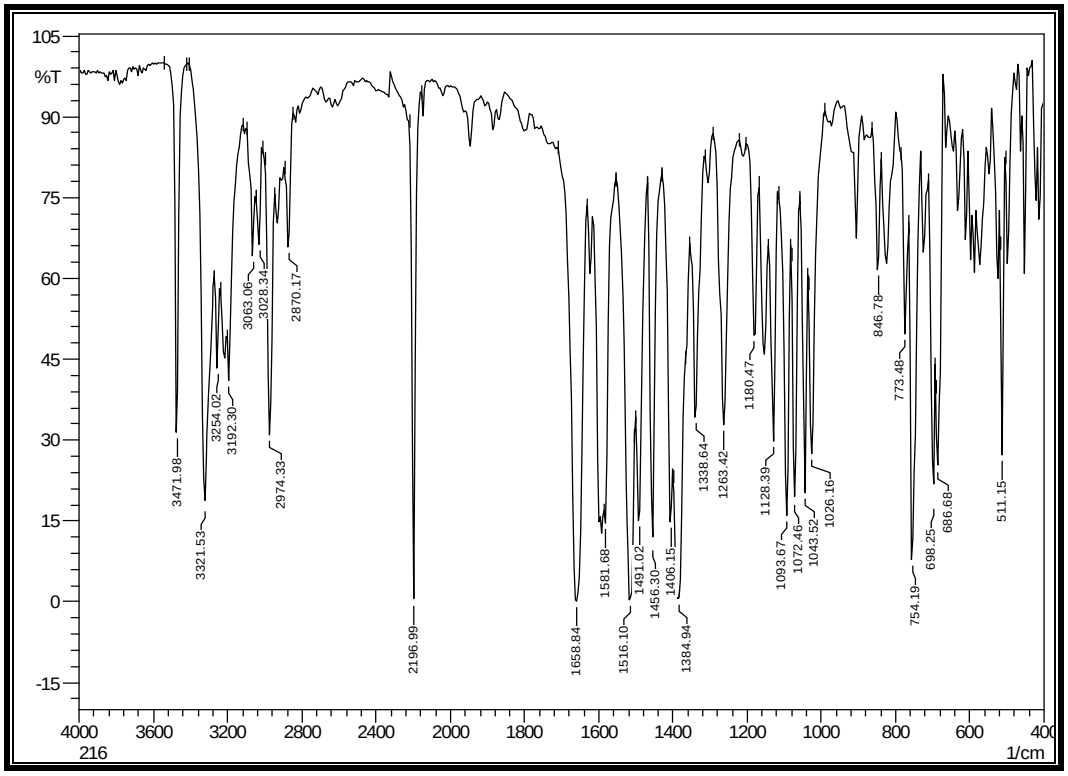
Expanded ^{1}H NMR spectrum of BHD-208



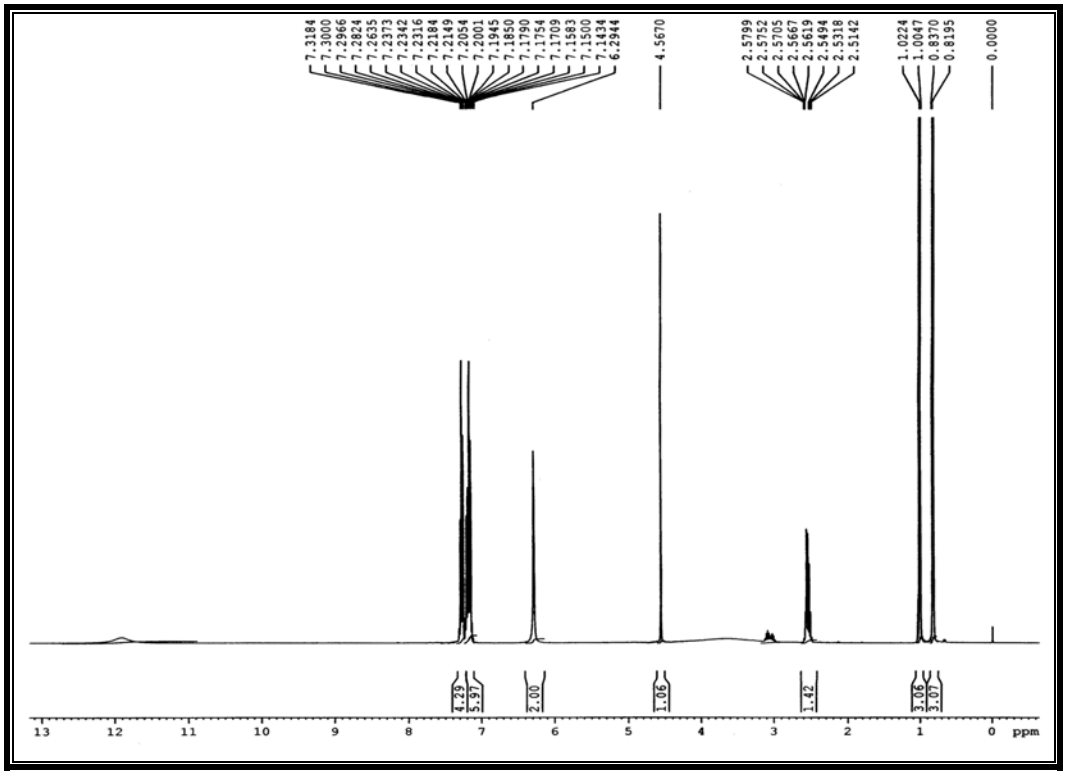
Mass spectrum of BHD-216



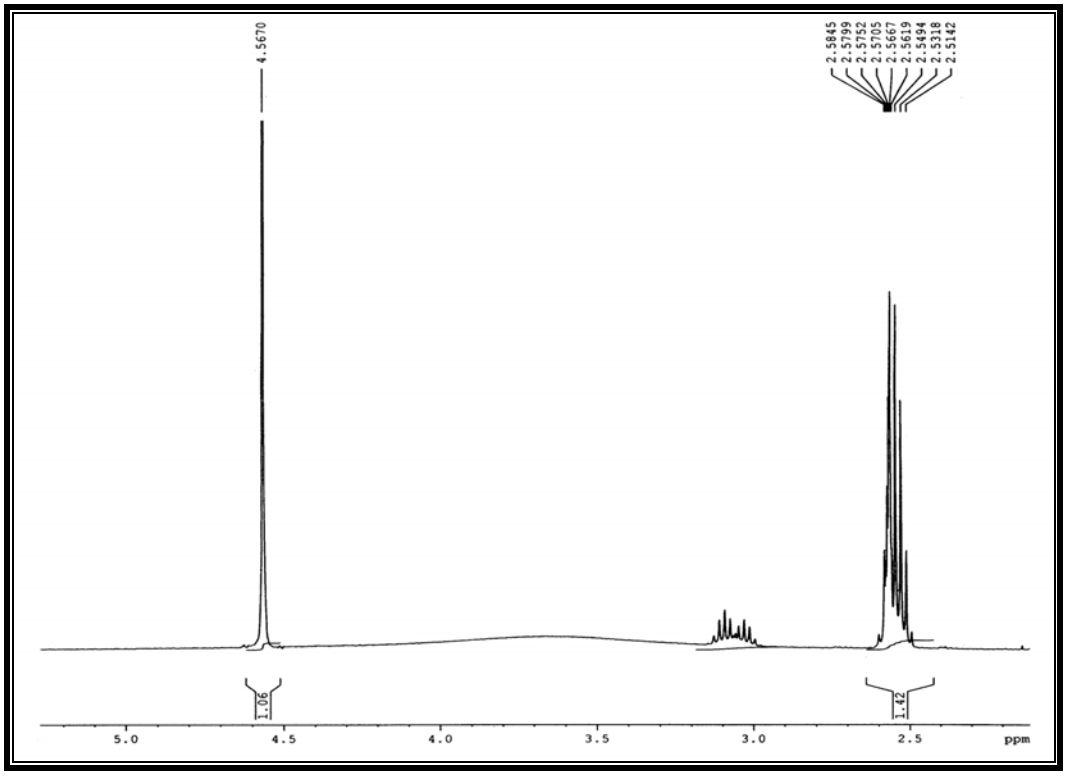
IR spectrum of BHD-216



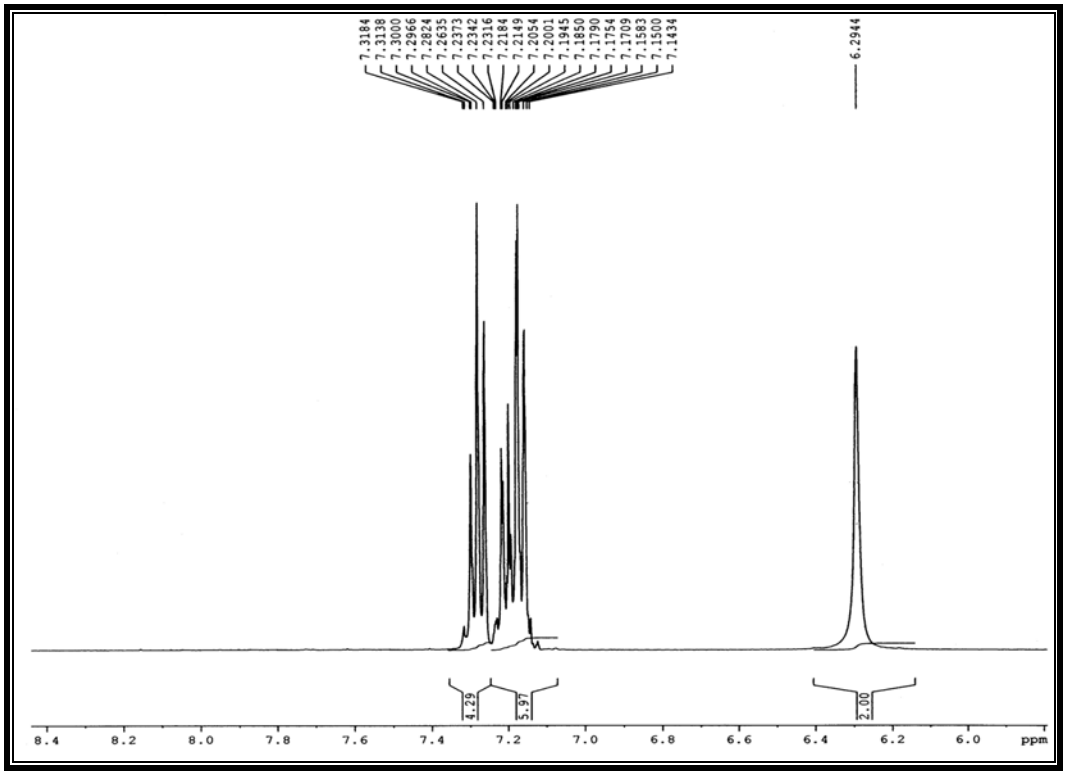
^{1}H NMR spectrum of BHD-216



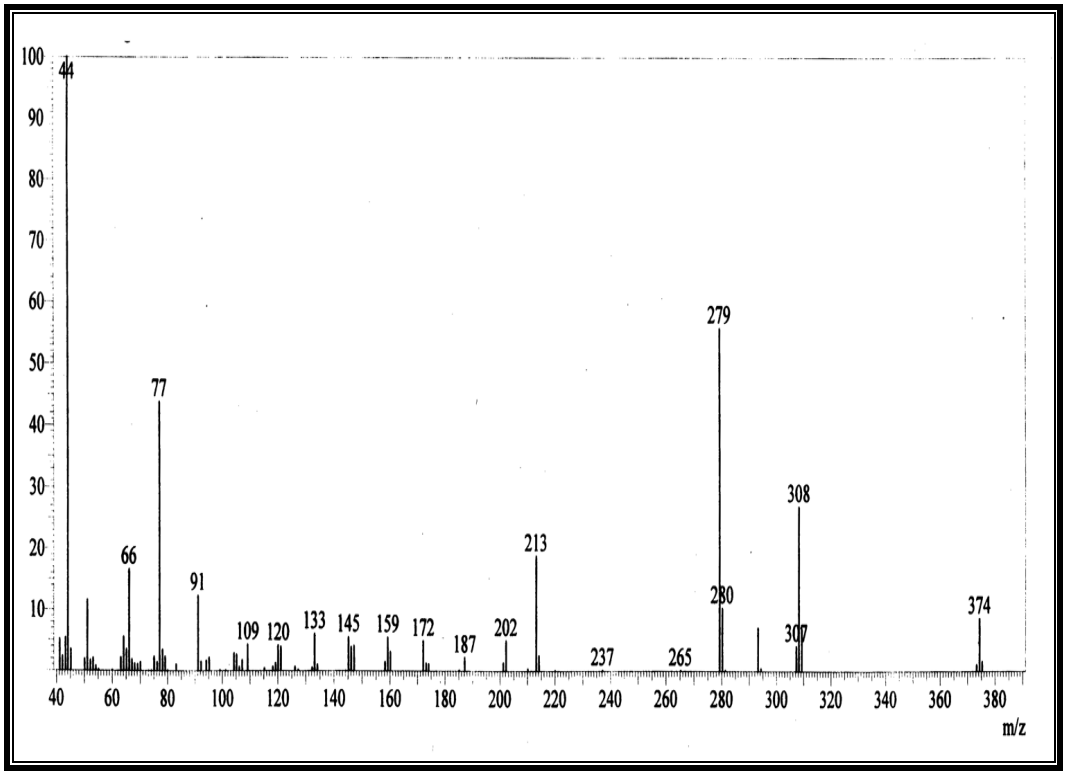
Expanded ^{1}H NMR spectrum of BHD-216



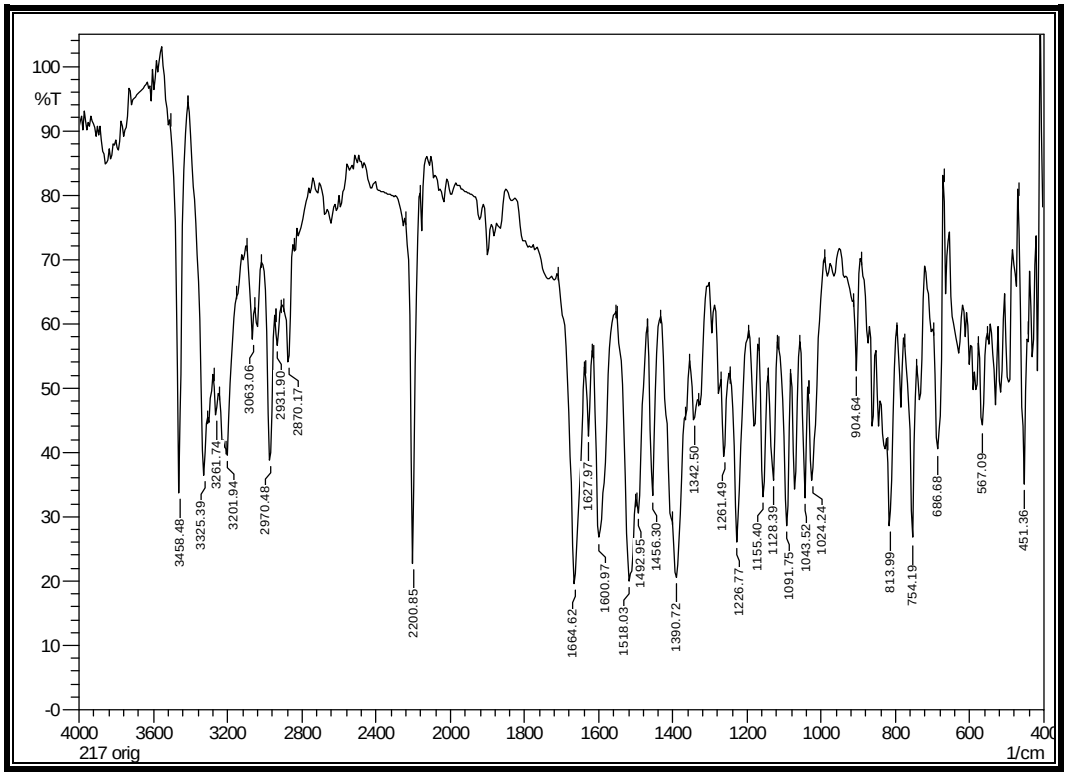
Expanded ^{1}H NMR spectrum of BHD-216



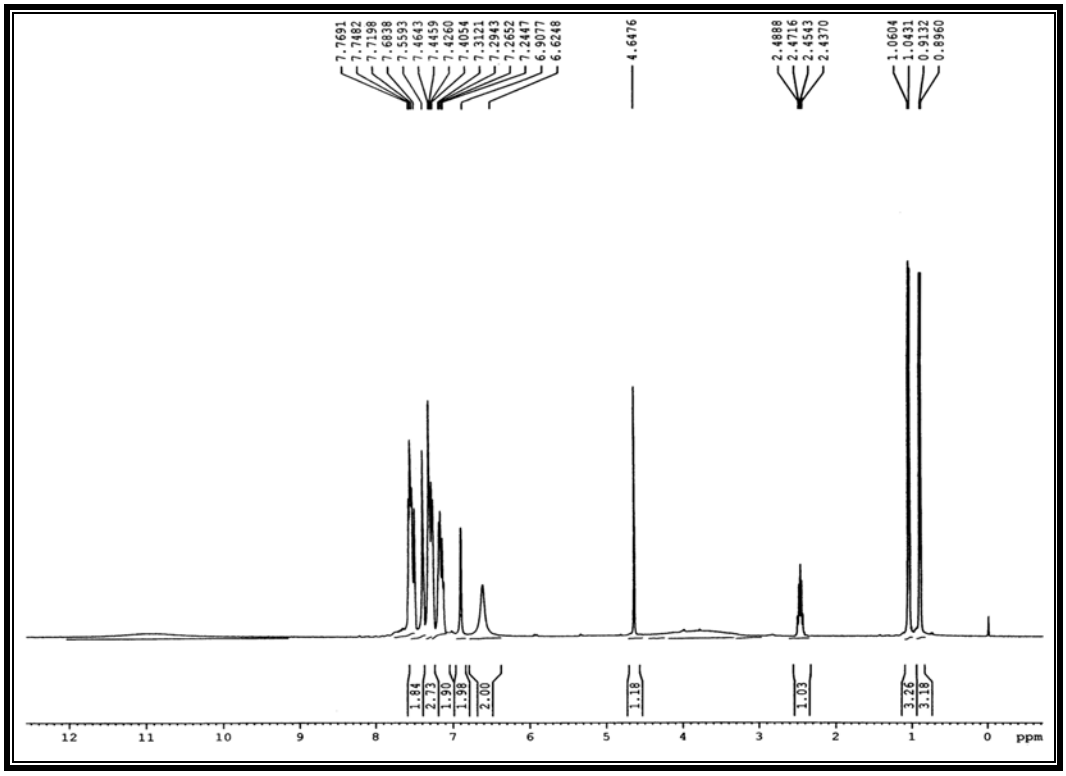
Mass spectrum of BHD-217



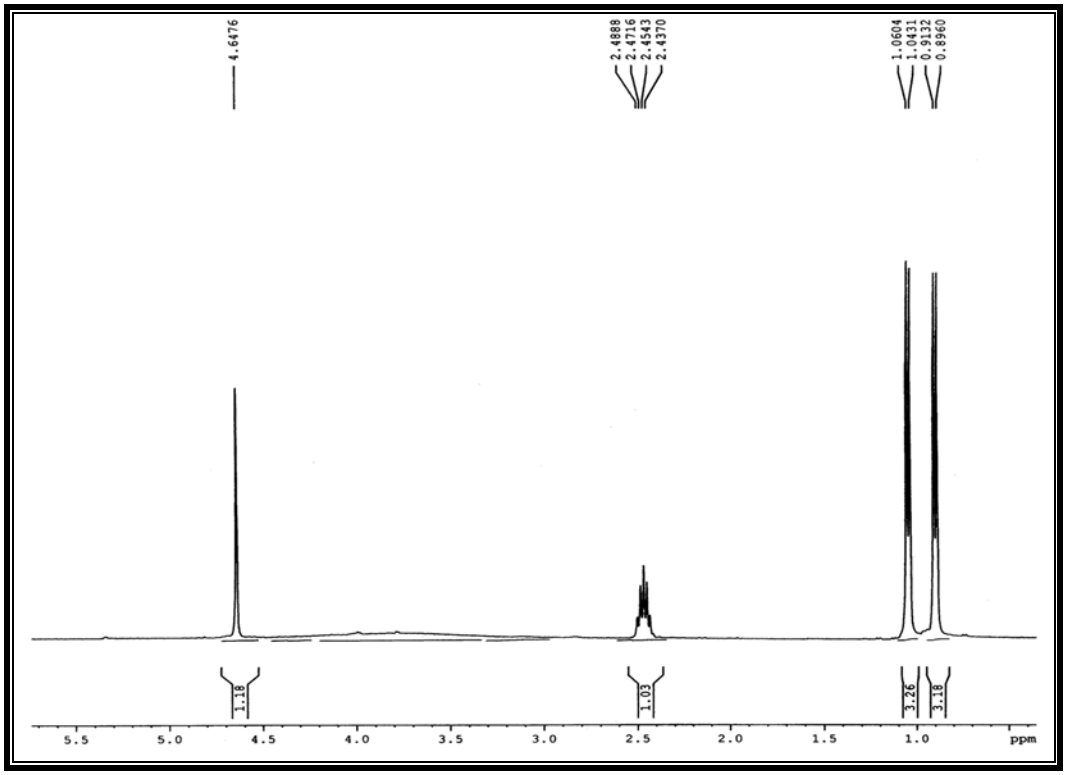
IR spectrum of BHD-217



^{1}H NMR spectrum of BHD-217



Expanded ^{1}H NMR spectrum of BHD-217



3.9 Biological evaluation

3.9.1 Antimicrobial evaluation

All the synthesized compounds (BHD-201 to BHD-230) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [123, 124] 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 greseofulvin 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 [123]. 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 200 μg mL^{-1}, 100 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1}, and 6.25 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 BHD-201 to 230

Code	Minimal 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.***
BHD-201	200	100	100	100	250	1000	250
BHD-202	500	500	250	250	250	200	200
BHD-203	500	500	100	250	500	500	>1000
BHD-204	500	500	250	500	500	>1000	1000
BHD-205	250	62.5	250	500	>1000	>1000	>1000
BHD-206	100	200	62.5	125	500	>1000	>1000
BHD-207	250	250	250	500	1000	500	>1000
BHD-208	200	500	62.5	500	1000	500	500
BHD-209	100	200	500	500	250	>1000	>1000
BHD-210	500	500	100	250	250	1000	250
BHD-211	500	62.5	250	250	250	200	200
BHD-212	100	250	100	250	500	500	>1000
BHD-213	500	250	250	500	500	>1000	1000
BHD-214	500	500	250	500	>1000	>1000	>1000
BHD-215	500	100	100	125	500	>1000	1000
BHD-216	200	500	250	500	1000	500	>1000
BHD-217	250	500	62.5	500	1000	500	500
BHD-218	250	500	500	500	250	>1000	>1000
BHD-219	500	500	1000	1000	500	1000	1000
BHD-220	200	100	100	500	500	1000	200
BHD-221	250	250	250	250	500	500	1000
BHD-222	100	500	500	1000	250	500	500
BHD-223	500	100	62.5	100	500	500	>1000
BHD-224	250	500	500	500	200	500	200
BHD-225	500	250	500	500	1000	1000	1000
BHD-226	500	100	500	250	1000	>1000	1000
BHD-227	250	62.5	100	125	250	1000	500
BHD-228	500	250	200	500	500	1000	>1000
BHD-229	100	250	500	1000	1000	>1000	>1000
BHD-230	500	62.5	62.5	100	250	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
Greseofulvin	-	-	-	-	500	100	100

3.9.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds BHD-201 to BHD-230 is currently under investigation and results are awaited.

3.10 References and notes

[1] Newton, R. sp2 **2002**, 16; (see: http://www.sp2.uk.com)

[2] (a) Teague, S. J.; Davis, A. M.; Leeson, P. D.; Oprea, T. I. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 3743; (b) Armstrong, R. W.; Combs, A. P.; Tempest, P. A.; Brown, S. D.; Keating, T. A. *Acc. Chem. Res.* **1996**, *29*, 123.

[3] Domling, A.; Ugi, I. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 3168.

[4] Bienayme, H.; Hulme, C.; Oddon, G.; Schmitt, P. *Chem. Eur. J.* **2000**, *6*, 3321.

[5] Orru, R. V. A.; de Greef, M. *Synthesis* **2003**, 1471.

[6] Tempest, P. A. *Curr. Opin. Drug Discovery Dev.* **2005**, *8*, 776.

[7] Weber, L. *Curr. Med. Chem.* **2002**, *9*, 1241.

[8] Schreiber, S. L. *Science* **2000**, *287*, 1964.

[9] Terrett, N. K. Combinatorial Chemistry. Oxford University Press, New York, NY, **1998**.

[10] Domling, A. *Curr. Opin. Chem. Biol.* **2002**, *6*, 306.

[11] Domling, A. *Chem. Rev.* **2006**, *106*, 17.

[12] Zhu, J.; Bienayme, H. Multicomponent Reactions. Wiley-VCH, Weinheim, **2005**.

[13] Tietze, L. F. *Chem. Rev.* **1996**, *96*, 115.

[14] Denmark, S. E.; Thorarensen, A. *Chem. Rev.* **2003**, *96*, 137.

[15] (a) Bravo, P. A.; Carrero, M. C. P.; Galan, E. R.; Blazquez, J. A. S. *Heterocycles* **2000**, *1*, 81; (b) Franz, A.; Eschler, P. Y.; Tharin, M.; Stoeckli-Evans, H.; Neier, R. *Synthesis* **1996**, 1239.

[16] Kraus, G. A.; Taschner, M. J. *J. Am. Chem. Soc.* **1980**, *102*, 1974.

[17] Danishefsky, S.; Schuda, P. F.; Kitahara, T.; Etheredge, S. J. *J. Am. Chem. Soc.* **1977**, *99*, 6066.

[18] Nishiyama, Y.; Katahira, C.; Sonoda, N. *Tetrahedron Lett.* **2004**, *45*, 8539.

[19] Portlock, D. E.; Naskar, D.; West, L.; Ostaszewski, R.; Chen, J. J. *Tetrahedron Lett.* **2003**, *44*, 5121.

[20] The corresponding starting materials of these reactions are commercially available, mostly in numbers exceeding 1000 (RCO_2H, RNH_2, RCHO, RCOR). However, less than 1000 boronic acids are available at the moment.

[21] Passerini, M. *Gazz. Chim. Ital.* **1921**, *51*, 121.

[22] Passerini, M. *Gazz. Chim. Ital.* **1921**, *51*, 181.

[23] Ugi, I.; Steinbrückner, C. DE-B1, **1959**, 103, 337.

[24] Ugi, I.; Steinbrückner, C. *Chem. Ber.* **1961**, *94*, 734.

[25] Ugi, I.; Dömling, A.; Hörl, W. *Endeavour* **1994**, *18*, 115.

[26] Strecker, S. *Liebigs Ann. Chem.* **1850**, 27.

[27] Hantzsch, A. Ber. *Dtsch. Chem. Ges.* **1890**, *75*, 1474.

[28] Biginelli, P. Ber. *Dtsch. Chem. Ges.* **1891**, *24*, 1317.

[29] Kappe, C. O. *Acc. Chem. Res.* **2000**, *33*, 879.

[30] Kappe, C. O. *Eur. J. Med. Chem.* **2000**, *35*, 1043.

[31] Dondoni, A.; Massi, A.; Sabbatini, S. *Tetrahedron Lett.* **2002**, *43*, 5913.

[32] Robinson, R. J. *Chem. Soc.* **1917**, 762.

[33] Domling, A.; Ugi, I. *Angew. Chem.* **2000**, *112*, 3300.

[34] Bergs, H., DE-B 566, **1929**, 094.

[35] Bucherer, T.; Barsch, H. *J. Prakt. Chem.* **1934**, *140*, 151.

[36] Chubb, F. L.; Edward, J. T.; Wong, S. C. *J. Org. Chem.* **1980**, *45*, 2315.

[37] Asinger, F. *Angew. Chem.* **1958**, *71*, 67.

[38] Sabnis, R. W.; Rangnekar, D. W.; Sonawane, N. D. *J. Heterocycl. Chem.* **1999**, *36*, 333.

[39] Furka, A. *Drug Dev. Res.* **1995**, *36*, 1.

[40] Merrifield, B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.

[41] Balkenhohl, F.; Buschen-Hünnefeld, C. V.; Lanshy, A.; Zechel. C.; *Angew. Chem.* **1996**, *108*, 3436; *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2288.

[42] (a) Armstrong, R. W. *J. Am. Chem. Soc.* **1995**, 117, 7842; (b) Stocker, A. M.; Keating, T. A.; Tempest, P. A.; Armstrong, R. W. *Tetrahedron Lett.* **1996**, *37*, 1149.

[43] (a) Weber, L.; Waltbaum, S.; Broger, C.; Gubernator, K. *Angew. Chem.* **1995**, *107*, 2452; (b) *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2280; (c) Lacke, O.; Weber, L. *Chimia* **1996**, *50*, 445; (d) Weber, L.; Illgen, K.; Almstetter, M. *Synlett* **1999**, *3*, 366.

[44] Lehmann, F.; Holm, M.; Laufer, S. *J. Comb. Chem.* **2008**, *10,* 364.

[45] Yuasa, Y.; Tsuruta, H.; Yuasa, Y. *Org. Process Res. Dev.* **1998**, *2*, 412.

[46] Mendes, E.; Vernieres, J. C.; Keane, P. E.; Bachy, A. Eur. Patent EP A1 346208, 19891213, **1989**.

[47] Litvinov, Y. M.; Shestopalov, A. A.; Rodinovskaya, L. A.; Shestopalov, A. M. *J. Comb. Chem.* **2009**, *11,* 914.

[48] Litvinov, Y. M.; Shestopalov, A. M.; Rodinovskaya, L. A.; Shestopalov, A. A.; Fedorov, A. E. Proceedings of the 12-th Blue Danube Symposium on Heterocyclic Chemistry, Tihany, Hungary, **2007**, BDSHC-12, Book of Abstracts; ISBN: 978-963-7067-15-0 (963-7067-15-9). Chemical Research Center of Hungarian Academy of Sciences: Budapest, Hungary, **2007**, 50.

[49] Shi, D.; Mou, J.; Zhuang, Q.; Niu, L.; Wu, N.; Wang, X. *Synth. Commun.* **2004**, *34,* 4557.

[50] Li, D.; Song, L.; Song's.; Zhu, S. *J. Fluorine Chem.* **2007**, *128*, 952.

[51] Yadav, J. S.; Kumar, S. P.; Kondaji, G.; Rao, R.S.; Nagaiah, K. *Chem. Lett.* **2004**, *33*, 1168.

[52] Mabry, J.; Ganem, B. *Tetrahedron Lett.* **2006**, *47*, 55.

[53] Lee, J. H. *Tetrahedron Lett.* **2005**, *46*, 7329.

[54] Mitsumori, S.; Zhang, H. L.; Cheong, P. H.; Houk, K. N.; Tanaka, F.; Barbas, C. F.; *J. Am. Chem. Soc.* **2006**, *128*, 1040.

[55] Sakamoto, R.; Ryoto, M.; Kume, S.; Sampei, H.; Sugimoto, M.; Nishihara, H. *Chem. Commun.* **2005**, *9*, 1214.

[56] Abdel-Rahman, M. A.; Khodairy, A.; Ghattas, A. B. A. G.; Younes, S. *J. Chin. Chem. Soc.* **2004**, *51*, 103.

[57] El-Saghier, A. M. M.; Khodairy, A. *Phosphorus, Sulphur and Silicon* **2000**, *160*, 105.

[58] Shestopalov, A. M.; Emeliyanova, Y. M.; Shestopalov, A. A.; Rodinovskaya, L. A.; Niazimbetova, Z. I.; Evans, D. H. *ORGANIC LETTERS* **2002**, *4*, 423.

[59] Atta, A. H. *J. Chin. Chem. Soc*. **2006**, *53*, 663.

[60] Thumar, N. J.; Patel, M. P. *ARKIVOC* **2009**, *13*, 363.

[61] Heravi, M. M.; Ghods, A.; Derikvand, F.; Bakhtiari, K.; Bamoharram, F. F. *J. Iran. Chem. Soc.* **2010**, *7*, 615.

[62] Nakamichi, N.; Kawashita, Y.; Hayashi, M. *Synthesis* **2004**, 1015.

[63] Heravi, M. M.; Zadsirjan, V.; Bakhtiari, K.; Oskooie, H. A.; Bamoharram, F. F. *Catal. Commun.* **2007**, *8*, 315.

[64] Karc, F. *Dyes and Pigments* **2008**, *76*, 97.

[65] Alizadeh, M. H.; Razavi, H.; Bamoharram, F. F.; Hassanzadeh, M. K. *Kinet. Catal.* **2003**, *44*, 524.

[66] Nagarajan, A. S.; Reddy, B. S. R. *Synlett* **2009**, *12*, 2002.

[67] Vasuki, G, Kumaravel, K. *Tetrahedron Lett.* **2008**, *49*, 5636.

[68] Kumaravel, K.; Vasuki, G. *Green Chem.* **2009**, *11*, 1945.

[69] (a) Singh, K.; Singh, J.; Singh, H. *Tetrahedron* **1996**, *52*, 14273; (b) Wang, X. S.; Shi, D. Q.; Tu, S. J.; Yao, C. S. *Synth. Commun.* **2003**, *33*, 119; (c) Wang, X. S.; Shi, D. Q.; Rong, L. C.; Yao, C. S.; Dai, G. Y. *Jie Gou Hua Xue* **2003**, *22*, 331; (d) Ismail, Z. H.; Aly, G. M.; El-Degwi, M. S.; Heiba, H. I.; Ghorab, M. M. *Egyptian. J. Biotechnol.* **2003**, *13*, 73; (e) Ipsita, D.; Pulak, J. B. *Tetrahedron Lett.* **2004**, *45*, 8625.

[70] (a) Tu, S. J.; Miao, C. B.; Gao, Y.; Feng, Y. J.; Feng, J. G. *Chin. J. Chem.* **2002**, *20*, 703; (b) Zhou, J. F.; Tu, T. J.; Gao, Y.; Ji, M. *Chin. J. Org. Chem.* **2001**, *21*, 742; (c) Tu, S. J.; Gao, Y.; Guo, C.; Shi, D. Q.; Lu, Z. S. *Synth. Commun.* **2002**, *32*, 2137; (d) Zhou, J. F.; Tu, S. J.; Zhu, H. Q.; Zhi, S. J. *Synth. Commun.* **2002**, *32*, 3363.

[71] Li, J. T.; Xu, W. Z.; Yang, L. C.; Li, T. S. *Synth. Commun.* **2004**, *34*, 4565.

[72] (a) Jin, T. S.; Wang, A. Q.; Wang, X.; Zhang, J. S.; Li, T. S. *Synlett.* **2004**, 871; (b) Jin, T. S.; Wang, A. Q.; Cheng, Z. L.; Zhang, J. S.; Li, T. S. *Synth. Commun.* **2005**, *35*, 137.

[73] (a) Shi, D. Q.; Mou, J.; Zhuang, Q. Y.; Wang, X. S. *J. Chem. Res.* **2004**, *12*, 821; (b) Shi, D. Q.; Zhang, Z.; Zhuang, Q. Y.; Tu, S. J.; Hu, H. W. *Chin. J. Org. Chem.* **2003**, *23*, 877.

[74] Kaupp, G.; Naimi-Jamal, M. R.; Schmeyers, J. *Tetrahedron* **2003**, *59*, 3753.

[75] Guo, S. B.; Wang, S. X.; Lai, J. T. *Synth. Commun.* **2007**, *37*, 2111.

[76] Suresh, P.; Pitchumani, K. *J. Org. Chem.* **2008**, *73*, 9121.

[77] Suresh, P.; Pitchumani, K. *Tetrahedron: Asymmetry* **2008**, *19*, 2037.

[78] Kanagaraj, K.; Pitchumani, K. *Tetrahedron Letters* **2010**, *51*, 3312.

[79] El-Ashry, E. S. H.; El-Rafi, M. E.; El-Nagdi, M. H.; Abou-Elnaga, H. H.; Abdel Azime, W. M. A.; Boghdadi, Y. M. *Jordan Journal of Chemistry* **2009**, *4*, 223.

[80] Mobinikhaledi, A.; Bodaghi Fard, M. A. *Acta Chim. Slov.* **2010,** *57,* 931-935.

[81] Rai, U. S.; Isloor, A. M.; Shetty, P.; Vijesh, A. M.; Prabhu, N.; Isloor, S.; Thiageeswaran, M.; Fun, H. K. *Eur. J. Med. Chem.* **2010**, *45*, 2695.

[82] Kumar, D.; Reddy, V. B.; Sharad, S.; Dube, U.; Kapur, S. *Eur. J. Med. Chem.* **2009**, *44*, 3805.

[83] Al-Matar, H. M.; Khalil, K. D.; Meier, H.; Kolshorn, H.; Elnagdi, M. H. *ARKIVOC* **2008**, *16*, 288.

[84] Pansuriya, A. M.; Savant, M. M.; Bhuva, C. V.; Singh, J.; Naliapara, Y. T. *ARKIVOC* **2009**, *12*, 254.

[85] Kidwai, M. *Pure Appl. Chem.* **2001**, *73*, 147.

[86] Chen, L.; Li, Y. Q.; Huang, X. J.; Zheng, W. J. *Heteroat. Chem.* **2009**, *20*, 91.

[87] Peng, Y. Q.; Song, G. H. *Catal. Commun.* **2007**, *8*, 111.

[88] Babu, N. S.; Pasha, N.; Rao, K. T. V.; Prasad, P. S. S.; Lingalah, N. *Tetrahedron Lett.* **2008**, *49*, 2730.

[89] Balalaie, S.; Bararjanian, M.; Amani, A. M.; Movassagh, B. *Synlett* **2006**, 263.

[90] Balalaie, S.; Sheikh-Ahmadi, M.; Bararjanian, M. *Catal. Commun.* **2007**, *8*, 1724.

[91] El-Agrody, A. M.; Abd El-Latif, M. S.; El-Hady, N. A.; Fakery, A. H.; Bedair, A. H. *Molecule* **2001**, *6*, 519.

[92] Bedair, A. H.; El-Hady, N.A.; Abd El-Latif, M. S.; Fakery, A. H.; El-Agrody, A. M.; *Il Farmaco* **2000**, *55*, 708.

[93] El-Agrody, A. M.; El-Hakim, M. H.; Abd El-Latif, M. S.; Fakery, A. H.; El-Sayed, E. M.; El-Ghareab, K. A, *Acta. Pharm.* **2000**, *50*, 111.

[94] Eid, F. A.; Abd El-Wahab, A. H. F.; El-Hag Ali, G. A. M.; Khafagy, M. M. *Acta. Pharm.* **2004**, *54*, 13.

[95] Perez-Perez, M.; Balzarini, J.; Rozenski, J.; De Clercq, E.; Herdewijn, P. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1115.

[96] Shamroukh, A. H.; Zaki, M. E. A.; Morsy. E. M. H.; Abdel-Motti, F. M.; Abdel-Megeid, F. M. E. *Arch. Pharm.* **2007**, *340*, 236.

[97] Aytemir, M. D.; Calis, U.; Ozalp, M. *Arch. Pharm*. **2004**, *337*, 281.

[98] Melliou, E.; Magiatis, P.; Mitaku, S.; Skaltsounis, A. L.; Pierre, A.; Atassi, G.; Renard, P.; *Bioorg. Med. Chem.* **2001**, *9*, 607.

[99] Chabchoub, F.; Messaad, M.; Mansour, H. B.; Chekir-Ghedira, L.; Salem, M. *Eur. J. Med. Chem.* **2007**, *42*, 715.

[100] Regaila, H. A.; El-Bayonk, A. K. Hammad, M. *Egypt J. Chem.* **1979**, *20*,197.

[101] Shafiee, A.; Bagheri, M.; Shekarchi, M.; Abdollahi, M.; *J. Pharm. Sci*. **2003**, *6*, 360.

[102] Ren, X. L.; Li, H. B.; Wu, C.; Yang, H. Z. *ARKIVOC* **2005**, *15*, 59.

[103] Almansa, C.; Gomez, L. A.; Cavalcanti, F. L.; de Arriba, A. F.; Garcia-Rafanell, J.; Forn, J. *J. Med. Chem.* **1997**, *40*, 547.

[104] Park, H. J.; Lee, K.; Park, S. J.; Ahn. B.; Lee, J. C.; Cho, H.; Lee, K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3307.

[105] Cali, P.; Naerum, L.; Mukhija, S.; Hjelmencrantz, A. *Biorg. Med. Chem. Lett.* **2004**, *14*, 5997.

[106] Prasad. Y. R.; Rao, A. L.; Prasoona, L.; Murali, K.; Kumar, P. R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5030.

[107] Mojtahedi, M. M.; Jalali, M. R.; Bolourtchian, M. *Synth. Commun.* **2006**, *36*, 51.

[108] Pavlik, J. W.; Ervithayasuporn, V.; MacDonald, J. C.; Tantayanon, S. *ARKIVOC* **2009**, *8*, 57.

[109] Guard, J. A. M.; Steel, P. J. *ARKIVOC* **2001**, *7*, 32.

[110] Kanwal, P.; Gupta, V. K.; Brahmbhatt, D. I.; Patel, M. A. *Analytical Sci*. **2007**, *23*, 237.

[111] Jin, T.; Zhao, R.; Li, T. *ARKIVOC* **2006**, *11*, 176.

[112] El-Tamany, E. S.; El-Shahed, F. A.; Mohamed, B. H. *J. Serb. Chem. Soc.* **1999**, *64*, 9.

[113] Ismail, Z. H.; Aly, G. M.; El-Degwi, M. S.; Heiba, H. I.; Ghorab, M. M. *Egyp. J. Biot.* **2003**, *13*, 73.

[114] Zaki, M. E. A.; Soliman, H. A.; Hiekal, O. A.; Rashad, A. E. Z. *Naturforsch C.* **2006**, *61*, 1.

[115] Abdelrazek, F. M.; Metz, P.; Metwally, N. H.; El-Mahrouky, S. F. *Arch. Pharm.* **2006**, *339*, 456.

[116] Abdelrazek, F. M.; Metz, P.; Kataeva, O.; Jaeger, A.; El-Mahrouky, S. F. *Arch. Pharm.* **2007**, *340*, 543.

[117] Foloppe, N.; Fisher, L. M.; Howes, R.; Potter, A.; Robertson Alan, G. S.; Surgenor, A. E. *Bioorg. Med. Chem.* **2006**, *14*, 4792.

[118] Otto, H. H. *Arch. Pharm.* **1974**, *307*, 444.

[119] Otto, H. H.; Schmelz, H. *Arch. Pharm.* **1979**, *312*, 478.

[120] Klokol, G. V.; Krivokolysko, S. G.; Dyachenko, V. D.; Litvinov, V. P. *Chem. Heterocycl. Compd.* **1999**, *35*, 1183.

[121] Ren, Z.; Cao, W.; Tong, W.; Jin, Z. *Synth. Commun.* **2005**, *35*, 2509.

[122] Vogel's Textbook of Practical Organic Chemistry, Fifth Edition, ELBS, Longman Scientific and Technical: England. **1989**, Reprinted 1994, 1150.

[123] National Committee for Clinical and Laboratory Standards, Method for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically Approved Standard, fourth ed. NCCLS, Villanova, Italy, **1997**, Document M 100-S7. S100-S157.

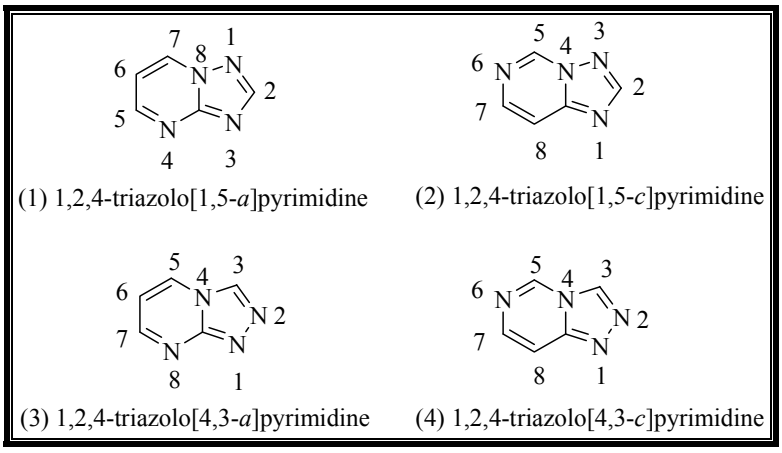
[124] (a) D.H. Isenberg, Essential Procedure for Clinical Microbiology, *American Society for Microbiology*, Washington, **1998**; (b) Zgoda, J. R.; Porter, J. R. *Pharm. Biol.* **2001**, *39*, 221.

Chapter 4

Microwave assisted synthesis and biological evaluation of 1,2,4-triazolo [1,5-*a*]pyrimidines

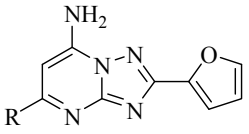
4.1 Introduction

The condensation of a ring of 1,2,4-triazole and another one of pyrimidine gives rise to the formation of bicyclic heterocycles known as 1,2,4-triazolopyrimidines. Four different possibilities exist for the relative orientation of both rings, so four different isomeric families of compounds are defined: 1,2,4-triazolo[1,5-*a*]pyrimidine (1), 1,2,4-triazolo[1,5-*c*]pyrimidine (2), 1,2,4-triazolo[4,3-*a*]pyrimidine (3) and 1,2,4-triazolo[4,3-*c*]pyrimidine (4).

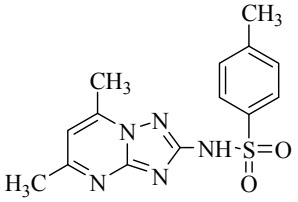


Among these isomeric families of compounds, 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives are thermodynamically more stable and, thus, the most studied ones [1], a few of them being commercially available. Revisions surveying the synthesis, reactivity, spectroscopic characterization and crystallographic studies of 1,2,4-triazolo[1,5-*c*]pyrimidines [2], 1,2,4-triazolo[4,3-*a*]pyrimidines [3] and 1,2,4-triazolo [4,3-*c*]pyrimidines [4] have also been published.

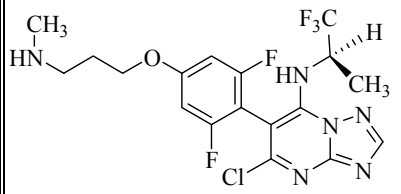
From the standpoint of biological activity, fused heteroaromatic systems are often of much greater interest than the constituent monocyclic compounds. Recently, 1,2,4-triazolo[1,5-*a*]pyrimidines have aroused increasing attention from the chemical and biological view points, due to their diverse pharmacological activities, such as antitumor potency [5, 6], inhibition of KDR kinase [7], antifungal effect [8] and macrophage activation [9]. They have proved to be promising anticancer agents with dual mechanisms of tubulin polymerization promotion [5, 6] as well as cyclin dependent kinases 2 inhibition [10]. Some examples of published derivatives of 1,2,4-triazolo[1,5-*a*]pyrimidine with their biological activities are as following.



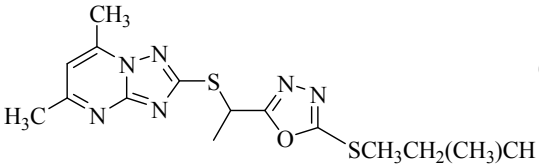
(5) Activity: A_{2A} adenosine receptor antagonists [11]



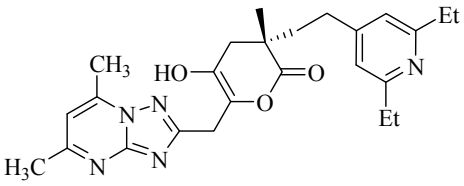
(6) Activity: latent leishmanicidal activity [12]



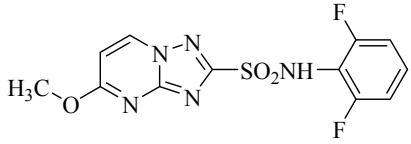
(7) Activity: Anticancer acticity [13]



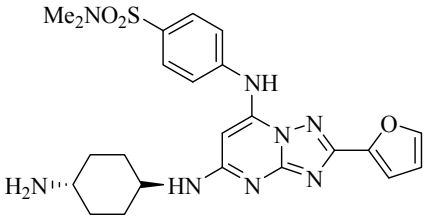
(8) Activity: antifungal activity [14]



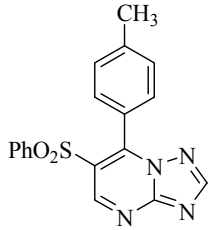
(9) Activity: Hepatitis C virus polymerase inhibitor [15]



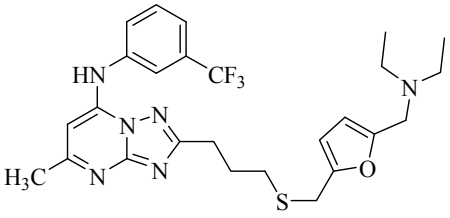
(10) Activity: Acetohydroxyacid synthase inhibitor [16]



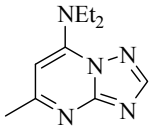
(11) Activity: CDK2 inhibitors [17]



(12) Activity: Anti-inflammatory [18]



(13) Activity: Antiproliferative activity [19]

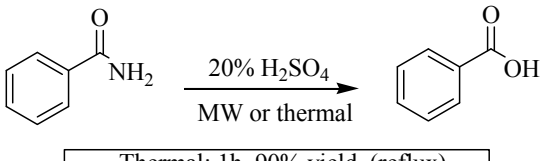


(14) Trapidil: Antiischemic and cardiatonic agent [20]

4.2 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 [21, 22].

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) [23] to biochemistry (protein hydrolysis, sterilization) [23], pathology (histoprocessing, tissue fixation) [24] and medical treatments (diathermy) [25]. 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 4.1) [26] and Raymond J. Giguere/George Majetich [27] in 1986.



Thermal: 1h, 90% yield (reflux)
MW: 10 min, 99% yield (sealed vessel)

Scheme 4.1 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 [28]. 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 [28], 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 [29]. 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 [30] 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 [31]. 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 [32], 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.

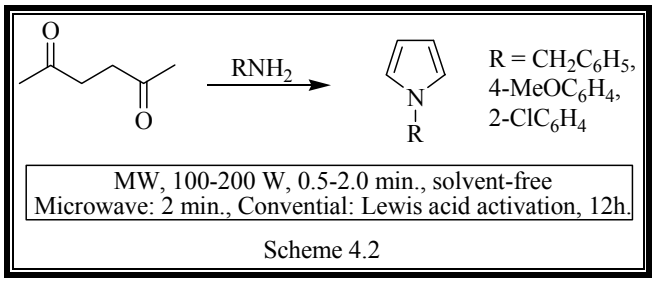
The initially slow uptake of the technology in the late 1980s and 1990s has been attributed to its lack of controllability and reproducibility, coupled with a general lack of understanding of the basics of microwave dielectric heating. The risks associated with the flammability of organic solvents in 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 [21, 22]. 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.

4.3 Applications of microwaves in heterocyclic ring formation

4.3.1 Five-membered heterocyclic rings

4.3.1.1 Pyrroles

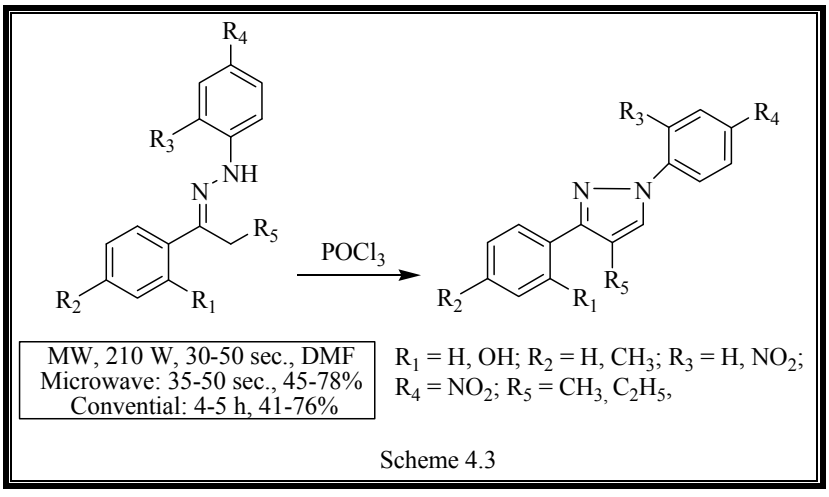
The classical Paal-Knorr cyclization of 1,4-diketones to give pyrroles is dramatically speeded-up under microwave irradiation and high yields are obtained as shown in Scheme 4.2 [33].



Scheme 4.2

4.3.1.2 Pyrazoles

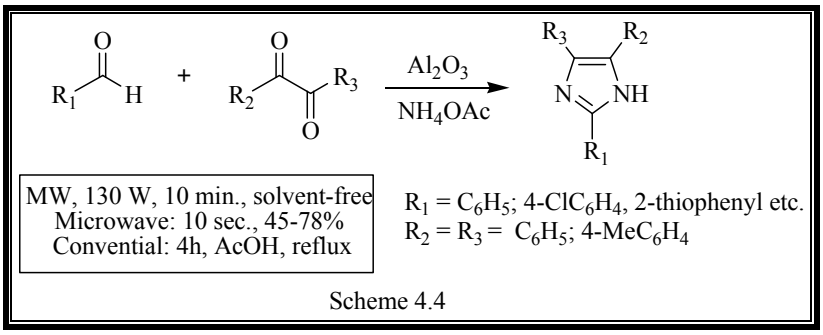
Another recent application of microwaves in cyclization is the preparation of pyrazoles from hydrazones using the Vilsmeier cyclization method by treatment with $POCl_3$ and DMF [34]. As shown in Scheme 4.3, once again the reaction is speeded-up by factors of several 100-fold.



Scheme 4.3

4.3.1.3 Imidazoles

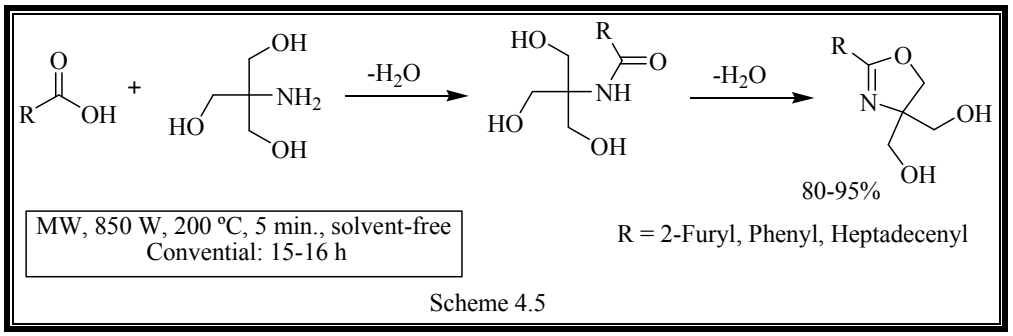
An important classical preparation of imidazoles is from an α-diketone, an aldehyde and ammonia. Here again, excellent yields can be obtained in reaction times of a few minutes as shown in Scheme 4.4 [35].



Scheme 4.4

4.3.1.4 Oxazolines

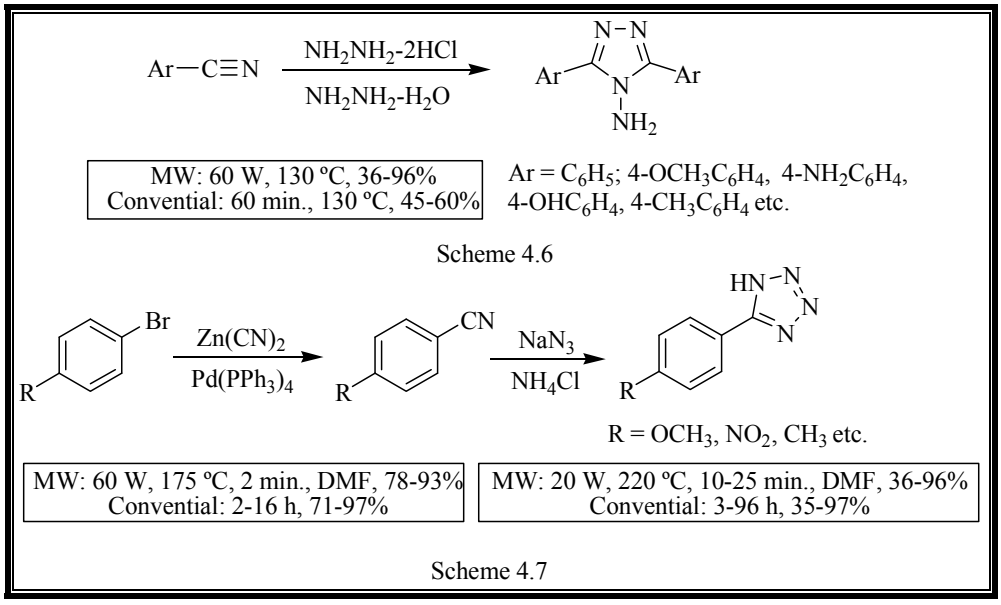
The example of Scheme 4.5, the preparation of oxazolines shows that partially saturated five-membered rings can also be prepared advantageously using microwaves [36].



Scheme 4.5

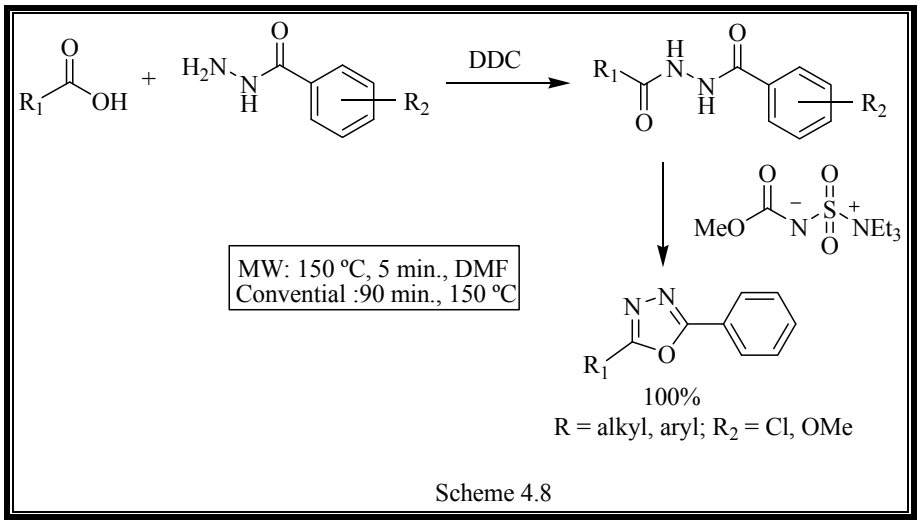
4.3.1.5 Triazoles and Tetrazoles

Schemes 4.6 and 4.7 continue the overview of five-membered rings with illustrations of the advantageous preparation of 1,2,4-triazoles (Scheme 4.6) [37] and tetrazoles (Scheme 4.7) [38] using microwaves. Notice that in Scheme 4.6 the starting aryl cyanides are also made by a Pd-catalyzed but microwave-enhanced replacement of aryl bromides using zinc cyanide.



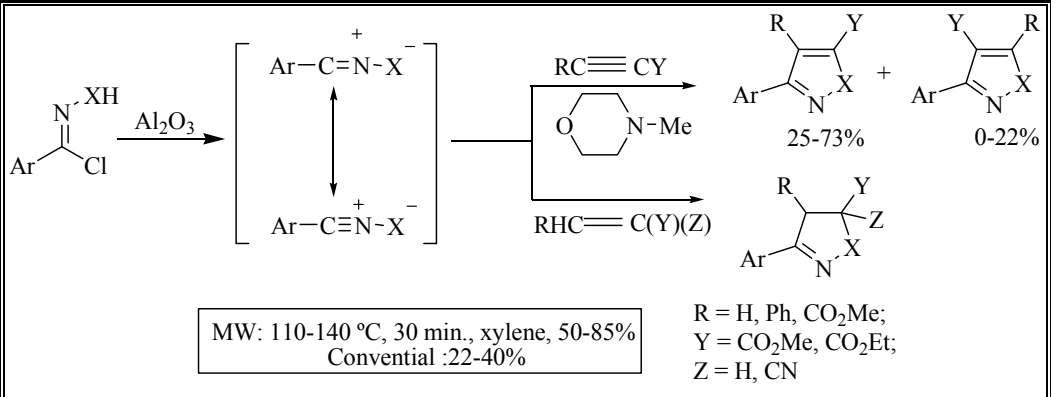
4.3.1.6 Oxadiazoles

The dehydration of unsymmetrical diacylhydrazines (themselves prepared by a conventional Mitsunobu reaction) using Burgess's reagent is shown in Scheme 4.8 to give 1,3,4-oxadiazoles rapidly under microwave irradiation [39].



4.3.1.7 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 4.9; the resulting compounds are obtained in far high yield than under conventional conditions [40].

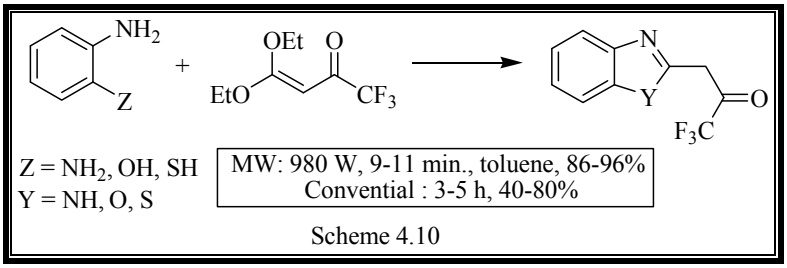


Scheme 4.9

4.3.2 Benzo-derivatives of five-membered rings

4.3.2.1 Benz-imidazoles, -oxazoles, and –thiazoles

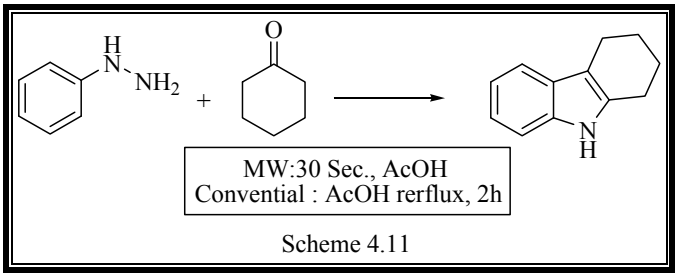
Ring closure reactions of appropriate *o*-substituted anilines to give benzimidazoles, benzoxazoles, and benzthiazoles takes place much faster and in significantly high yield under microwave conditions than conventionally [41] as shown in Scheme 4.10.



Scheme 4.10

4.3.2.2 Indoles

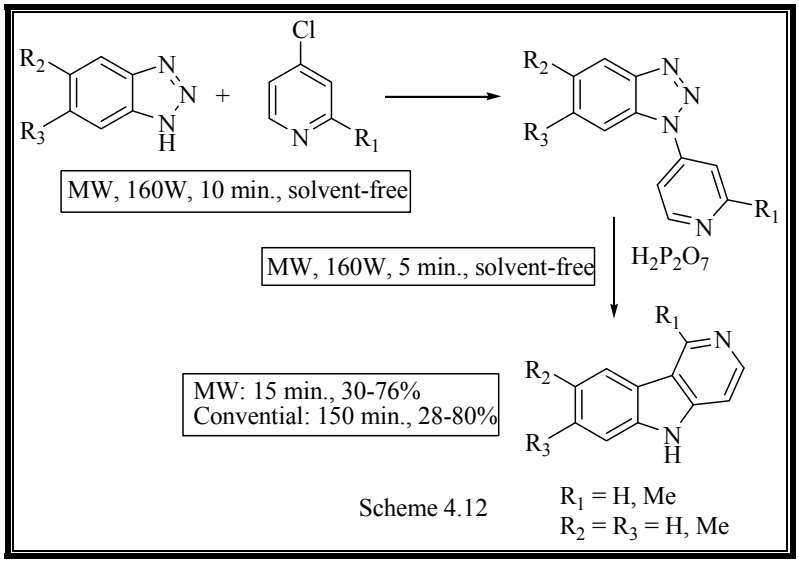
The classical Fischer-indole synthesis from an aryl hydrazine and a ketone is speeded-up by several 100-fold as documented in Scheme 4.11 [42].



Scheme 4.11

4.3.2.3 *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 4.12 where the 1-(4-pyridyl)benzotriazole is converted into a *γ*-carboline [43].



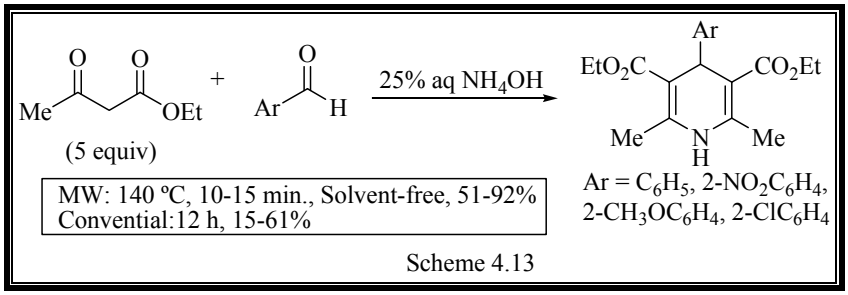
Scheme 4.12

R_1 = H, Me
R_2 = R_3 = H, Me

4.3.3 Six-membered rings

4.3.3.1 Dihydropyridines

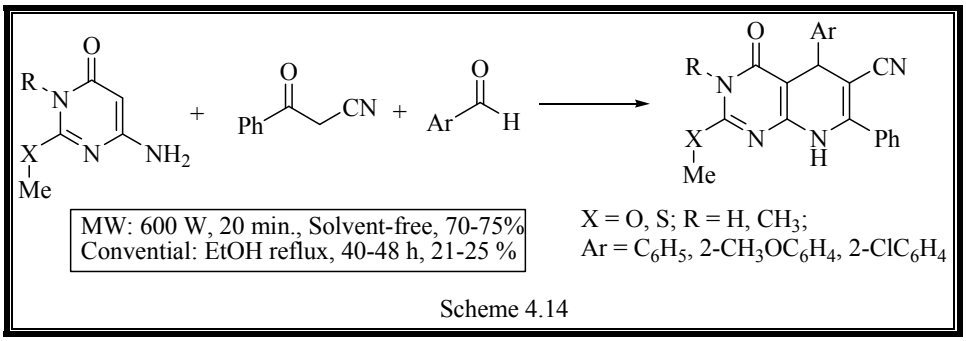
The Hantzsch dihydropyridine synthesis remains one of the most important routes to pyridine ring systems. Under conventional conditions long periods of heating are required and yields are poor to moderate. Microwaves dramatically reduce the heating times and also significantly increase the yields as shown in Scheme 4.13 [44].



Scheme 4.13

4.3.3.2 Dihydropyridopyrimidinones

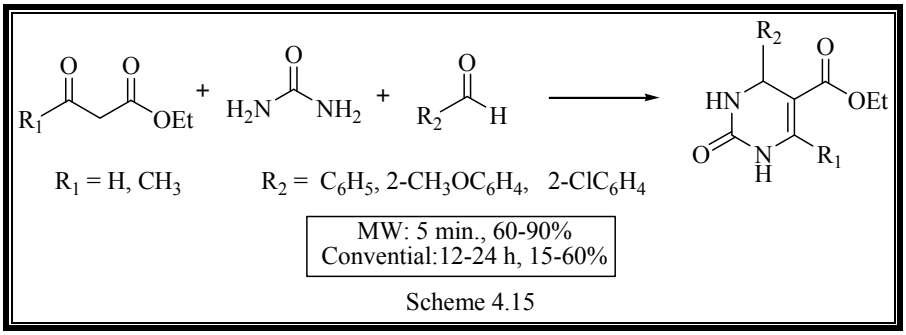
Dihydropyridopyrimidinones have been produced by ring annulations of aminopyrimidinones. Once again the reaction time is dramatically reduced and yields are much better with the solvent-free microwave conditions (Scheme 4.14) [45].



Scheme 4.14

4.3.3.3 Dihydropyrimidines

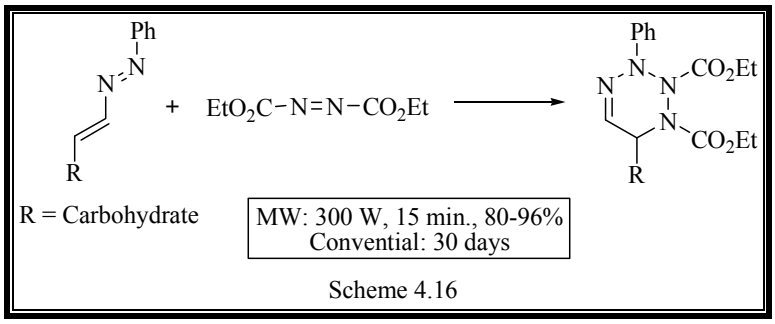
The Biginelli reaction is important for the preparation of dihydropyrimidine derivatives and excellent results are found for reactions carried out with microwave enhancement (Scheme 4.15) [39].



Scheme 4.15

4.3.3.4 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 4.16 [46].

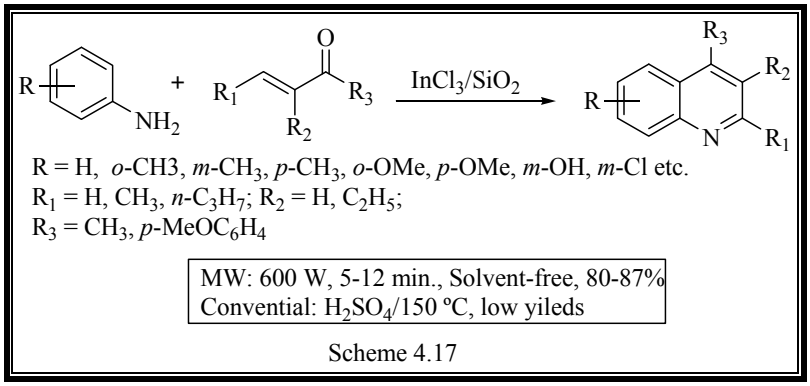


Scheme 4.16

4.3.4 Polycyclic six-membered rings

4.3.4.1 Quinolines

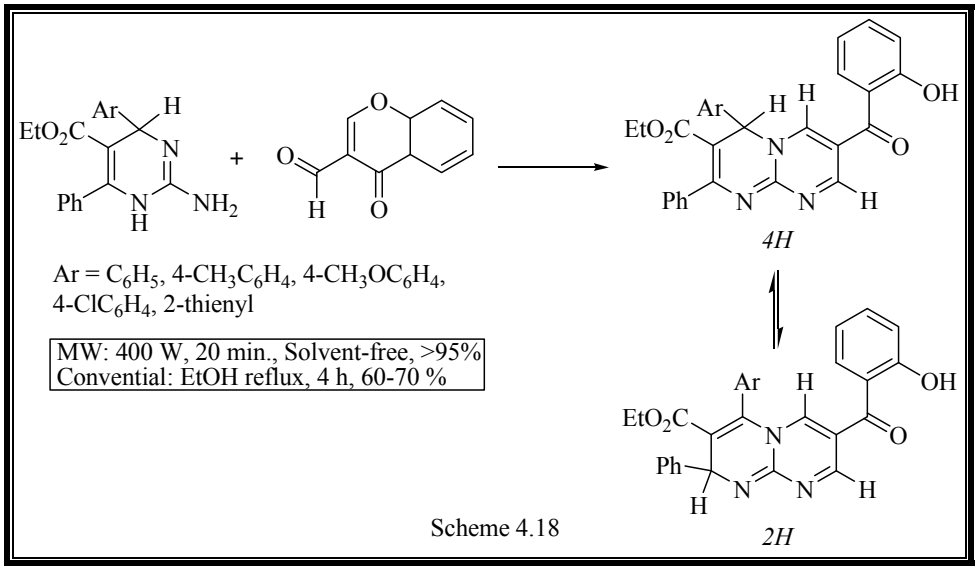
The Skraup synthesis has a bad reputation as it involves very messy conditions and gives only low yields of quinolines when carried out conventionally. Recently, it has been reported that microwave enhancement reduces the reaction time to a few minutes and allows high yields to be isolated (Scheme 4.17) [47].



Scheme 4.17

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

Pyrimido[1,2-*a*]pyrimidines are prepared from dihydroaminopyrimidines and chromone-3-aldehydes as is shown in Scheme 4.18 [48]. Although the conventional reaction must proceed in refluxing ethanol, reactions are much faster and better yields have been obtained with microwaves.

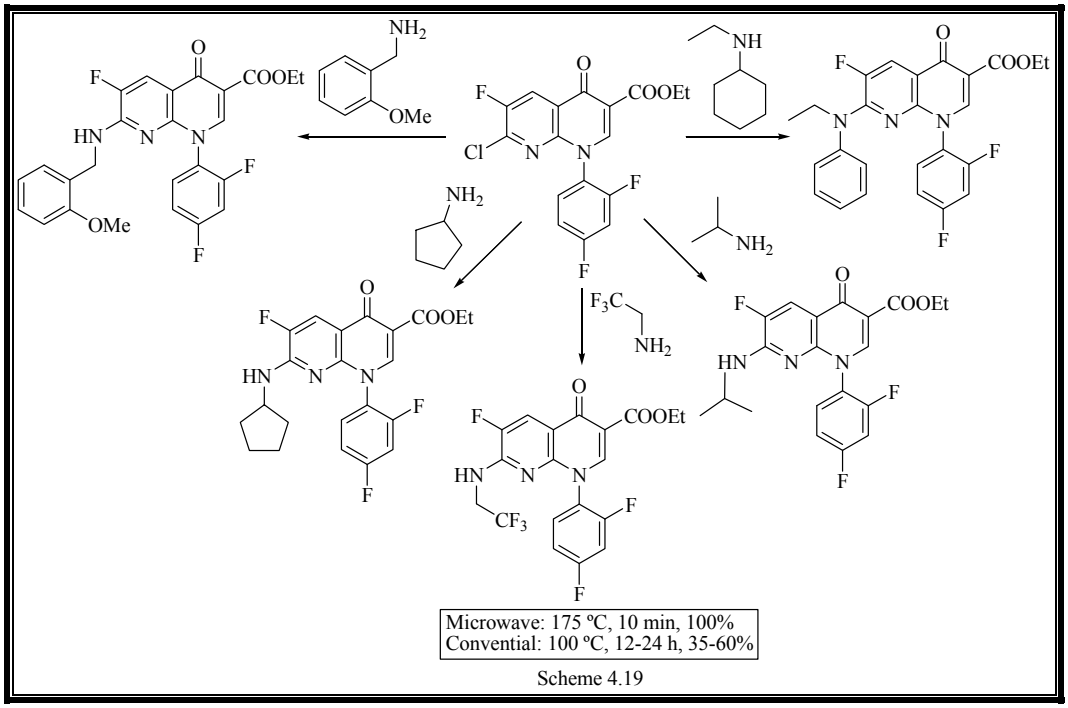


Scheme 4.18

4.3.5 Nucleophilic Substitutions

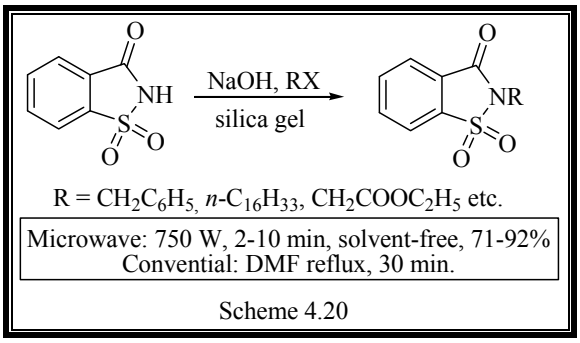
4.3.5.1 Heterocyclic *C*-alkylations

Nucleophilic substitution reactions can be speeded-up very considerably as is illustrated in Scheme 4.19 for a chloro-naphthyridine derivative [49].



Scheme 4.19

4.3.5.2 Heterocyclic *N*-alkylations

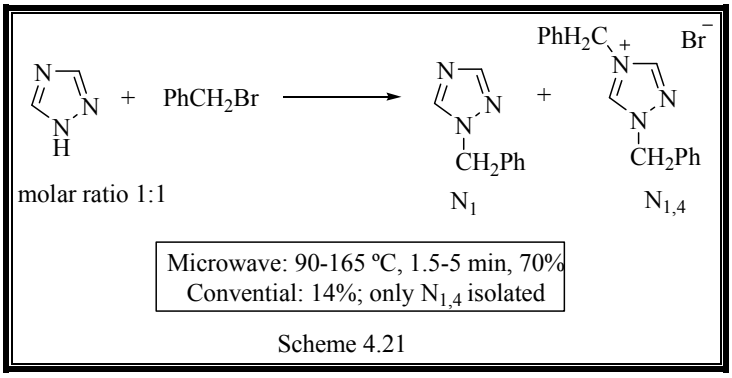


Scheme 4.20

Another class of nucleophilic substitution is involved in heterocyclic *N*-alkylation which we have illustrated in Scheme 4.20. This shows that nucleophilic substitution on the nitrogen atom of saccharin is significantly speeded-up by microwave irradiation [39].

4.3.5.3 Selective-alkylation

In Scheme 4.21, 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-substituted compound [50].

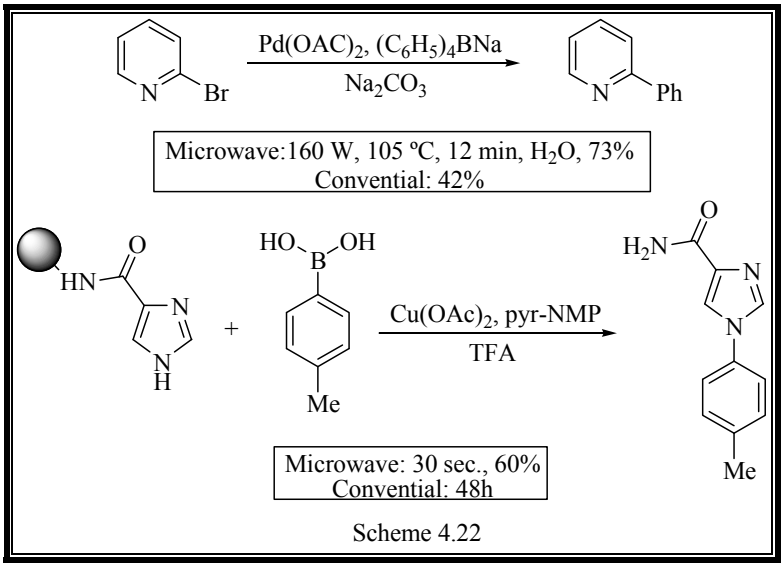


Scheme 4.21

4.3.5.4 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

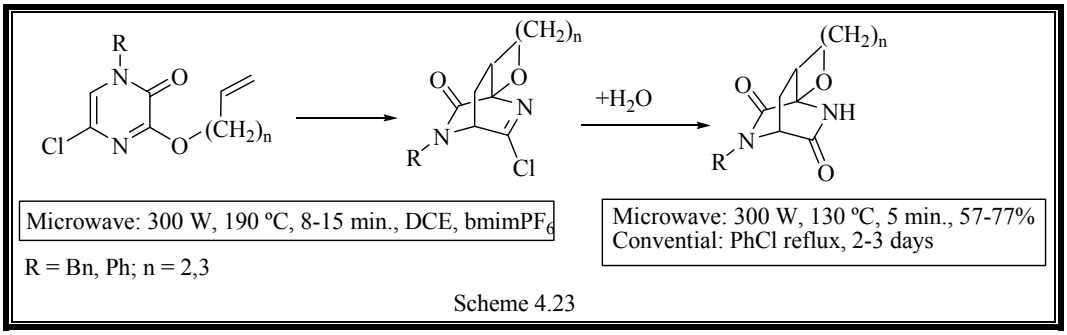
shown at the top of Scheme 4.22 to give significantly better yield in the presence of microwave irradiation [51]. At the bottom of Scheme 4.22 another Suzuki coupling is speeded-up by a factor of 100 [52].



Scheme 4.22

4.3.6 Hetero-Diels-Alder reactions

4.3.6.1 Intramolecular reactions

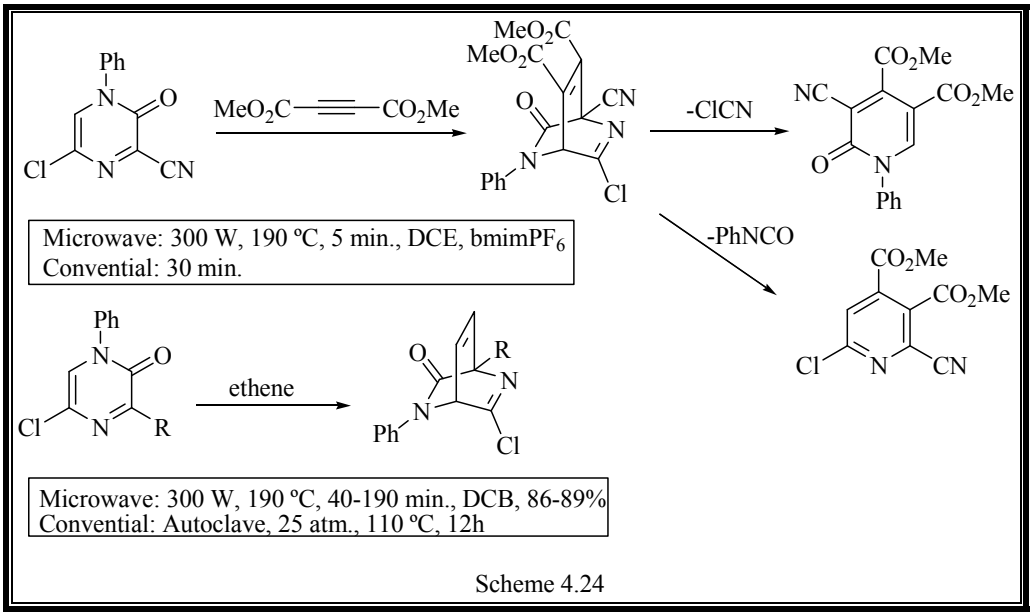


Scheme 4.23

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 4.23 indicates that the reaction was accelerated by a factor of around 1000 by microwave irradiation [53].

4.3.6.2 Intermolecular reactions

Scheme 4.24 shows two impressive examples of rate enhancement for intermolecular hetero-Diels-Alder reactions [53]. In the first example on the top of Scheme 4.24 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 4.24 where an autoclave is required under conventional conditions but which can be dispensed with when microwave acceleration is utilized.

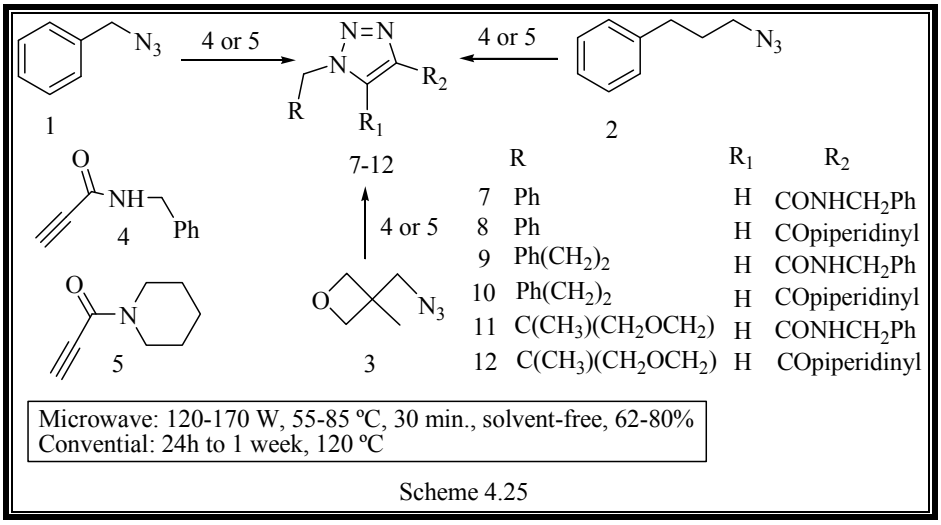


Scheme 4.24

4.3.7 1,3-Dipolar cycloaddition reactions

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

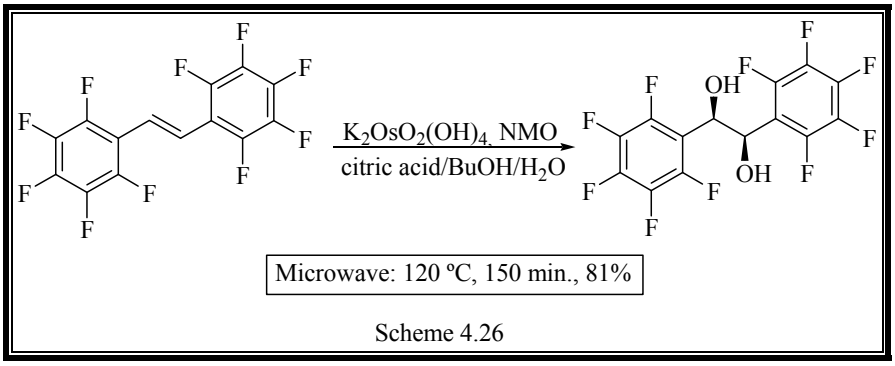
We now turn to some of our own recent work which has involved microwave induced 1,3-dipolar cycloaddition of organic azides to acetylenic amides. As shown in Scheme 4.25, we were able to achieve these reactions under microwave conditions in a reasonable time at temperatures of around 70±15 ºC [54]. Under conventional conditions the times were roughly 100 times as long and the temperature had to be taken up to 120 ºC [55].



Scheme 4.25

4.3.8 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 [56] has uncovered that electron-deficient olefins can be converted into the corresponding diols much more efficiently when the reaction medium is kept acidic (Scheme 4.26).



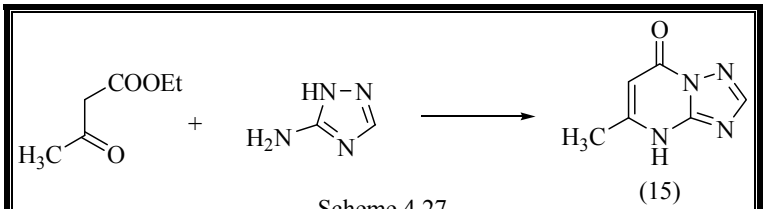
Scheme 4.26

4.4 Reported synthetic strategies

4.4.1 Amino-1,2,4-triazole and 1,3-bifunctional synthons

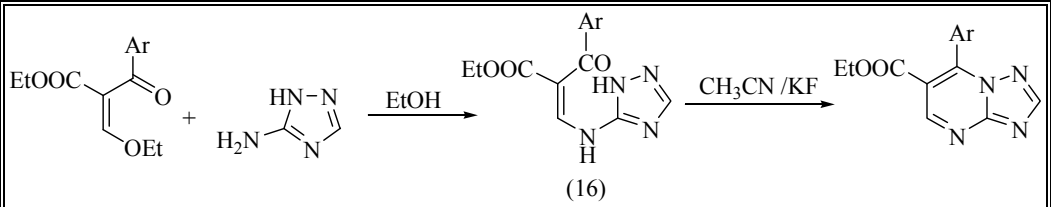
4.4.1.1 Principle and Conditions

By far the most triazolo[1,5-*a*]pyrimidine synthesis are condensations of dinucleophilic 5-amino-1,2,4-triazoles with 1,3-bifunctional synthons as shown in the formation of triazolo[1,5-*a*]pyrimidine (15) (Scheme 4.27) [57-60]. New synthetic conditions recently described involve melting under microwave irradiation, a reaction that is environmental friendly and gives higher yields than conventional heating in solvent [61]. Furthermore, certain lithium 1,3-diketonates have proven to be better synthons than the corresponding diketones [62].



Scheme 4.27

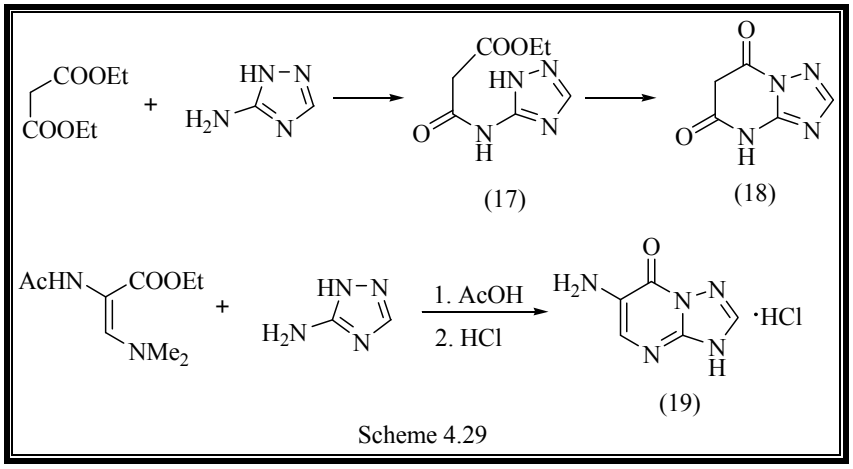
Previous mechanistic conclusions have been confirmed by isolating stable intermediate 5-amino-1,2,4-triazole derivatives such as enamine (16) (Scheme 4.28) on reacting 5-amino-1,2,4-triazoles with 3-ketovinyl ethers [63], 3-ketoenamines [64], 3-ketoaldehydes [65], enamine-2-carboxylic esters [66] or ethoxymethylene malonates [67].



Scheme 4.28

That means, the overall reaction starts with the interaction of the amino-1,2,4-triazole amino group and the enolic (or analogous) functionality of the three-carbon synthon. In the two-step examples, just mentioned, the first step proceeds under milder conditions (sometimes just in ethanol at room temperature), but the final

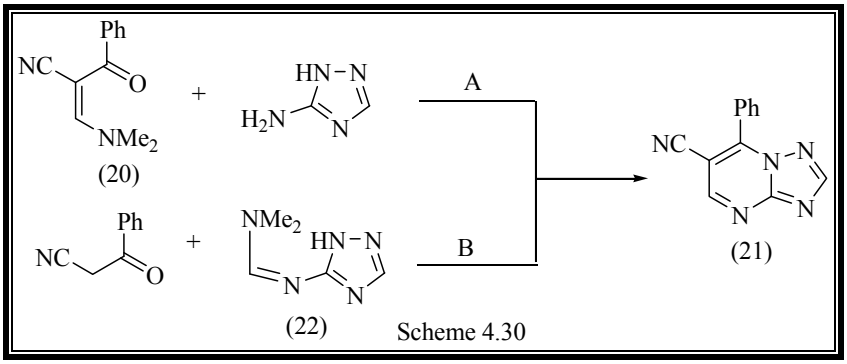
cyclization (or the one-step reaction, if the intermediate is not trapped) requires stronger means (e.g., polyphosphoric acid or boiling acetic acid). Under extreme conditions, triazolylamide (17) was subject to flash vacuum pyrolysis between 300 and 450 ºC to give about 50% triazolo[1,5-*a*]pyrimidine (18) (Scheme 4.29) [68]. Libraries of fused 3-aminopyrimidin-4-ones (19) and other compounds were just recently prepared by the solid-phase and by the solution-phase parallel synthesis [69]. The latter method turned out to be advantageous with respect to yield and purity.



Scheme 4.29

4.4.1.2 Use of Modified 5-amino-1,2,4-triazoles

Scheme 4.30 shows two parallel paths of pyrimidine ring annulation: the conventional method, route A and a route B using a reactive amino-1,2,4-triazole derivative [70]. Amidine (22), formed from 5-amino-1,2,4-triazole and DMF dimethylacetal, can be regarded as the result of incorporating one carbon of the three-carbon synthon (20) into the 5-amino-1,2,4-triazole molecule; condensation with a reactive two-carbon component leads to target triazolo[1,5-*a*]pyrimidine (21).



Scheme 4.30

Path B also serves in confirming the structure of product (21). Similar syntheses of 7-aryl and 7-heterocyclyl-triazolo[1,5-*a*]pyrimidines have been described [71-73], for example, that of an antipyrine derivative [74].

4.4.1.3 The diversity of 1,3-bifunctional synthons

Examples of triazolo[1,5-*a*]pyrimidine synthesis published in the relevant period are listed in Table 1, arranged according to the bifunctional synthons used and to the substituents entering the positions 5 and 7. Triazolo[1,5-*a*]pyrimidines are included in reviews dealing with heterocyclic synthesis by the use of enamines [75], enamine-2-carboxylic esters [76] and ketene mercaptals [77].

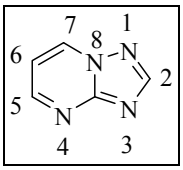


Table 1. Syntheses of triazolo[1,5-*a*]pyrimidines from 1,3-bifunctional synthons and 5-amino-1,2,4-triazoles

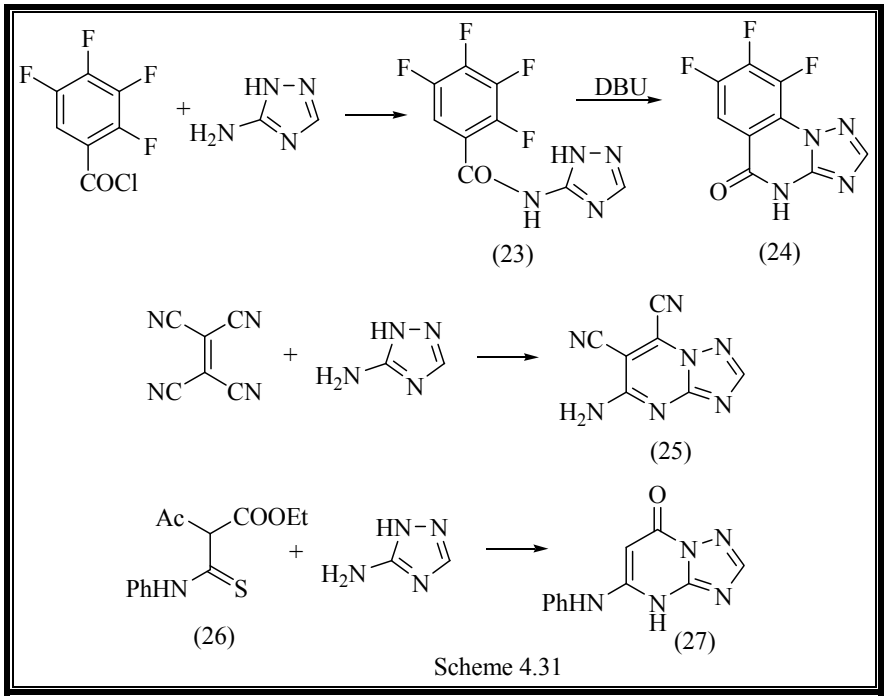
Bifunctional Synthons	R-5[b]	R-7[b]	Bifunctional Synthons	R-5[b]	R-7[b]
1,3-Dialdehyde [78]	H	H	Enamine-2-carboxylate [95]	H	OH
2-Formylacetal [79]	H	H	Acetylenedicarboxylate [96]	CO_2Me	OH
1,3-Diacetal [80]	H	H	3-Ketocarboxylate [97]	R	OH
2-Formylvinyl ether [81]	H	H	3-Alkoxyacrylate [98]	OH	R
2-Formylvinylchloride [82]	H	R	Alkoxyalkylene malonate [99]	R	OH
3-Iminiovinylchloride [83]	H	R	2-Chloroacrylate [100]	OH	R
2-Formylenamine [84]	H	R	Malonic ester [101]	OH	OH
3-Iminioenamine [85]	H	R	Malonyl chloride [102]	OH	OH
3-Ketoaldehyde [86]	R	H	2-Acylketene mercaptal [103]	SR	R’
3-Ketoacetal [87]	R	H	2-Cyanoketene mercaptal [104]	SR	NH_2
3-Ketovinyl ether [88]	H	R	Alkoxyalkylene cyanoacetate [105]	R	NH_2
3-Ketovinyl sulfone [89][c]	R	H	Alkoxyalkylene malonitrile [106]	R	NH_2
3-Ketoenamine [90]	H	R	2-Formylnitrile [107]	H	NH_2
1,3-Diketone [91]	R	R’	2-Cyanoenamine [108]	H	NH_2
3-Ketoalkyne [92]	R[d]	H	Malonitrile [109]	NH_2	NH_2
2-Formylcarboxylate [93]	R	OH	2-Thiocarbamylcarboxylate [110]	NHR	OH
2-Alkoxycarbonylacetal [94]	OH	H			

[a]or tautomeric form.
[b]Substituents on C-5 and C-7, respectively; R and R’ mean (possibly substituted) alkyl, aryl, heterocyclyl and H; OH means hydroxy or tautomeric oxo form.
[c]And regioisomeric 7-R compound.
[d]Deoxyaltrose derivative relating C-glycosides [111].

In recent years, 3-ketoenamines have growing interest as building blocks for 7-aryl-triazolo[1,5-*a*]pyrimidines (Scheme 4.30, Path A) [112, 113]. They also serve to synthesize 7-heterocyclyl-triazolo[1,5-*a*]pyrimidines [114, 115]. In addition to

usual *N*,*N*-dimethyl compounds also analogues having a free amino group can be used as in the synthesis of 7-trifluoromethyl derivatives [116]. Enaminones can be formed in situ, for instance, from dimedone and DMF dimethylacetal [117].

In the course of the cyclization of the stable tetrafluorobenzoyl derivative (23) (Scheme 4.31) fluorine at the *o*-position is involved in the reaction and is replaced to give trifluorobenzo-triazolo[1,5-*a*]pyrimidine (24) [118]. Acetonyl is introduced as substituent into the 7-position by the use of triketone-heptan-2,4,6-trione [119].

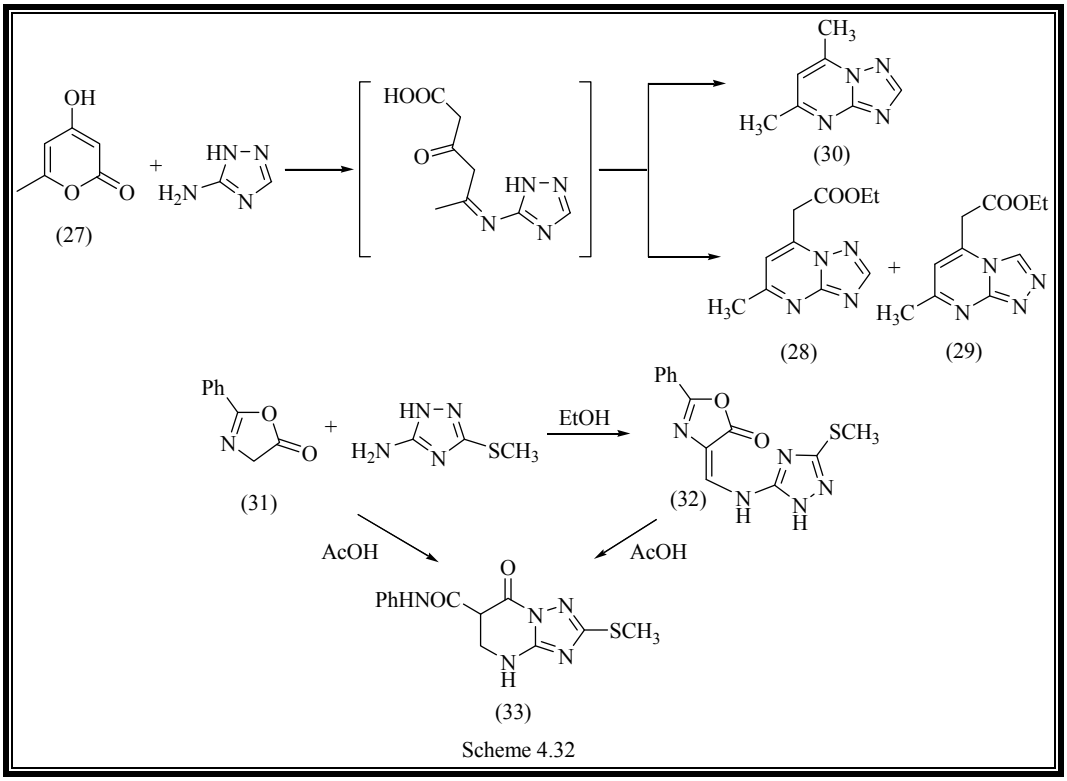


Scheme 4.31

The electron acceptor tetracyanoethylene on interaction with amino-1,2,4-triazole first forms a charge transfer complex that after loss of hydrocyanic acid is transformed into dicyano-triazolo[1,5-*a*]pyrimidine (25) [120]. Fusion of 1,4-naphthoquinone or indenone onto triazolo[1,5-*a*]pyrimidine can in a similar way be performed by the use of 2,3-dicyano-1,4-naphthoquinone or dicyanomethylene indane-1,3-dione, respectively. Another indeno-triazolo[1,5-*a*]pyrimidine is accessible from triketone-2-acetylindane-1,3-dione [121]. On the other hand, acetoacetic ester (26) with 5-amino-1,2,4-triazole suffers ester group cleavage to form anilino-triazolo[1,5-*a*]pyrimidine (27) [122].

4.4.2 Other pyrimidine ring synthesis

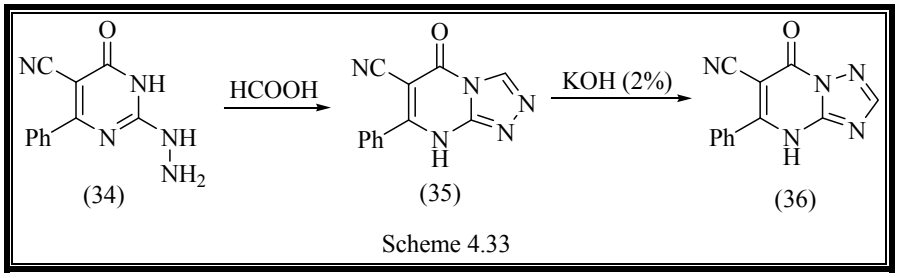
The annulation of pyrimidine onto the triazole ring can be accomplished by the use of heterocyclic precursors that can be regarded as masked 1,3-bifunctional reagents. This way, triacetic acid lactone (27) (Scheme 4.32) reacts as a masked 1,3-diketone and transforms 5-amino-1,2,4-triazole to triazolo[1,5-*a*]pyrimidine (28) together with ring isomer (29) and decarboxylation product (30) [123]. Oxazolones play a similar part [124-126]. Thus, enol ether (31) behaves as a masked 3-ethoxyacrylate and yields, through intermediate (32), benzamido-triazolo1,5-*a*]pyrimidine (33) that, under harsher conditions, directly forms from compound (31).



Scheme 4.32

4.4.3 2-Hydrazinopyrimidines and one-carbon synthons

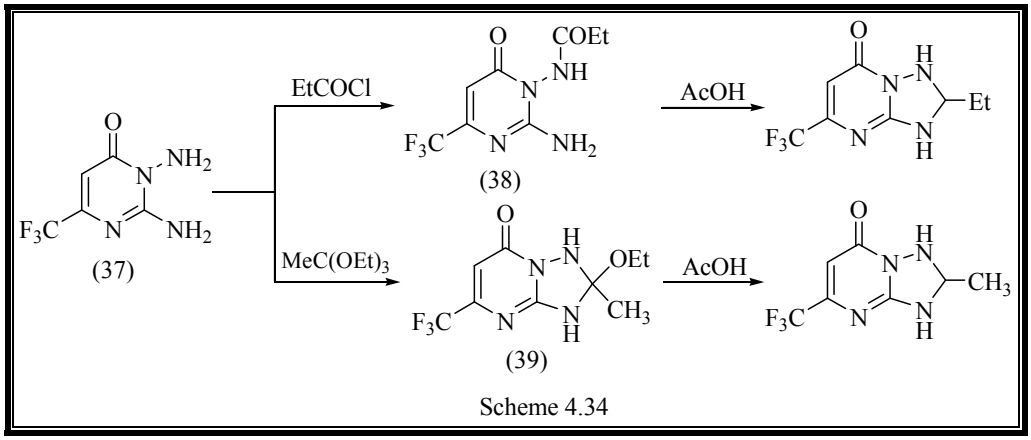
A second common triazolo[1,5-*a*]pyrimidine synthesis consists in the condensation of a C_1-synthon with a 2-hydrazinopyrimidine derivative (e.g., 34, Scheme 4.33). A triazolo[4,3-*a*]-pyrimidine (35) initially forms that often can be isolated [127]. Harsher conditions allow it to isomerize to the target triazolo[1,5-*a*]pyrimidine (36) by Dimroth rearrangement.



Scheme 4.33

4.4.4 Other triazole ring synthesis

Most cyclization of 2,3-diaminopyrimidones (37) [128] or corresponding quinazolones proceed with the participation of carboxylic acids or their derivatives (esters, anhydrides, chlorides, or orthoesters) as shown in Scheme 4.34. Noncyclized or saturated intermediates (38, 39) can frequently be found during synthesis of triazolo[1,5-*a*]pyrimidines.



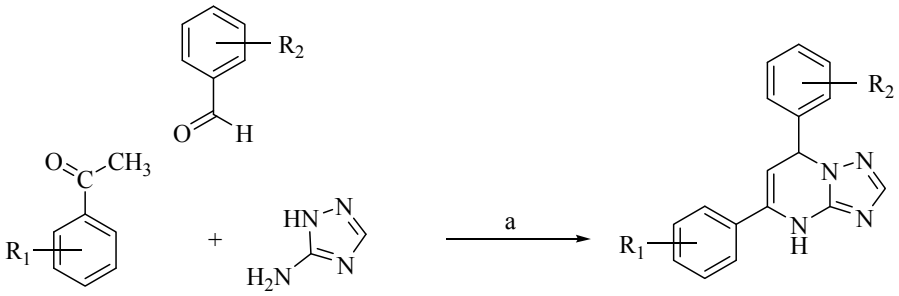
Scheme 4.34

4.5 Current work

The biological importance of 1,2,4-triazolo[1,5-*a*]pyrimidines is well documented. Over the years, various substituted derivatives of these heterocycles have shown utility against a range of biological targets. For example, they have demonstrated activity against malaria and bronchospasm and shown activity as coronary vasodilators, antihypertensive agents, leishmanicides, antibiotics, adenosine A_{2a} antagonists, immunosuppressants, antitumor agents, fungicides, xanthine oxidase inhibitors and phosphodiesterase inhibitors.

Recognizing these facts, we have synthesized four new series of 1,2,4-triazolo[1,5-*a*]pyrimidines (BHD-301 to 340). It was achieved by one-pot, microwave -assisted condensation reaction of aromatic aldehyde, corresponding acetophenone and 5-amino-1,2,4-triazole using glacial acetic acid as a solvent. The structures of all the newly synthesized compounds were elucidated by FT-IR, mass spectra, ^{1}H NMR and elemental analysis. The newly synthesized compounds were subjected to various biological activities *viz*., antimicrobial, antimycobacterial, anticancer and antiviral.

4.6 Reaction Scheme



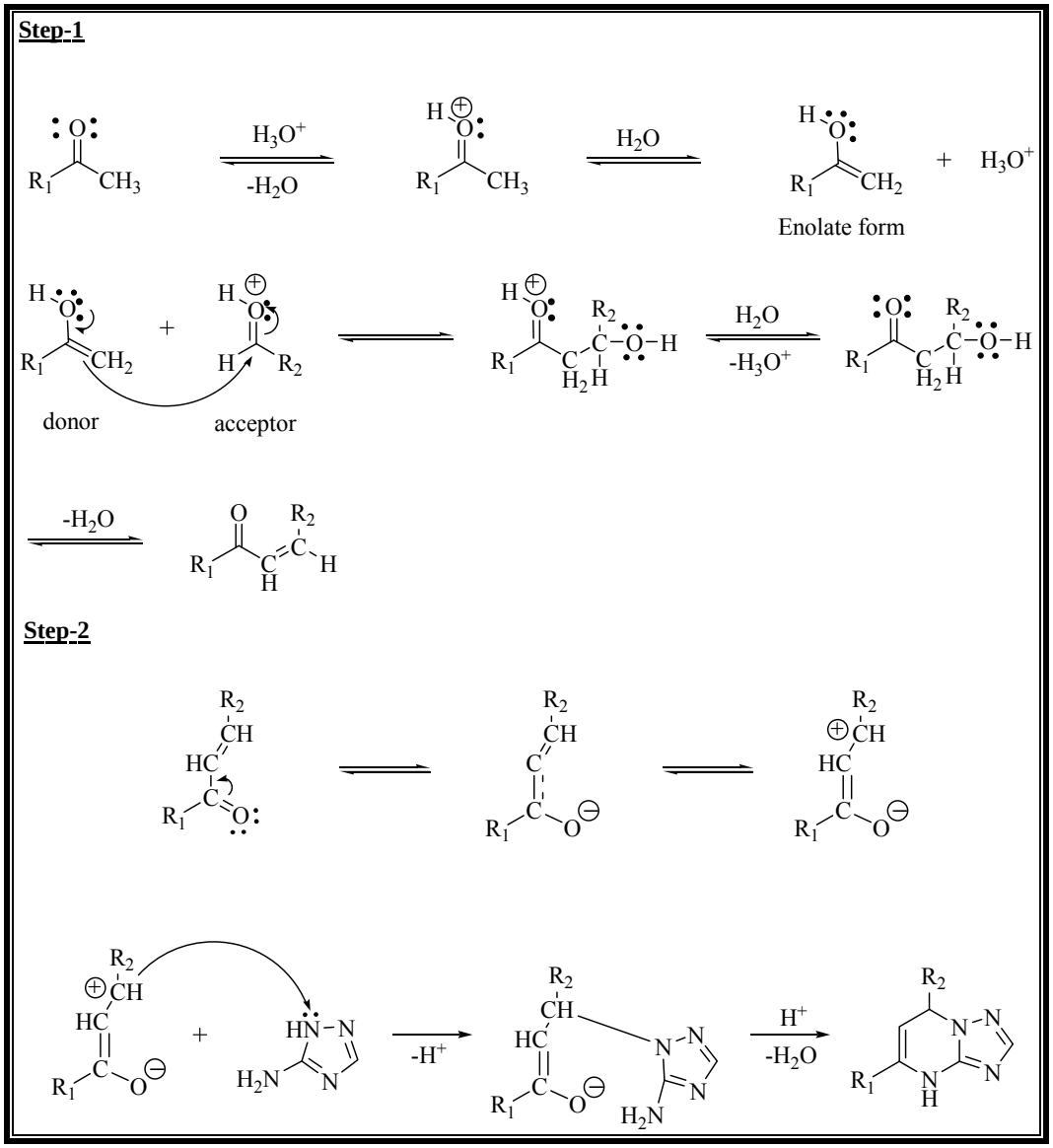
BHD 301 to **340**

Reagents and conditions: (a) gl. AcOH, MW, 120 ºC, 10-12 min

Code	R_1	R_2	M.F.	M.W.	M.P. ºC	Yield %	R_{f1}	R_{f2}
BHD-301	4-F	H	$C_{17}H_{13}FN_4$	292	175-177	70	0.45	0.67
BHD-302	4-F	4-F	$C_{17}H_{12}F_2N_4$	310	158-160	76	0.41	0.61
BHD-303	4-F	4-Cl	$C_{17}H_{12}ClFN_4$	326	231-233	62	0.50	0.60
BHD-304	4-F	4-NO_2	$C_{17}H_{12}FN_5O_2$	337	165-167	78	0.54	0.69
BHD-305	4-F	4-CH_3	$C_{18}H_{15}FN_4$	306	185-187	65	0.51	0.74
BHD-306	4-F	4-OCH_3	$C_{18}H_{15}FN_4O$	322	217-219	72	0.57	0.78
BHD-307	4-F	3,4-OCH_3	$C_{19}H_{17}FN_4O_2$	352	204-206	60	0.53	0.70
BHD-308	4-F	3-NO_2	$C_{17}H_{12}FN_5O_2$	337	191-193	62	0.42	0.63
BHD-309	4-F	3-Cl	$C_{17}H_{12}ClFN_4$	326	224-226	71	0.46	0.67
BHD-310	4-F	2-Cl	$C_{17}H_{12}ClFN_4$	326	199-201	79	0.40	0.60
BHD-311	4-CH_3	H	$C_{18}H_{16}N_4$	288	214-216	68	0.48	0.66
BHD-312	4-CH_3	4-F	$C_{18}H_{15}FN_4$	306	201-203	80	0.53	0.71
BHD-313	4-CH_3	4-Cl	$C_{18}H_{15}ClN_4$	322	179-181	73	0.43	0.69
BHD-314	4-CH_3	4-NO_2	$C_{18}H_{15}N_5O_2$	333	197-199	70	0.49	0.73
BHD-315	4-CH_3	4-CH_3	$C_{19}H_{18}N_4$	302	185-187	75	0.54	0.72
BHD-316	4-CH_3	4-OCH_3	$C_{19}H_{18}N_4O$	318	224-226	60	0.60	0.64
BHD-317	4-CH_3	3,4-OCH_3	$C_{20}H_{20}N_4O_2$	348	161-163	72	0.63	0.75
BHD-318	4-CH_3	3-NO_2	$C_{18}H_{15}N_5O_2$	333	148-150	64	0.50	0.70
BHD-319	4-CH_3	3-Cl	$C_{18}H_{15}ClN_4$	322	166-168	72	0.52	0.62
BHD-320	4-CH_3	2-Cl	$C_{18}H_{15}ClN_4$	322	210-212	76	0.48	0.61
BHD-321	4-CF_3	H	$C_{18}H_{13}F_3N_4$	342	169-171	80	0.51	0.56
BHD-322	4-CF_3	4-F	$C_{18}H_{12}F_4N_4$	360	217-219	71	0.54	0.62
BHD-323	4-CF_3	4-Cl	$C_{18}H_{12}ClF_3N_4$	376	231-233	82	0.45	0.70
BHD-324	4-CF_3	4-NO_2	$C_{18}H_{12}F_3N_5O_2$	387	188-190	78	0.50	0.72
BHD-325	4-CF_3	4-CH_3	$C_{19}H_{15}F_3N_4$	356	152-154	85	0.58	0.68
BHD-326	4-CF_3	4-OCH_3	$C_{19}H_{15}F_3N_4O$	372	172-174	80	0.61	0.75
BHD-327	4-CF_3	3,4-OCH_3	$C_{20}H_{17}F_3N_4O_2$	402	227-229	68	0.60	0.71
BHD-328	4-CF_3	3-NO_2	$C_{18}H_{12}F_3N_5O_2$	387	137-139	72	0.49	0.62
BHD-329	4-CF_3	3-Cl	$C_{18}H_{12}ClF_3N_4$	376	212-214	74	0.47	0.68
BHD-330	4-CF_3	2-Cl	$C_{18}H_{12}ClF_3N_4$	376	191-193	69	0.50	0.60
BHD-331	3-CF_3	H	$C_{18}H_{13}F_3N_4$	342	156-158	80	0.49	0.59
BHD-332	3-CF_3	4-F	$C_{18}H_{12}F_4N_4$	360	222-224	77	0.43	0.63
BHD-333	3-CF_3	4-Cl	$C_{18}H_{12}ClF_3N_4$	376	244-246	82	0.50	0.60
BHD-334	3-CF_3	4-NO_2	$C_{18}H_{12}F_3N_5O_2$	387	198-200	69	0.56	0.67
BHD-335	3-CF_3	4-CH_3	$C_{19}H_{15}F_3N_4$	356	205-207	65	0.61	0.74
BHD-336	3-CF_3	4-OCH_3	$C_{19}H_{15}F_3N_4O$	372	143-145	75	0.56	0.75
BHD-337	3-CF_3	3,4-OCH_3	$C_{20}H_{17}F_3N_4O_2$	402	175-177	68	0.59	0.65
BHD-338	3-CF_3	3-NO_2	$C_{18}H_{12}F_3N_5O_2$	387	164-166	77	0.41	0.62
BHD-339	3-CF_3	3-Cl	$C_{18}H_{12}ClF_3N_4$	376	217-219	65	0.45	0.68
BHD-340	3-CF_3	2-Cl	$C_{18}H_{12}ClF_3N_4$	376	187-189	70	0.57	0.64

TLC Solvent system R_{f1}: Hexane: Ethyl acetate - 6:4; TLC Solvent system R_{f2}: Chloroform: Methanol - 9:1

4.6.1 Plausible Reaction Mechanism



4.7 Experimental

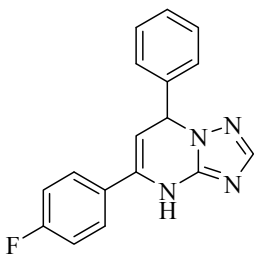
4.7.1 Materials and Methods

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 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 agreement with the structures assigned.

4.7.2 General procedure for the synthesis of 5-(4-fluorophenyl)-4,7-dihydro-7-(aryl)-[1,2,4]triazolo[1,5-a]pyrimidines (BHD 301-310)

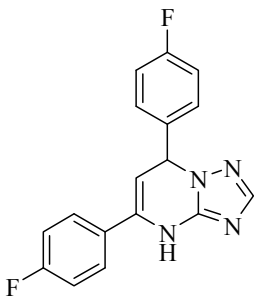
A mixture of the 5-amino-1,2,4-triazole (0.01 mol), 4-fluoroacetophenone (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) in glacial acetic acid (10 mL) was irradiated under microwave conditions at 120 °C for 10-12 min. The microwave irradiation was operated in 30-second cycles. After cooling, methanol (10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products BHD 301-310, which were recrystallized from ethanol.

4.7.2.1 5-(4-fluorophenyl)-4,7-dihydro-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-301)

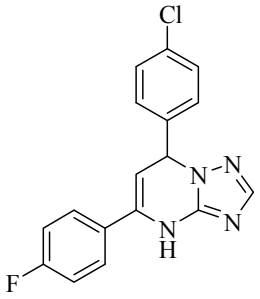


Yield: 70%; mp 175-177 ºC; MS: *m/z* 292; Anal. Calcd. for $C_{17}H_{13}FN_4$: C, 69.85; H, 4.48; N, 19.17. Found: C, 69.75; H, 4.43; N, 19.11%.

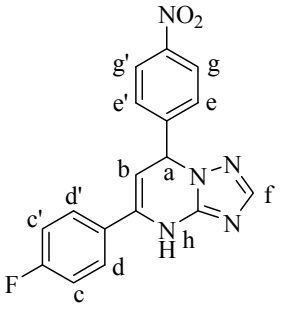
4.7.2.2 5,7-bis(4-fluorophenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-302) Yield: 76%; mp 158-160 ºC; MS: *m/z* 310; Anal. Calcd. for $C_{17}H_{12}F_2N_4$: C, 65.80; H, 3.90; N, 18.06. Found: C, 65.68; H, 3.82; N, 17.97%.



4.7.2.3 5-(4-fluorophenyl)-4,7-dihydro-7-(4-chlorophenyl)-[1,2,4]triazolo[1,5-a] pyrimidine (BHD-303) Yield: 62%; mp 231-233 ºC; MS: *m/z* 326; Anal. Calcd. for $C_{17}H_{12}ClFN_4$: C, 62.49; H, 3.70; N, 17.15. Found: C, 62.37; H, 3.64; N, 17.05%.



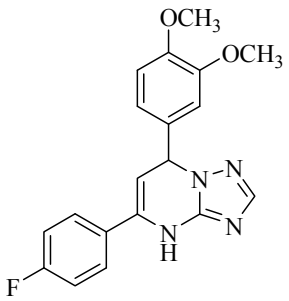
4.7.2.4 5-(4-fluorophenyl)-4,7-dihydro-7-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a] pyrimidine (BHD-304) Yield: 78%; mp 165-167 ºC; IR (cm^{-1}): 3281 (N-H stretching of secondary amine), 3109 (C-H stretching of aromatic ring), 1626 (C=N stretching of triazole ring), 1552 and 1498 (C=C stretching of aromatic ring), 1377 (NO_2 stretching), 1294 (C-N stretching), 1269 (C-H in plane deformation of aromatic ring), 1197 (C-F stretching), 804 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 5.08-5.09 (d, 1H, H_a, J = 2.3 Hz), 6.37-6.38 (d, 1H, H_b, J = 3.6 Hz), 7.13-7.18 (t, 2H, $H_{cc'}$, J = 8.6 Hz), 7.53-7.55 (d, 2H, $H_{dd'}$, J = 8.6 Hz), 7.60-7.65 (m, 3H, $H_{ee'-f}$), 8.21-8.23 (d, 2H, $H_{gg'}$, J = 8.6 Hz), 10.15 (s, 1H, H_h); MS: *m/z* 337; Anal. Calcd. for $C_{17}H_{12}FN_5O_2$: C, 60.53; H, 3.59; N, 20.76. Found: C, 60.40; H, 3.53; N, 20.68%.



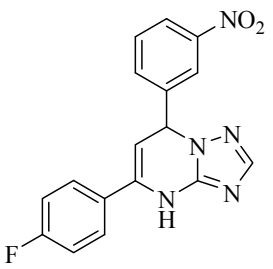
4.7.2.5 5-(4-fluorophenyl)-4,7-dihydro-7-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-305) Yield: 65%; mp 185-187 ºC; IR (cm^{-1}): 3282 (N-H stretching of secondary amine), 3107 (C-H stretching of aromatic ring), 2956 (C-H symmetrical stretching of CH_3 group), 2839 (C-H asymmetrical stretching of CH_3 group), 1626 (C=N stretching of triazole ring), 1552 and 1498 (C=C stretching of aromatic ring), 1292 (C-N stretching), 1269 (C-H in plane deformation of aromatic ring), 1197 (C-F stretching), 804 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.35 (s, 3H, H_a), 5.03-5.04 (d, 1H, H_b, J = 3.6 Hz), 6.10-6.11 (d, 1H, H_c, J = 3.6 Hz), 7.13-7.21 (m, 6H, $H_{d\text{-}f'}$), 7.47-7.49 (d, 2H, $H_{gg'}$, J = 8.2 Hz), 7.54 (s, 1H, H_h), 9.90 (s, 1H, H_i); MS: *m/z* 306; Anal. Calcd. for $C_{18}H_{15}FN_4$: C, 70.57; H, 4.94; N, 18.29. Found: C, 70.41; H, 4.90; N, 18.21%.

4.7.2.6 5-(4-fluorophenyl)-4,7-dihydro-7-(4-methoxyphenyl)-[1,2,4]triazolo[1,5-a] pyrimidine (BHD-306) Yield: 72%; mp 217-219 ºC; IR (cm^{-1}): 3281 (N-H stretching of secondary amine), 3111 (C-H stretching of aromatic ring), 1624 (C=N stretching of triazole ring), 1552 and 1498 (C=C stretching of aromatic ring), 1427 (C-H asymmetrical deformation of CH_3 group), 1294 (C-N stretching), 1269 (C-H in plane deformation of aromatic ring), 1197 (C-F stretching), 1058 (C-O-C symmetrical stretching of ether linkage), 804 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 3.76 (s, 3H, H_a), 5.02 (d, 1H, H_b, J = 2.4 Hz), 6.10 (d, 1H, H_c, J = 3.4 Hz), 6.86-6.88 (d, 2H, $H_{dd'}$, J = 8.5 Hz), 7.10-7.14 (t, 2H, $H_{ee'}$, J = 8.6 Hz), 7.25-7.27 (d, 2H, $H_{ff'}$, J = 8.5 Hz), 7.50 (s, 1H, H_g), 7.61-7.64 (t, 2H, $H_{hh'}$), 10.04 (s, 1H, H_i); MS: *m/z* 322; Anal. Calcd. for $C_{18}H_{15}FN_4O$: C, 67.07; H, 4.69; N, 17.38. Found: C, 66.90; H, 4.63; N, 17.30%.

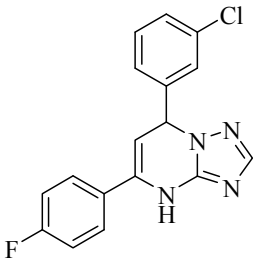
4.7.2.7 5-(4-fluorophenyl)-4,7-dihydro-7-(3,4-dimethoxyphenyl)-[1,2,4]triazolo[1,5-a] pyrimidine (BHD-307) Yield: 60%; mp 204-206 ºC; MS: *m/z* 352; Anal. Calcd. for $C_{19}H_{17}FN_4O_2$: C, 64.76; H, 4.86; N, 15.90. Found: C, 64.61; H, 4.81; N, 15.79%.



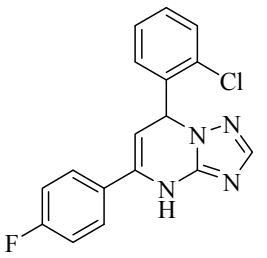
4.7.2.8 5-(4-fluorophenyl)-4,7-dihydro-7-(3-nitrophenyl)-[1,2,4]triazolo[1,5-a] pyrimidine (BHD-308) Yield: 62%; mp 191-193 ºC; MS: *m/z* 337; Anal. Calcd. for $C_{17}H_{12}FN_5O_2$: C, 60.53; H, 3.59; N, 20.76. Found: C, 60.38; H, 3.51; N, 20.65%.



4.7.2.9 5-(4-fluorophenyl)-4,7-dihydro-7-(3-chlorophenyl)-[1,2,4]triazolo[1,5-a] pyrimidine (BHD-309) Yield: 71%; mp 224-226 ºC; MS: *m/z* 326; Anal. Calcd. for $C_{17}H_{12}ClFN_4$: C, 62.49; H, 3.70; N, 17.15. Found: C, 62.34; H, 3.65; N, 17.08%.



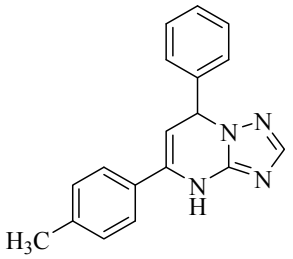
4.7.2.10 5-(4-fluorophenyl)-4,7-dihydro-7-(2-chlorophenyl)-[1,2,4]triazolo[1,5-a] pyrimidine (BHD-310) Yield: 79%; mp 199-201 ºC; MS: *m/z* 326; Anal. Calcd. for $C_{17}H_{12}ClFN_4$: C, 62.49; H, 3.70; N, 17.15. Found: C, 62.36; H, 3.66; N, 17.10%.



4.7.3 General procedure for the synthesis of 4,7-dihydro-7-(aryl)-5-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidines (BHD 311-320)

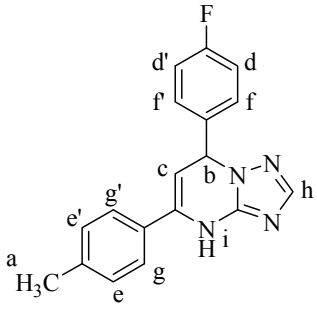
A mixture of the 5-amino-1,2,4-triazole (0.01 mol), 4-methylacetophenone (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) in glacial acetic acid (10 mL) was irradiated under microwave conditions at 120 °C for 10-12 min. The microwave irradiation was operated in 30-second cycles. After cooling, methanol (10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products BHD 311-320, which were recrystallized from ethanol.

4.7.3.1 4,7-dihydro-7-phenyl-5-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-311)



Yield: 68%; mp 214-216 ºC; MS: *m/z* 288; Anal. Calcd. for $C_{18}H_{16}N_4$: C, 74.98; H, 5.59; N, 19.43. Found: C, 74.83; H, 5.52; N, 19.31%.

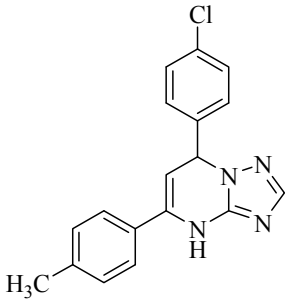
4.7.3.2 4,7-dihydro-7-(4-fluorophenyl)-5-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-312)



Yield: 80%; mp 201-203 ºC; IR (cm^{-1}): 3194 (N-H stretching of secondary amine), 3090 (C-H stretching of aromatic ring), 2972 (C-H symmetrical stretching of CH_3 group), 2860 (C-H asymmetrical stretching of CH_3 group), 1658 (N-H deformation of pyrimidine ring), 1597 (C=N stretching of triazole ring), 1550 and 1475 (C=C stretching of aromatic ring), 1330 (C-N stretching), 1070 (C-F stretching), 941 (C-H in plane deformation of aromatic ring), 825 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.35 (s, 3H, H_a), 5.04-5.05 (d, 1H, H_b, J = 3.6 Hz), 6.18 (d, 1H, H_c, J = 3.6 Hz), 7.07-7.11 (t, 2H, $H_{dd'}$, J = 8.7 Hz), 7.19-7.21 (d, 2H, $H_{ee'}$, J = 8.0 Hz), 7.32-7.36 (t, 2H, $H_{ff'}$), 7.48-7.50 (d, 2H, $H_{gg'}$, J = 8.2 Hz), 7.55 (s, 1H, H_h), 9.96 (s,

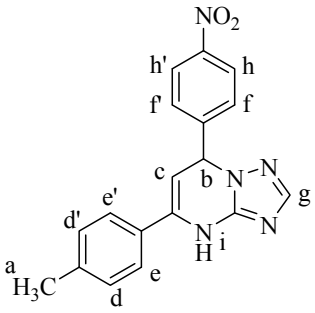
1H, H_i); MS: *m/z* 306; Anal. Calcd. for $C_{18}H_{15}FN_4$: C, 70.57; H, 4.94; N, 18.29. Found: C, 70.64; H, 4.90; N, 18.20%.

4.7.3.3 4,7-dihydro-7-(4-chlorophenyl)-5-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-313)



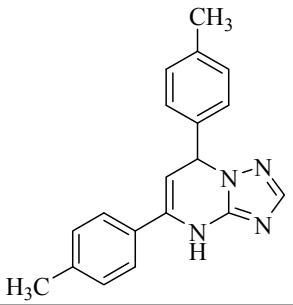
Yield: 73%; mp 179-181 ºC; MS: *m/z* 322; Anal. Calcd. for $C_{18}H_{15}ClN_4$: C, 66.98; H, 4.68; N, 17.36. Found: C, 66.87; H, 4.65; N, 17.29%.

4.7.3.4 4,7-dihydro-7-(4-nitrophenyl)-5-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-314)



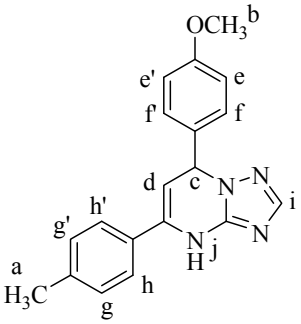
Yield: 70%; mp 197-199 ºC; IR (cm^{-1}): 3192 (N-H stretching of secondary amine), 3093 (C-H stretching of aromatic ring), 2976 (C-H symmetrical stretching of CH_3 group), 2862 (C-H asymmetrical stretching of CH_3 group), 1658 (N-H deformation of pyrimidine ring), 1593 (C=N stretching of triazole ring), 1548 (NO_2 stretching), 1523 and 1473 (C=C stretching of aromatic ring), 1352 (C-N stretching), 943 (C-H in plane deformation of aromatic ring), 823 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.37 (s, 3H, H_a), 5.02-5.03 (d, 1H, H_b, J = 3.0 Hz), 6.33-6.34 (d, 1H, H_c, J = 3.2 Hz), 7.20-7.22 (d, 2H, $H_{dd'}$, J = 7.8 Hz), 7.47-7.49 (d, 2H, $H_{ee'}$, J = 7.6 Hz), 7.52-7.55 (d, 2H, $H_{ff'}$, J = 8.1 Hz), 7.58 (s, 1H, H_g), 8.20-8.22 (d, 2H, $H_{hh'}$, J = 8.0 Hz), 9.96 (s, 1H, H_i); MS: *m/z* 333; Anal. Calcd. for $C_{18}H_{15}N_5O_2$: C, 64.86; H, 4.54; N, 21.01. Found: C, 64.69; H, 4.49; N, 20.91%.

4.7.3.5 4,7-dihydro-5,7-di-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-315)



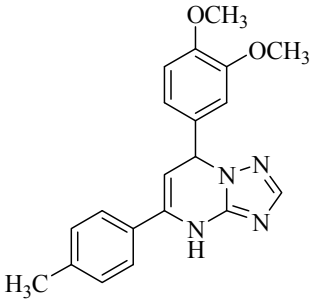
Yield: 75%; mp 185-187 ºC; MS: *m/z* 302; Anal. Calcd. for $C_{19}H_{18}N_4$: C, 75.47; H, 6.00; N, 18.53. Found: C, 75.31; H, 5.95; N, 18.43%.

4.7.3.6 4,7-dihydro-7-(4-methoxyphenyl)-5-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine



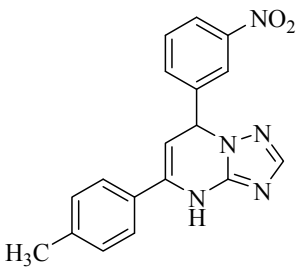
(BHD-316) Yield: 60%; mp 224-226 ºC; IR (cm^{-1}): 3196 (N-H stretching of secondary amine), 3086 (C-H stretching of aromatic ring), 3020 (C-H symmetrical stretching of CH_3 group), 2858 (C-H asymmetrical stretching of CH_3 group), 1662 (N-H deformation of pyrimidine ring), 1595 (C=N stretching of triazole ring), 1550 and 1467 (C=C stretching of aromatic ring), 1429 (C-H asymmetrical deformation of CH_3 group), 1330 (C-N stretching), 1176 (C-O-C asymmetrical stretching of ether linkage), 941 (C-H in plane deformation of aromatic ring), 829 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.36 (s, 3H, H_a), 3.76 (s, 3H, H_b), 5.04-5.05 (d, 1H, H_c, J = 3.4 Hz), 6.10-6.11 (d, 1H, H_d, J = 3.5 Hz), 6.86-6.88 (d, 2H, $H_{ee'}$, J = 8.6 Hz), 7.19-7.21 (d, 2H, $H_{ff'}$, J = 8.1 Hz), 7.24-7.26 (d, 2H, $H_{gg'}$, J = 8.6 Hz), 7.48-7.50 (d, 2H, $H_{hh'}$, J = 8.1 Hz), 7.56 (s, 1H, H_i), 9.92 (s, 1H, H_j); MS: *m/z* 318; Anal. Calcd. for $C_{19}H_{18}N_4O$: C, 71.68; H, 5.70; N, 17.60. Found: C, 71.68; H, 5.70; N, 17.60%.

4.7.3.7 4,7-dihydro-7-(3,4-dimethoxyphenyl)-5-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine



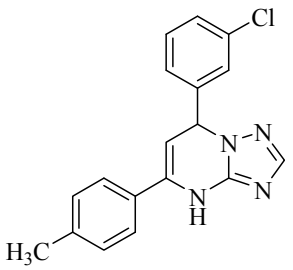
(BHD-317) Yield: 72%; mp 161-163 ºC; MS: *m/z* 348; Anal. Calcd. for $C_{20}H_{20}N_4O_2$: C, 68.95; H, 5.79; N, 16.08. Found: C, 68.81; H, 5.76; N, 16.00%.

4.7.3.8 4,7-dihydro-7-(3-nitrophenyl)-5-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine

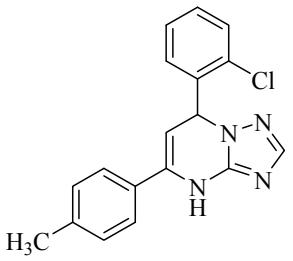


(BHD-318) Yield: 64%; mp 148-150 ºC; MS: *m/z* 333; Anal. Calcd. for $C_{18}H_{15}N_5O_2$: C, 64.86; H, 4.54; N, 21.01. Found: C, 64.71; H, 4.49; N, 20.91%.

4.7.3.9 4,7-dihydro-7-(3-chlorophenyl)-5-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-319) Yield: 72%; mp 166-168 ºC; MS: *m/z* 322; Anal. Calcd. for $C_{18}H_{15}ClN_4$: C, 66.98; H, 4.68; N, 17.36. Found: C, 66.84; H, 4.63; N, 17.25%.



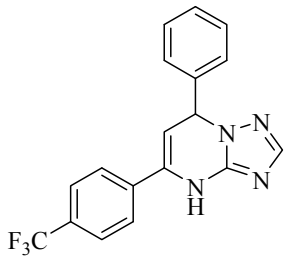
4.7.3.10 4,7-dihydro-7-(2-chlorophenyl)-5-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-320) Yield: 76%; mp 210-212 ºC; MS: *m/z* 322; Anal. Calcd. for $C_{18}H_{15}ClN_4$: C, 66.98; H, 4.68; N, 17.36. Found: C, 66.86; H, 4.67; N, 17.27%.



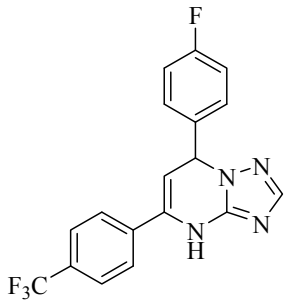
4.7.4 General procedure for the synthesis of 5-(4-(trifluoromethyl)phenyl)-4,7-dihydro-7-(aryl)-[1,2,4]triazolo[1,5-a]pyrimidines (BHD 321-330)

A mixture of the aminoazole (0.01 mol), 4-trifluoromethylacetophenone (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) in glacial acetic acid (10 mL) was irradiated under microwave conditions at 120 °C for 10-12 min. The microwave irradiation was operated in 30-second cycles. After cooling, methanol (10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products BHD 321-330, which were recrystallized from ethanol.

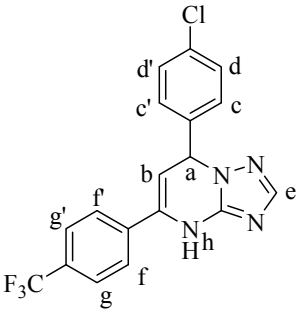
4.7.4.1 5-(4-(trifluoromethyl)phenyl)-4,7-dihydro-7-phenyl-[1,2,4]triazolo[1,5-a] pyrimidine (BHD-321) Yield: 80%; mp 169-171 ºC; MS: *m/z* 342; Anal. Calcd. for $C_{18}H_{13}F_3N_4$: C, 63.16; H, 3.83; N, 16.37. Found: C, 63.02; H, 3.77; N, 16.29%.



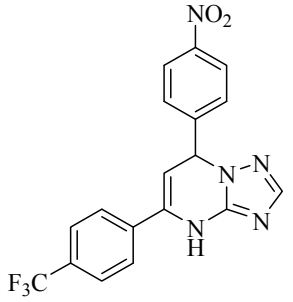
4.7.4.2 5-(4-(trifluoromethyl)phenyl)-4,7-dihydro-7-(4-fluorophenyl)-[1,2,4]triazolo [1,5-a]pyrimidine (BHD-322) Yield: 71%; mp 217-219 ºC; MS: *m/z* 360; Anal. Calcd. for $C_{18}H_{12}F_4N_4$: C, 60.00; H, 3.36; N, 15.55. Found: C, 59.87; H, 3.29; N, 15.47%.

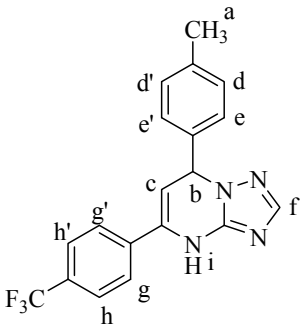


4.7.4.3 5-(4-(trifluoromethyl)phenyl)-4,7-dihydro-7-(4-chlorophenyl)-[1,2,4]triazolo [1,5-a]pyrimidine (BHD-323) Yield: 82%; mp 231-233 ºC; IR (cm^{-1}): 3196 (N-H stretching of secondary amine), 3088 (C-H stretching of aromatic ring), 1656 (N-H deformation of pyrimidine ring), 1597 (C=N stretching of triazole ring), 1548 and 1491 (C=C stretching of aromatic ring), 1330 (C-N stretching), 1132 (C-F stretching), 941 (C-H in plane deformation of aromatic ring), 804 (C-H out of plane deformation of 1,4-disubstitution), 723 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 5.24-5.25 (d, 1H, H_a, J = 3.6 Hz), 6.23-6.24 (d, 1H, H_b, J = 3.6 Hz), 7.30-7.33 (d, 2H, $H_{cc'}$, J = 8.6 Hz), 7.35-7.39 (d, 2H, $H_{dd'}$, J = 8.4 Hz), 7.59 (s, 1H, H_e), 7.69-7.72 (d, 2H, $H_{ff'}$, J = 8.3 Hz), 8.80-8.83 (d, 2H, $H_{gg'}$, J = 8.2 Hz), 10.23 (s, 1H, H_h); MS: *m/z* 376; Anal. Calcd. for $C_{18}H_{12}ClF_3N_4$: C, 57.38; H, 3.21; N, 14.87. Found: C, 57.21; H, 3.16; N, 14.80%.

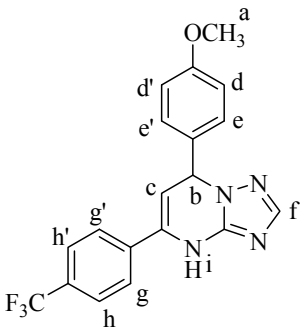


4.7.4.4 5-(4-(trifluoromethyl)phenyl)-4,7-dihydro-7-(4-nitrophenyl)-[1,2,4]triazolo [1,5-a]pyrimidine (BHD-324) Yield: 78%; mp 188-190 ºC; IR (cm^{-1}): MS: *m/z* 387; Anal. Calcd. for $C_{18}H_{12}F_3N_5O_2$: C, 55.82; H, 3.12; N, 18.08. Found: C, 55.69; H, 3.05; N, 17.99%.



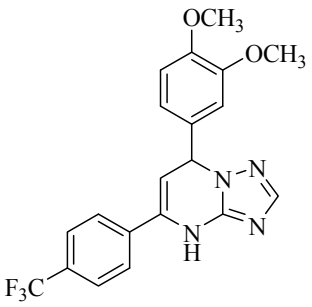
4.7.4.5 5-(4-(trifluoromethyl)phenyl)-4,7-dihydro-7-p-tolyl-[1,2,4]triazolo[1,5-a] pyrimidine (BHD-325) 

Yield: 85%; mp 152-154 ºC; IR (cm^{-1}): 3196 (N-H stretching of secondary amine), 3093 (C-H stretching of aromatic ring), 3032 (C-H symmetrical stretching of CH_3 group), 2904 (C-H asymmetrical stretching of CH_3 group), 1656 (N-H deformation of pyrimidine ring), 1595 (C=N stretching of triazole ring), 1548 and 1514 (C=C stretching of aromatic ring), 1323 (C-N stretching), 1116 (C-F stretching), 941 (C-H in plane deformation of aromatic ring), 804 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.31 (s, 3H, H_a), 5.21-5.22 (d, 1H, H_b, J = 3.5 Hz), 6.15 (d, 1H, H_c, J = 3.6 Hz), 7.14-7.16 (d, 2H, $H_{dd'}$, J = 8.0 Hz), 7.19-7.21 (d, 2H, $H_{ee'}$, J = 8.1 Hz), 7.54 (s, 1H, H_f), 7.67-7.69 (d, 2H, $H_{gg'}$, J = 8.4 Hz), 7.79-7.81 (d, 2H, $H_{hh'}$, J = 8.2 Hz), 10.12 (s, 1H, H_i); MS: *m/z* 356; Anal. Calcd. for $C_{19}H_{15}F_3N_4$: C, 64.04; H, 4.24; N, 15.72. Found: C, 63.91; H, 4.17; N, 15.63%.

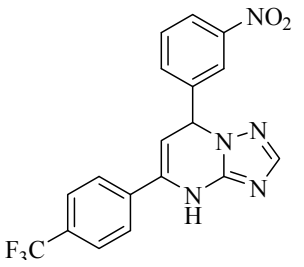
4.7.4.6 5-(4-(trifluoromethyl)phenyl)-4,7-dihydro-7-(4-methoxyphenyl)-[1,2,4]triazolo [1,5-a]pyrimidine (BHD-326) 

Yield: 80%; mp 172-174 ºC; IR (cm^{-1}): 3194 (N-H stretching of secondary amine), 3090 (C-H stretching of aromatic ring), 1656 (N-H deformation of pyrimidine ring), 1595 (C=N stretching of triazole ring), 1550 and 1512 (C=C stretching of aromatic ring), 1329 (C-N stretching), 1192 (C-O-C asymmetrical stretching of ether linkage), 1130 (C-F stretching), 939 (C-H in plane deformation of aromatic ring), 808 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 3.77 (s, 3H, H_a), 5.21-5.22 (d, 1H, H_b, J = 2.8 Hz), 6.14-6.15 (d, 1H, H_c, J = 3.6 Hz), 6.87-6.89 (d, 2H, $H_{dd'}$, J = 8.6 Hz), 7.25-7.27 (d, 2H, $H_{ee'}$, J = 8.6 Hz), 7.56 (s, 1H, H_f), 7.68-7.70 (d, 2H, $H_{gg'}$, J = 8.3 Hz), 7.80-7.82 (d, 2H, $H_{hh'}$, J = 8.2 Hz), 10.11 (s, 1H, H_i); MS: *m/z* 372; Anal. Calcd. for $C_{19}H_{15}F_3N_4O$: C, 61.29; H, 4.06; N, 15.05. Found: C, 61.19; H, 4.01; N, 14.98%.

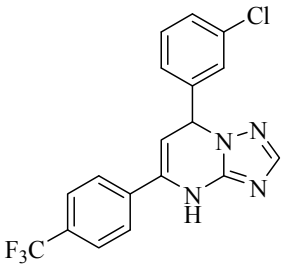
4.7.4.7 5-(4-(trifluoromethyl)phenyl)-4,7-dihydro-7-(3,4-dimethoxyphenyl)-[1,2,4] triazolo[1,5-a]pyrimidine (BHD-327) Yield: 68%; mp 227-229 ºC; MS: *m/z* 402; Anal. Calcd. for $C_{20}H_{17}F_3N_4O_2$: C, 59.70; H, 4.26; N, 13.92. Found: C, 59.70; H, 4.26; N, 13.92%.



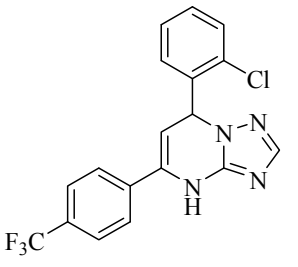
4.7.4.8 5-(4-(trifluoromethyl)phenyl)-4,7-dihydro-7-(3-nitrophenyl)-[1,2,4]triazolo [1,5-a]pyrimidine (BHD-328) Yield: 72%; mp 137-139 ºC; MS: *m/z* 387; Anal. Calcd. for $C_{18}H_{12}F_3N_5O_2$: C, 55.82; H, 3.12; N, 18.08. Found: C, 55.71; H, 3.07; N, 17.95%.



4.7.4.9 5-(4-(trifluoromethyl)phenyl)-4,7-dihydro-7-(3-chlorophenyl)-[1,2,4]triazolo [1,5-a]pyrimidine (BHD-329) Yield: 74%; mp 212-214 ºC; MS: *m/z* 376; Anal. Calcd. for $C_{18}H_{12}ClF_3N_4$: C, 57.38; H, 3.21; N, 14.87. Found: C, 57.25; H, 3.18; N, 14.78%.



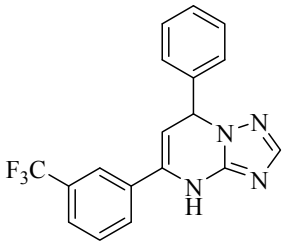
4.7.4.10 5-(4-(trifluoromethyl)phenyl)-4,7-dihydro-7-(2-chlorophenyl)-[1,2,4]triazolo [1,5-a]pyrimidine (BHD-330) Yield: 69%; mp 191-193 ºC; MS: *m/z* 376; Anal. Calcd. for $C_{18}H_{12}ClF_3N_4$: C, 57.38; H, 3.21; N, 14.87. Found: C, 57.22; H, 3.15; N, 14.79%.



4.7.5 General procedure for the synthesis of 5-(3-(trifluoromethyl)phenyl)-4,7-dihydro-7-(aryl)-[1,2,4]triazolo[1,5-a]pyrimidines (BHD 331-340)

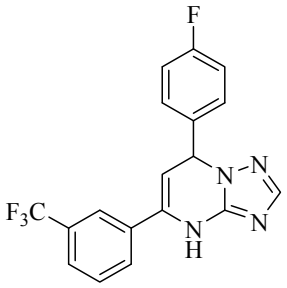
A mixture of the aminoazole (0.01 mol), 3-trifluoromethylacetophenone (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) in glacial acetic acid (10 mL) was irradiated under microwave conditions at 120 °C for 10-12 min. The microwave irradiation was operated in 30-second cycles. After cooling, methanol (10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products BHD 331-340, which were recrystallized from ethanol.

4.7.5.1 5-(3-(trifluoromethyl)phenyl)-4,7-dihydro-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-331)



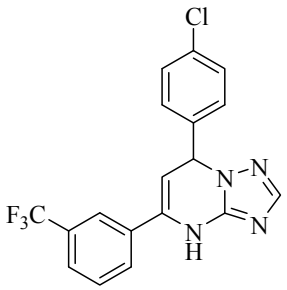
Yield: 80%; mp 156-158 ºC; MS: *m/z* 342; Anal. Calcd. for $C_{18}H_{13}F_3N_4$: C, 63.16; H, 3.83; N, 16.37. Found: C, 63.02; H, 3.77; N, 16.29%.

4.7.5.2 5-(3-(trifluoromethyl)phenyl)-4,7-dihydro-7-(4-fluorophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-332)



Yield: 77%; mp 222-224 ºC; MS: *m/z* 360; Anal. Calcd. for $C_{18}H_{12}F_4N_4$: C, 60.00; H, 3.36; N, 15.55. Found: C, 59.87; H, 3.29; N, 15.47%.

4.7.5.3 5-(3-(trifluoromethyl)phenyl)-4,7-dihydro-7-(4-chlorophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine (BHD-333)



Yield: 82%; mp 244-246 ºC; MS: *m/z* 376; Anal. Calcd. for $C_{18}H_{12}ClF_3N_4$: C, 57.38; H, 3.21; N, 14.87. Found: C, 57.21; H, 3.16; N, 14.80%.

4.7.5.4 5-(3-(trifluoromethyl)phenyl)-4,7-dihydro-7-(4-nitrophenyl)-[1,2,4]triazolo [1,5-a]pyrimidine (BHD-334) Yield: 69%; mp 198-200 ºC; IR (cm^{-1}): 3200 (N-H stretching of secondary amine), 3090 (C-H stretching of aromatic ring), 1660 (N-H deformation of pyrimidine ring), 1600 (C=N stretching of triazole ring), 1548 and 1519 (C=C stretching of aromatic ring), 1350 (C-N stretching), 1126 (C-F stretching), 950 (C-H in plane deformation of aromatic ring), 806 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 5.23-5.24 (d, 1H, H_a, J = 3.0 Hz), 6.40-6.41 (d, 1H, H_b, J = 3.4 Hz), 7.54-7.61 (m, 4H, $H_{c\text{-}e}$), 7.67-7.69 (d, 1H, H_f, J = 7.6 Hz), 7.85-7.87 (d, 1H, H_g, J = 7.6 Hz), 7.91 (s, 1H, H_h), 8.21-8.23 (d, 2H, $H_{ii'}$, J = 8.5 Hz), 10.36 (s, 1H, H_j); MS: *m/z* 387; Anal. Calcd. for $C_{18}H_{12}F_3N_5O_2$: C, 55.82; H, 3.12; N, 18.08. Found: C, 55.69; H, 3.05; N, 17.99%.

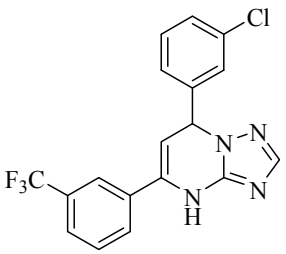
4.7.5.5 5-(3-(trifluoromethyl)phenyl)-4,7-dihydro-7-p-tolyl-[1,2,4]triazolo[1,5-a] pyrimidine (BHD-335) Yield: 65%; mp 205-207 ºC; IR (cm^{-1}): 3254 (N-H stretching of secondary amine), 3036 (C-H stretching of aromatic ring), 2978 (C-H symmetrical stretching of CH_3 group), 2870 (C-H asymmetrical stretching of CH_3 group), 1658 (N-H deformation of pyrimidine ring), 1599 (C=N stretching of triazole ring), 1552 and 1514 (C=C stretching of aromatic ring), 1433 (C-H asymmetrical deformation of CH_3 group),1348 (C-N stretching), 1134 (C-F stretching), 952 (C-H in plane deformation of aromatic ring), 800 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 2.31 (s, 3H, H_a), 5.21-5.22 (d, 1H, H_b, J = 3.2 Hz), 6.14-6.15 (d, 1H, H_c, J = 3.6 Hz), 7.14-7.16 (d, 2H, $H_{dd'}$, J = 8.1 Hz), 7.19-7.21 (d, 2H, $H_{ee'}$, J = 8.2 Hz), 7.54 (s, 1H, H_f), 7.57-7.61 (t, 1H, H_g, J = 7.8 Hz), 7.65-7.67 (d, 1H, H_h, J = 7.8 Hz), 7.85-7.87 (d, 1H, H_i, J = 7.8 Hz), 7.91 (s, 1H, H_j), 10.17 (s, 1H, H_k); MS: *m/z* 356; Anal. Calcd. for $C_{19}H_{15}F_3N_4$: C, 64.04; H, 4.24; N, 15.72. Found: C, 63.91; H, 4.17; N, 15.63%.

4.7.5.6 5-(3-(trifluoromethyl)phenyl)-4,7-dihydro-7-(4-methoxyphenyl)-[1,2,4]triazolo [1,5-a]pyrimidine (BHD-336) Yield: 75%; mp 143-145 ºC; IR (cm^{-1}): 3257 (N-H stretching of secondary amine), 3041 (C-H stretching of aromatic ring), 1662 (N-H deformation of pyrimidine ring), 1599 (C=N stretching of triazole ring), 1550 and 1510 (C=C stretching of aromatic ring), 1431 (C-H asymmetrical deformation of CH_3 group), 1346 (C-N stretching), 1120 (C-F stretching), 1159 (C-O-C asymmetrical stretching of ether linkage), 952 (C-H in plane deformation of aromatic ring), 804 (C-H out of plane deformation of 1,4-disubstitution); ^{1}H NMR (DMSO-d_6) δ ppm: 3.76 (s, 3H, H_a), 5.17 (s, 1H, H_b), 6.13-6.14 (d, 1H, H_c), 6.87-6.89 (d, 2H, $H_{dd'}$, J = 8.2 Hz), 7.26-7.28 (d, 2H, $H_{ee'}$, J = 8.2 Hz), 7.51 (s, 1H, H_f), 7.57-7.61 (t, 1H, H_g, J = 7.7 Hz), 7.65-7.67 (d, 1H, H_h, J = 7.5 Hz), 7.85-7.87 (d, 1H, H_i, J = 7.6 Hz), 7.91 (s, 1H, H_j), 10.22 (s, 1H, H_k); MS: *m/z* 372; Anal. Calcd. for $C_{19}H_{15}F_3N_4O$: C, 61.29; H, 4.06; N, 15.05. Found: C, 61.19; H, 4.01; N, 14.98%.

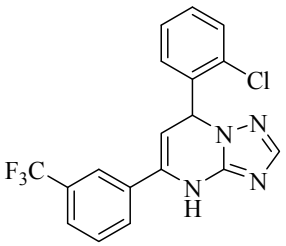
4.7.5.7 5-(3-(trifluoromethyl)phenyl)-4,7-dihydro-7-(3,4-dimethoxyphenyl)-[1,2,4] triazolo[1,5-a]pyrimidine (BHD-337) Yield: 68%; mp 175-177 ºC; MS: *m/z* 402; Anal. Calcd. for $C_{20}H_{17}F_3N_4O_2$: C, 59.70; H, 4.26; N, 13.92. Found: C, 59.70; H, 4.26; N, 13.92%.

4.7.5.8 5-(3-(trifluoromethyl)phenyl)-4,7-dihydro-7-(3-nitrophenyl)-[1,2,4]triazolo [1,5-a]pyrimidine (BHD-338) Yield: 77%; mp 164-166 ºC; MS: *m/z* 387; Anal. Calcd. for $C_{18}H_{12}F_3N_5O_2$: C, 55.82; H, 3.12; N, 18.08. Found: C, 55.71; H, 3.07; N, 17.95%.

4.7.5.9 5-(3-(trifluoromethyl)phenyl)-4,7-dihydro-7-(3-chlorophenyl)-[1,2,4]triazolo [1,5-a]pyrimidine (BHD-339) Yield: 65%; mp 217-219 ºC; MS: *m/z* 376; Anal. Calcd. for $C_{18}H_{12}ClF_3N_4$: C, 57.38; H, 3.21; N, 14.87. Found: C, 57.25; H, 3.18; N, 14.78%.



4.7.5.10 5-(3-(trifluoromethyl)phenyl)-4,7-dihydro-7-(2-chlorophenyl)-[1,2,4]triazolo [1,5-a]pyrimidine (BHD-340) Yield: 70%; mp 187-189 ºC; MS: *m/z* 376; Anal. Calcd. for $C_{18}H_{12}ClF_3N_4$: C, 57.38; H, 3.21; N, 14.87. Found: C, 57.22; H, 3.15; N, 14.79%.

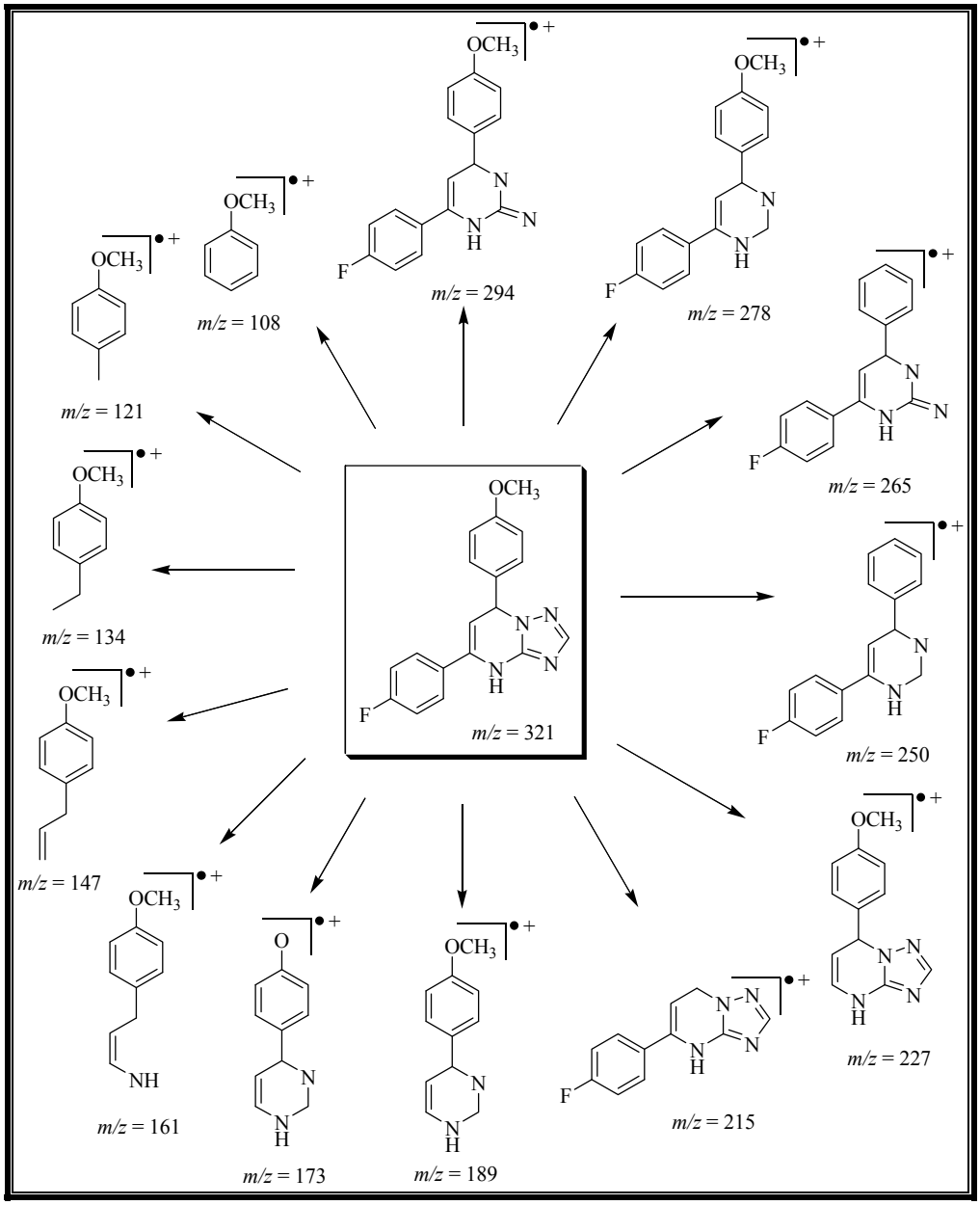


4.8 Spectral discussion

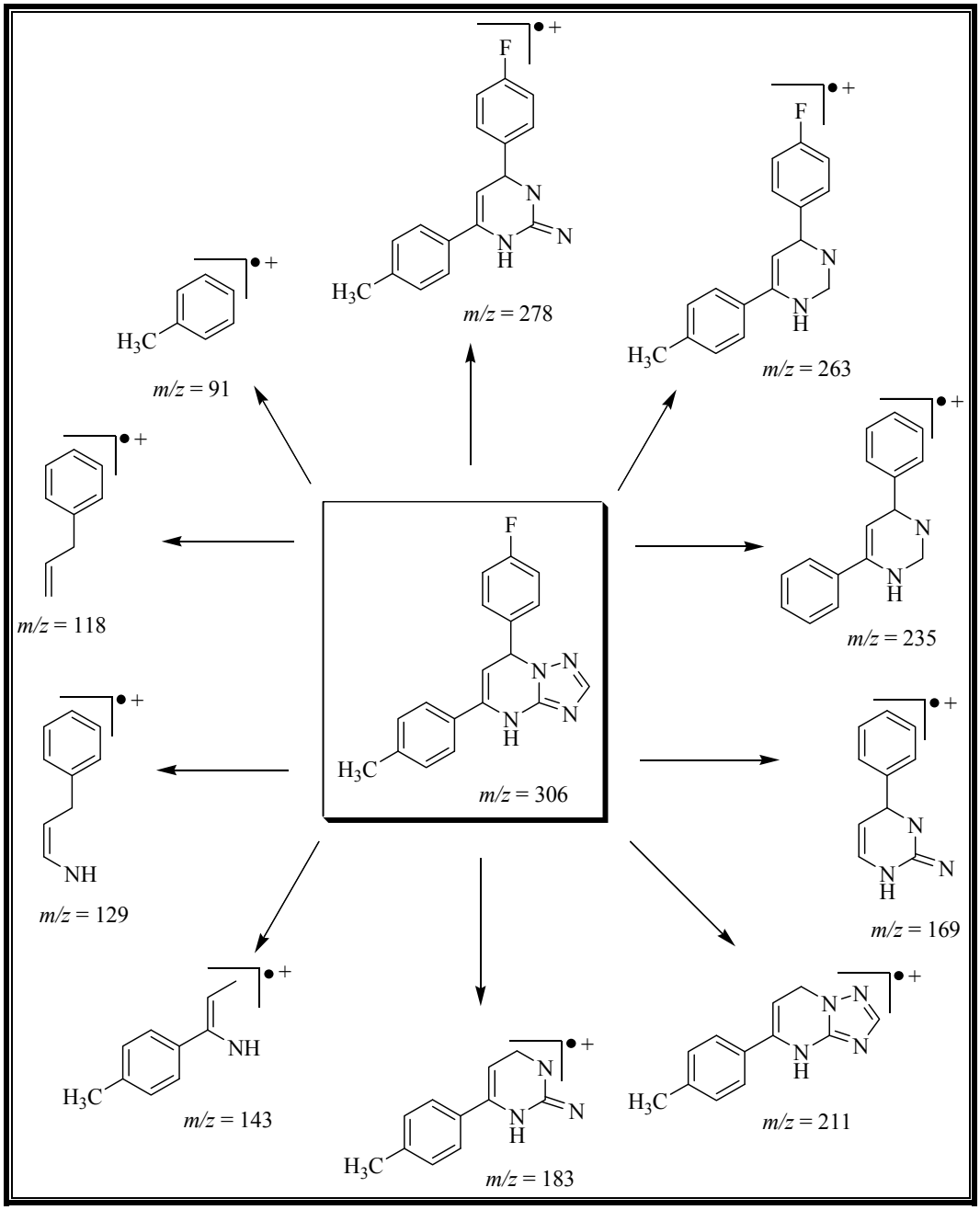
4.8.1 Mass spectral study

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.

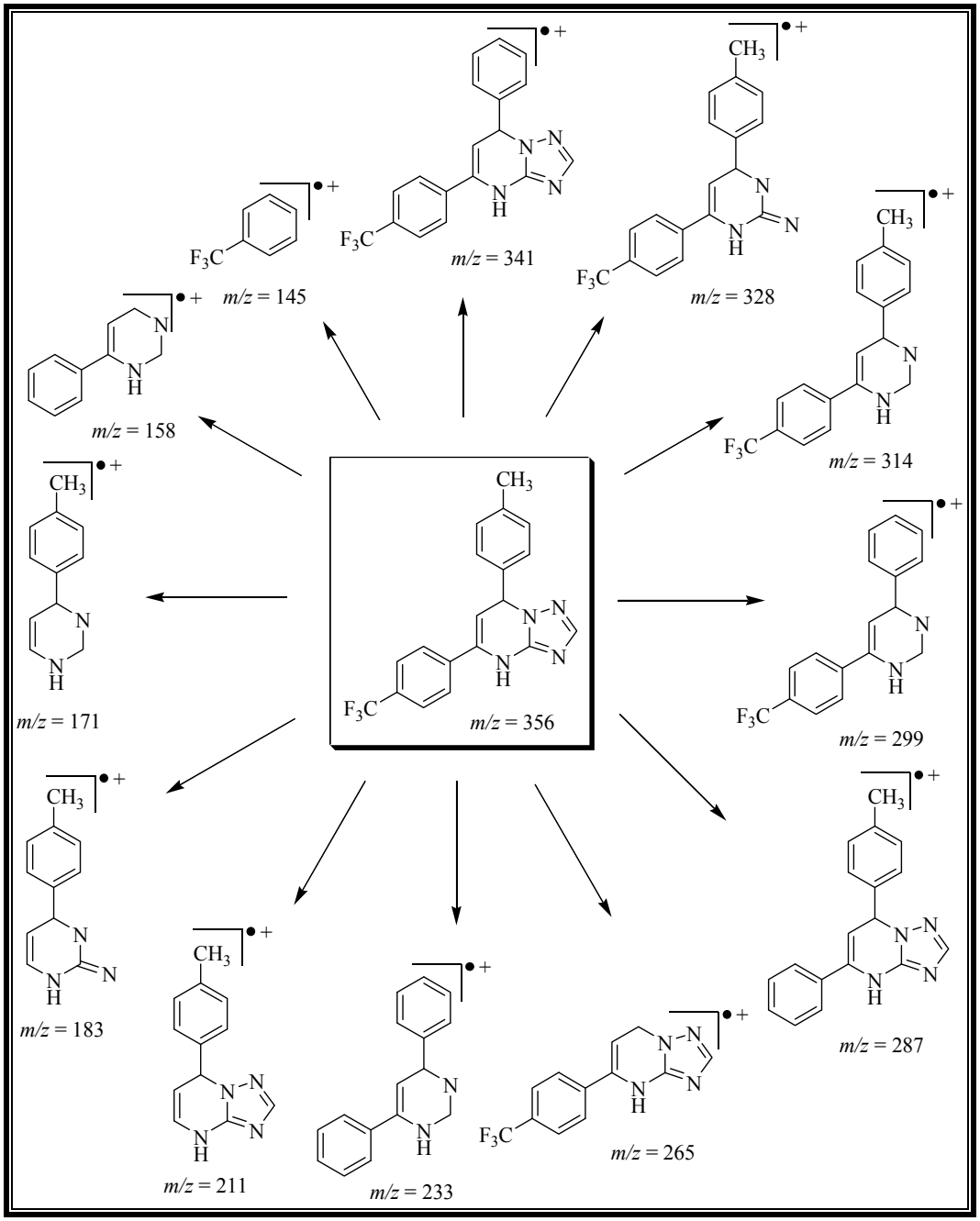
4.8.1.1 Mass fragmentation pattern for BHD-306



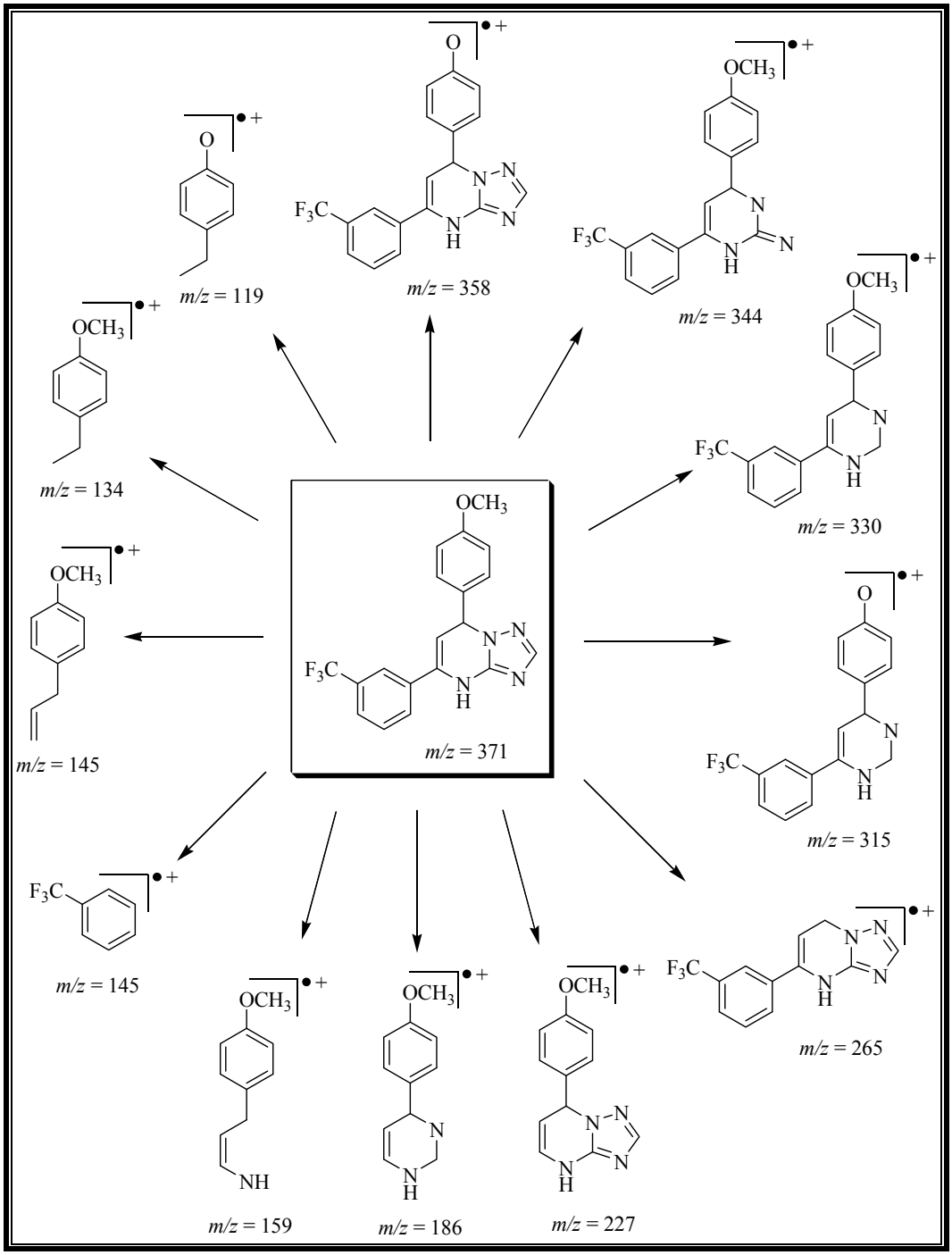
4.8.1.2 Mass fragmentation pattern for BHD-312



4.8.1.3 Mass fragmentation pattern for BHD-325



4.8.1.4 Mass fragmentation pattern for BHD-336



4.8.2 IR spectral study

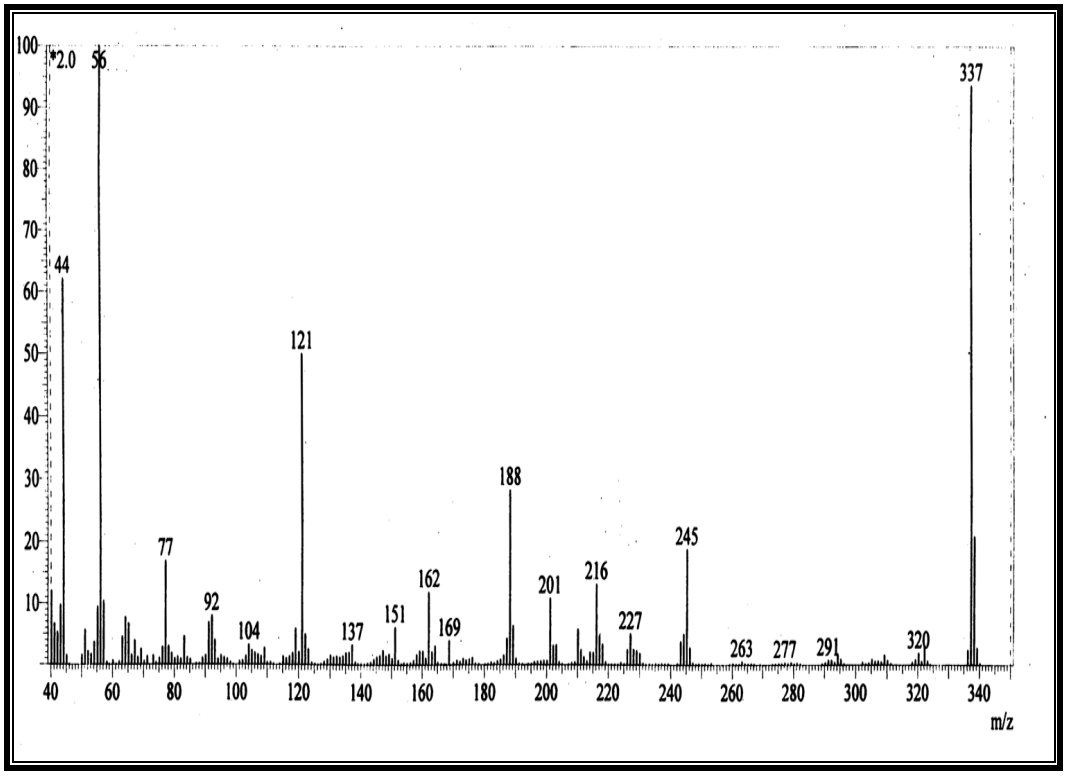
IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For 1,2,4-triazolo[1,5-*a*]pyrimidines (BHD-301 to 340), confirmatory bands for secondary amine (NH) and nitrile (C=N) stretching band were observed at 3190-3500 cm^{-1} and 1590-1650 cm^{-1} respectively. Another characteristic band for N-H deformation and C-N stretching were observed at 1600-1680 cm^{-1} and 1290-1350 cm^{-1} respectively, which suggested the formation of pyrimidine ring.

4.8.3 ^{1}H NMR spectral study

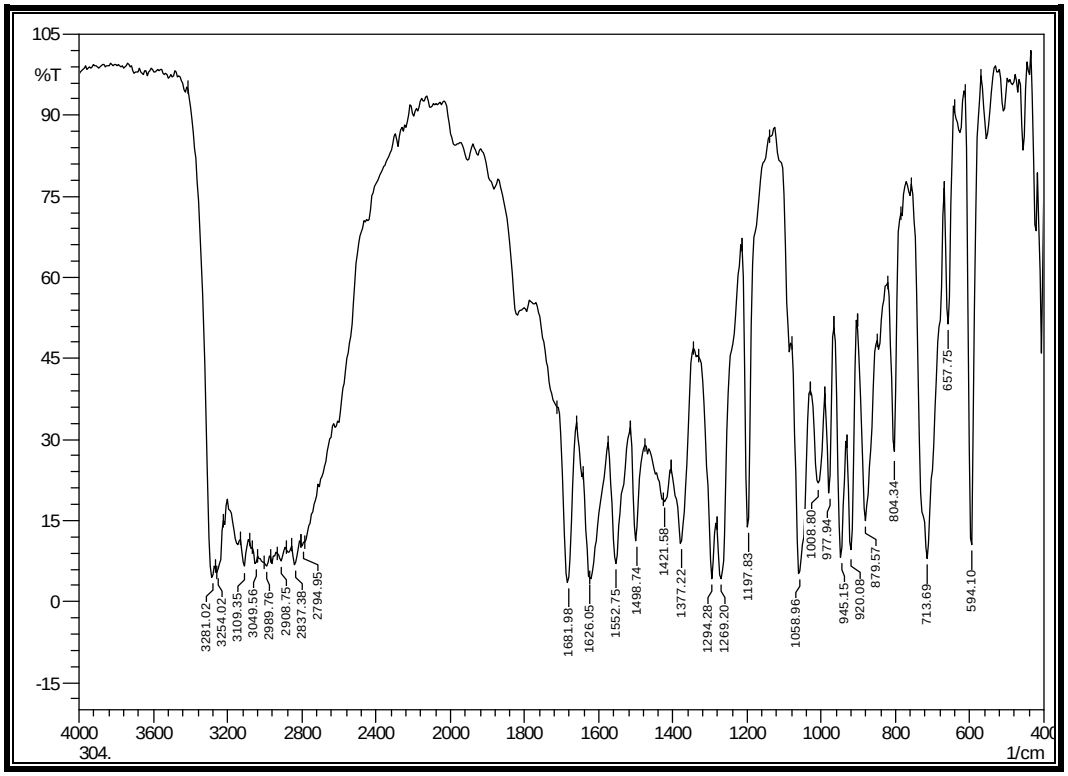
^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

^{1}H NMR spectra confirmed the structures of triazolopyrimidines BHD-301 to 340 on the basis of following signals: two characteristic peaks for the methine proton of pyrimidine ring and for the methine proton of triazole ring were observed at 5.02-5.25 δ ppm and 7.50-7.65 δ ppm respectively. And another singlet for amino group proton was observed at 9.90-10.36 δ ppm. The aromatic ring protons and *J* value were found to be in accordance with substitution pattern on phenyl ring.

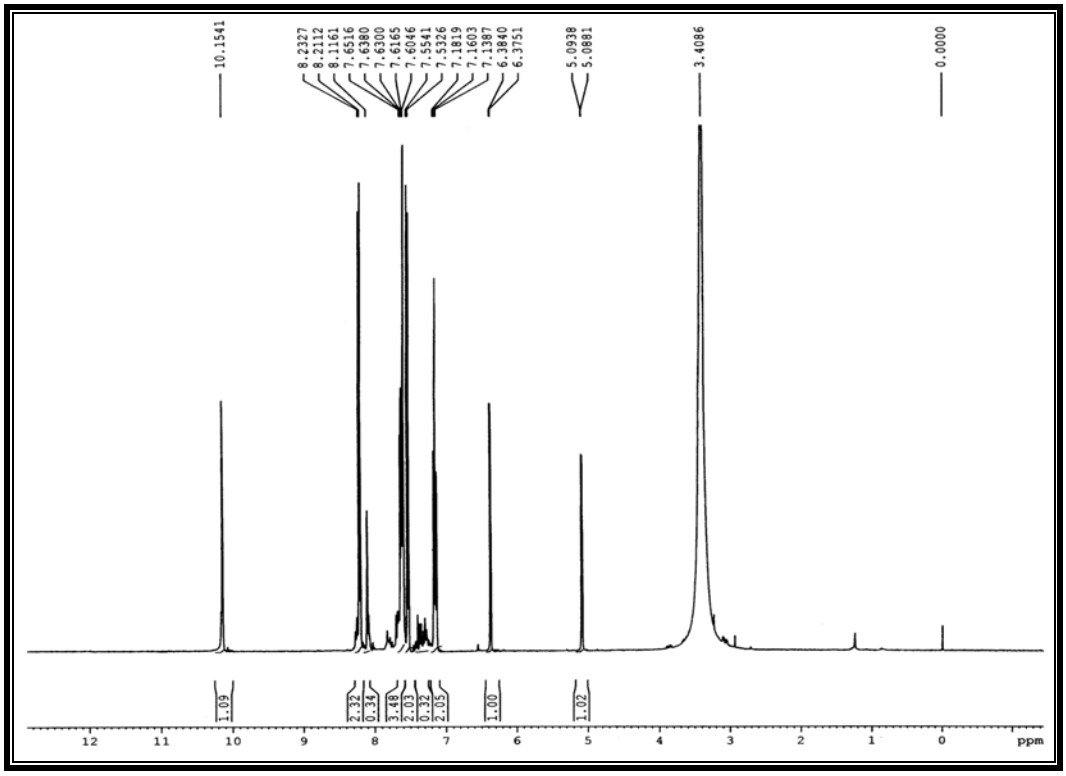
Mass spectrum of BHD-304



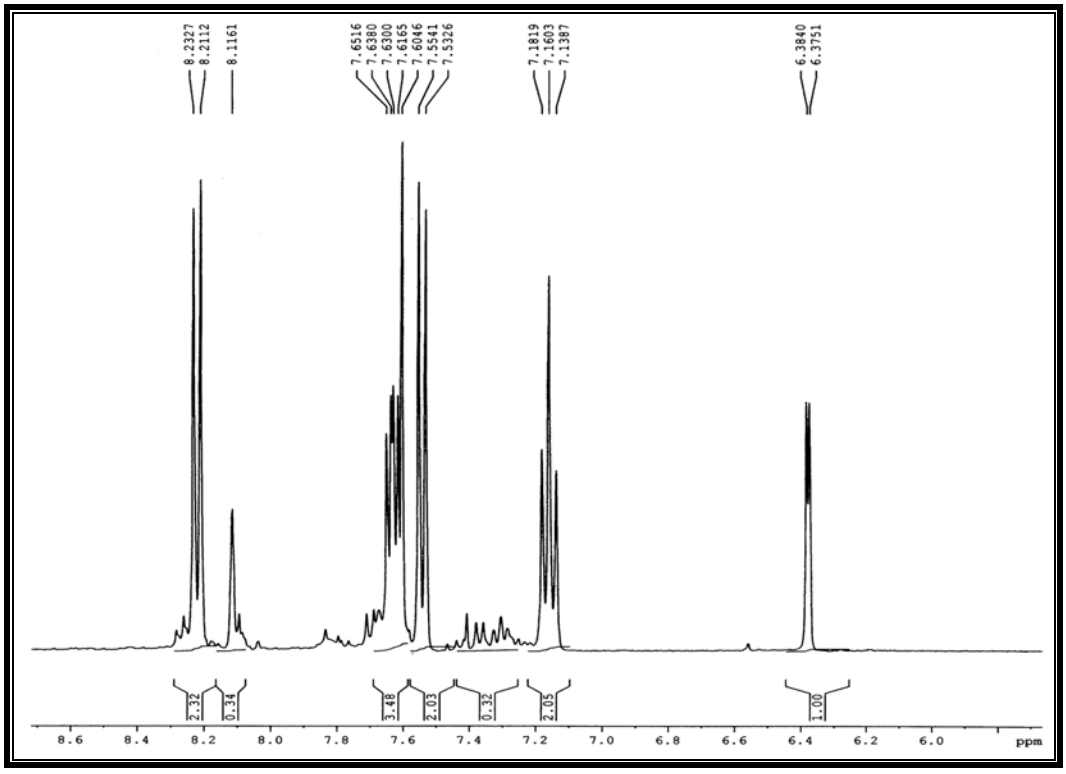
IR spectrum of BHD-304



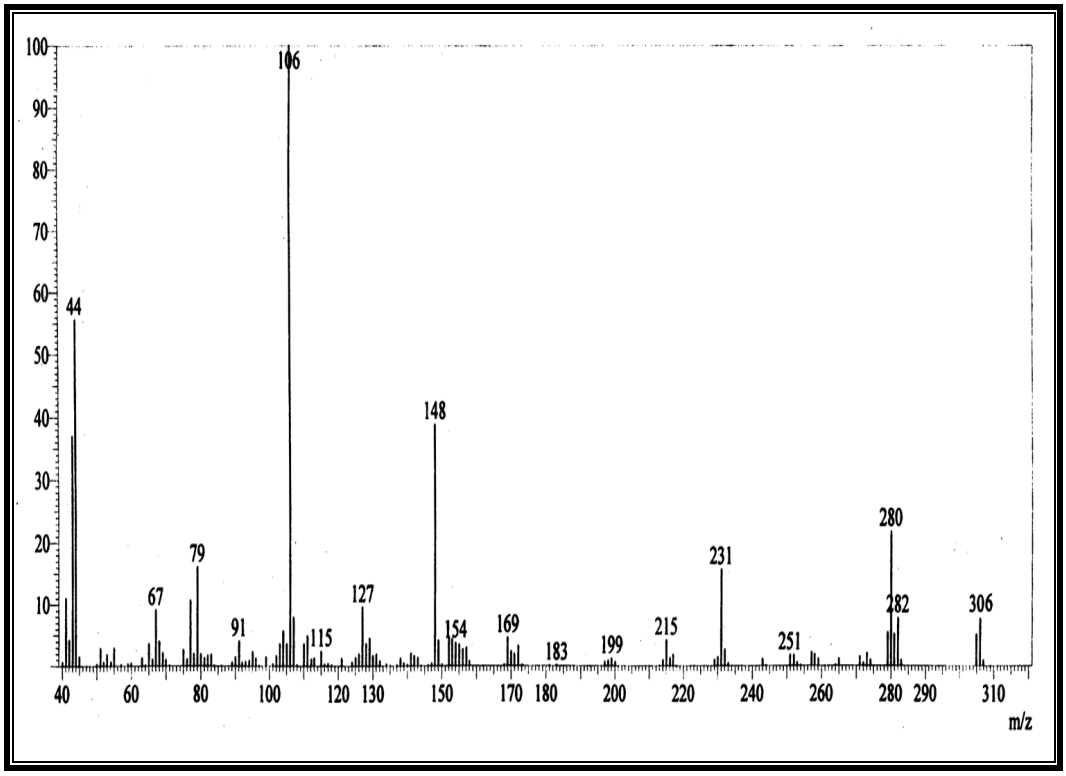
^{1}H NMR spectrum of BHD-304



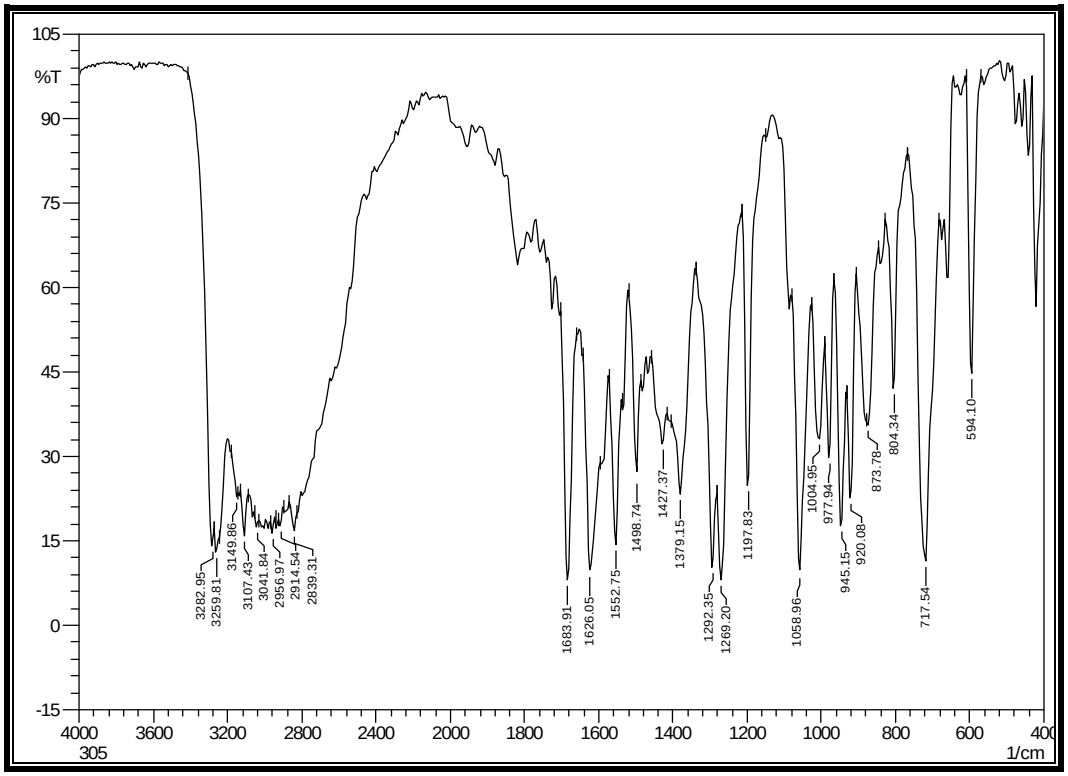
Expanded ^{1}H NMR spectrum of BHD-304



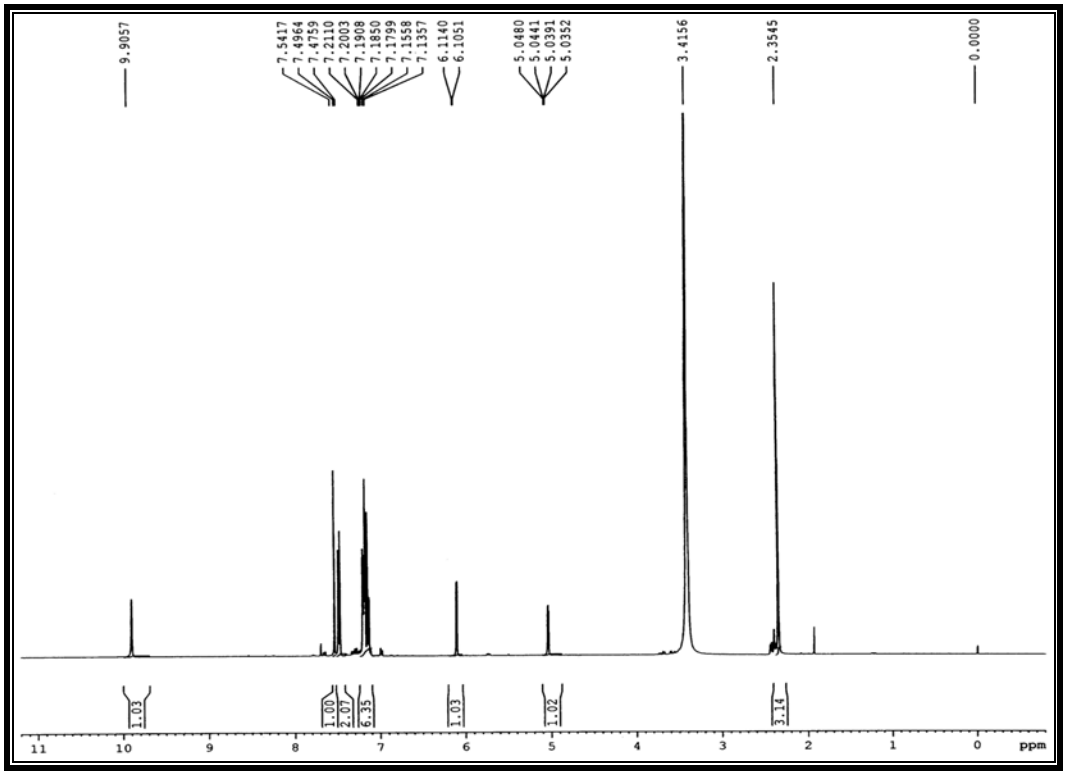
Mass spectrum of BHD-305



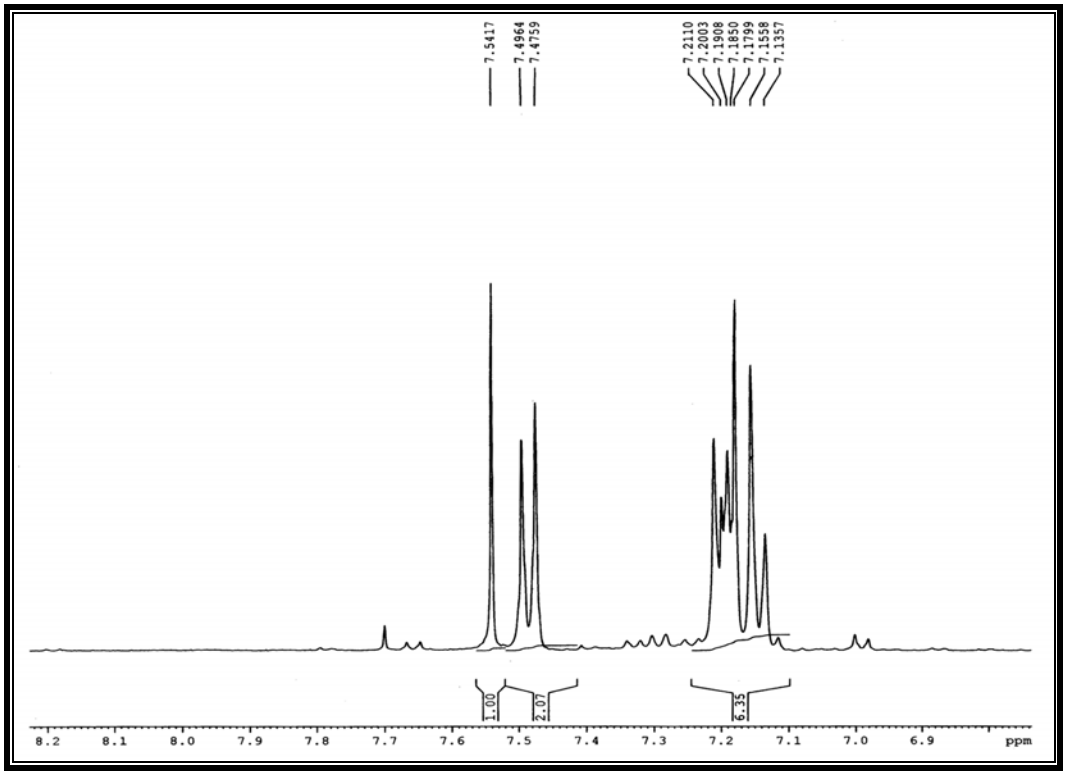
IR spectrum of BHD-305



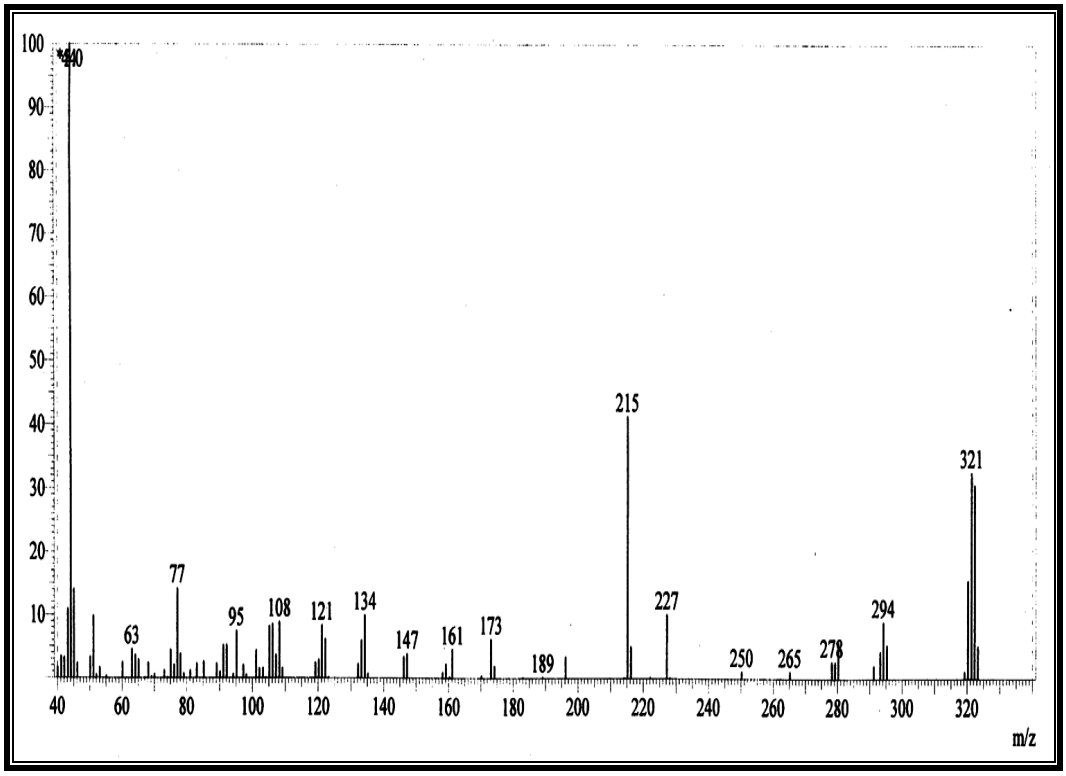
^{1}H NMR spectrum of BHD-305



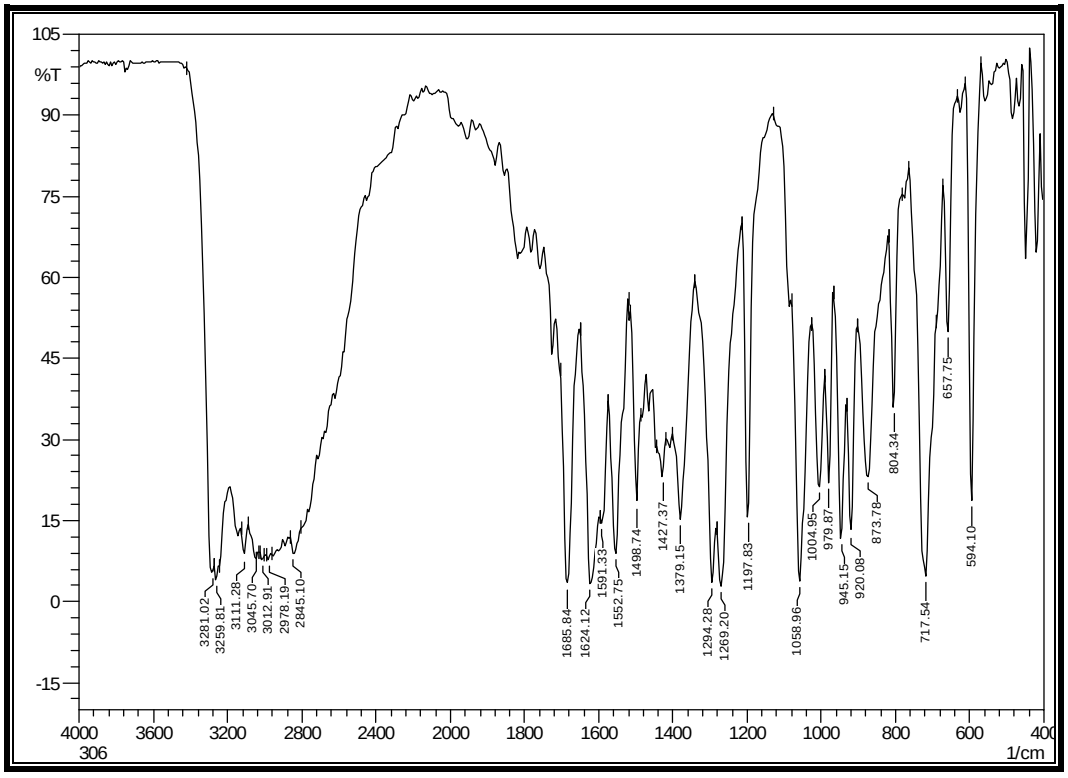
Expanded ^{1}H NMR spectrum of BHD-305



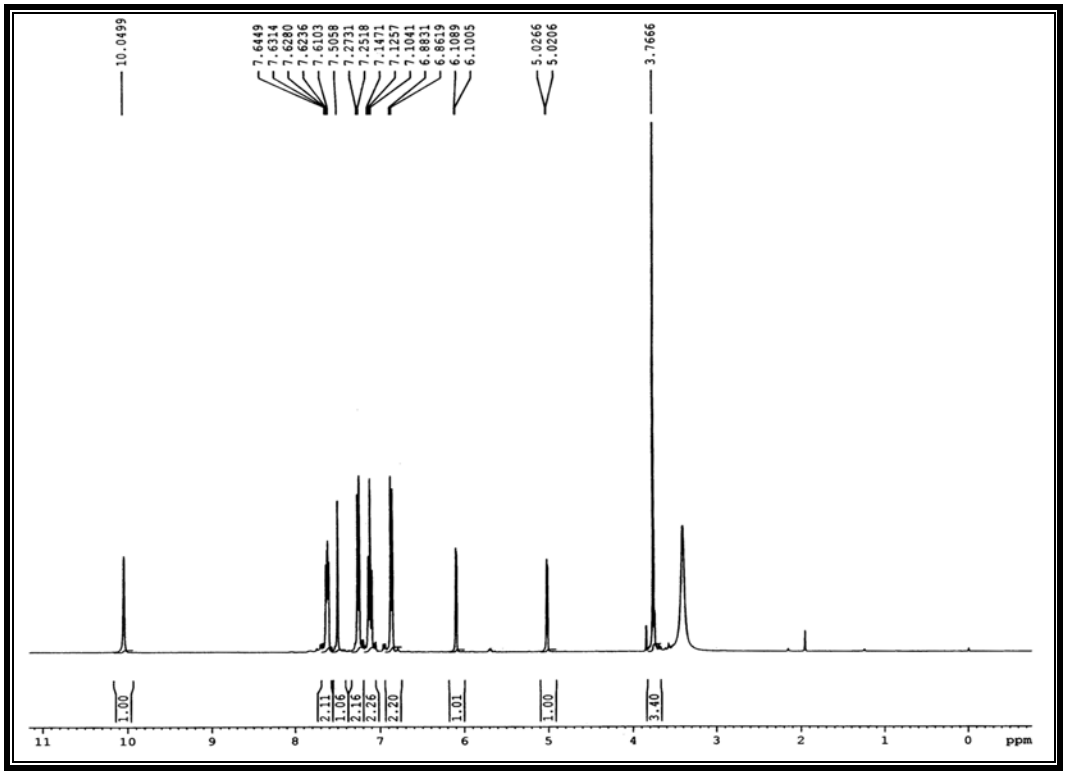
Mass spectrum of BHD-306



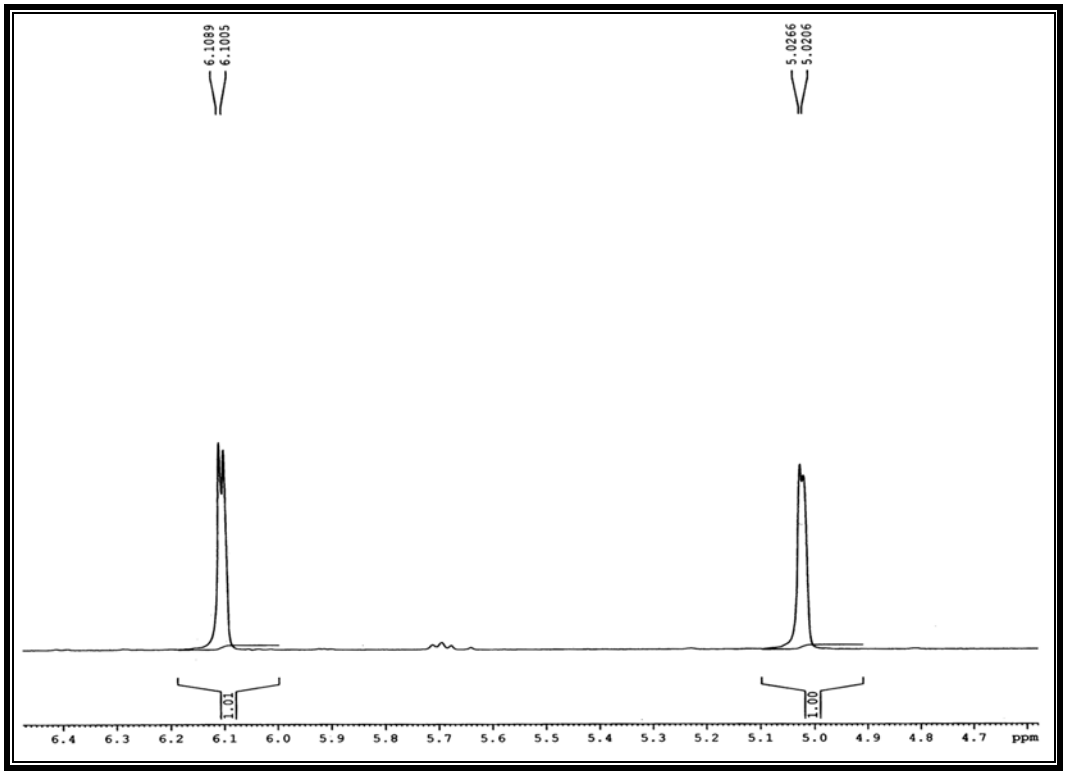
IR spectrum of BHD-306



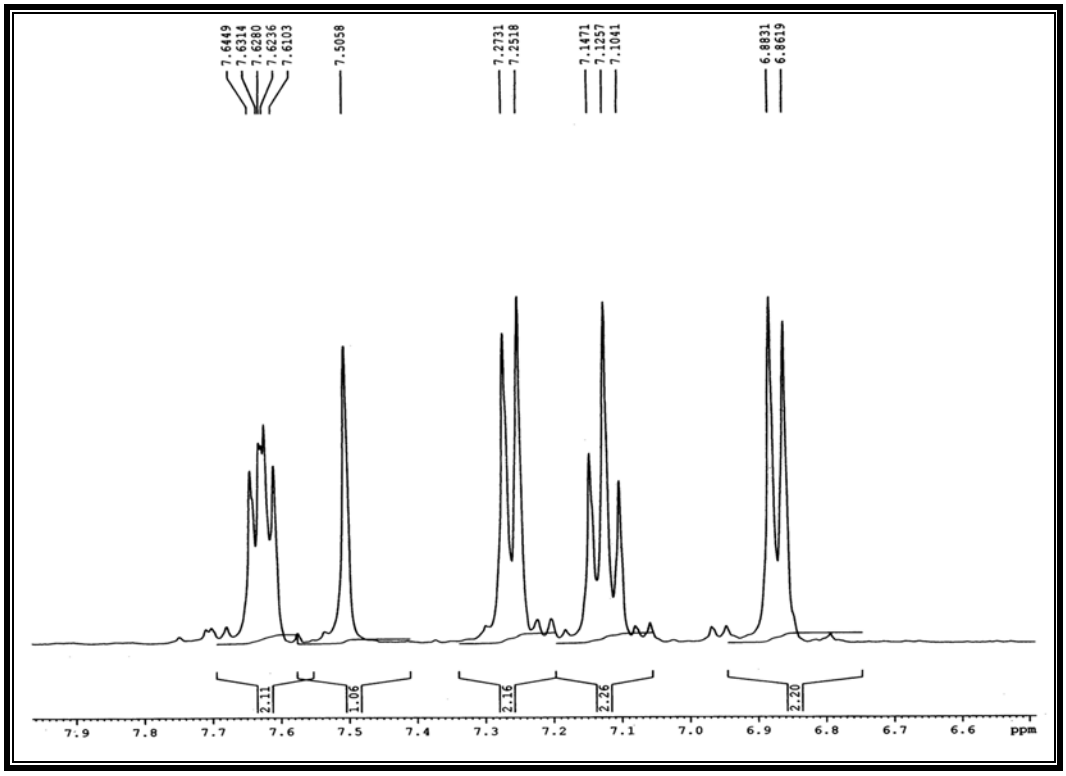
^{1}H NMR spectrum of BHD-306



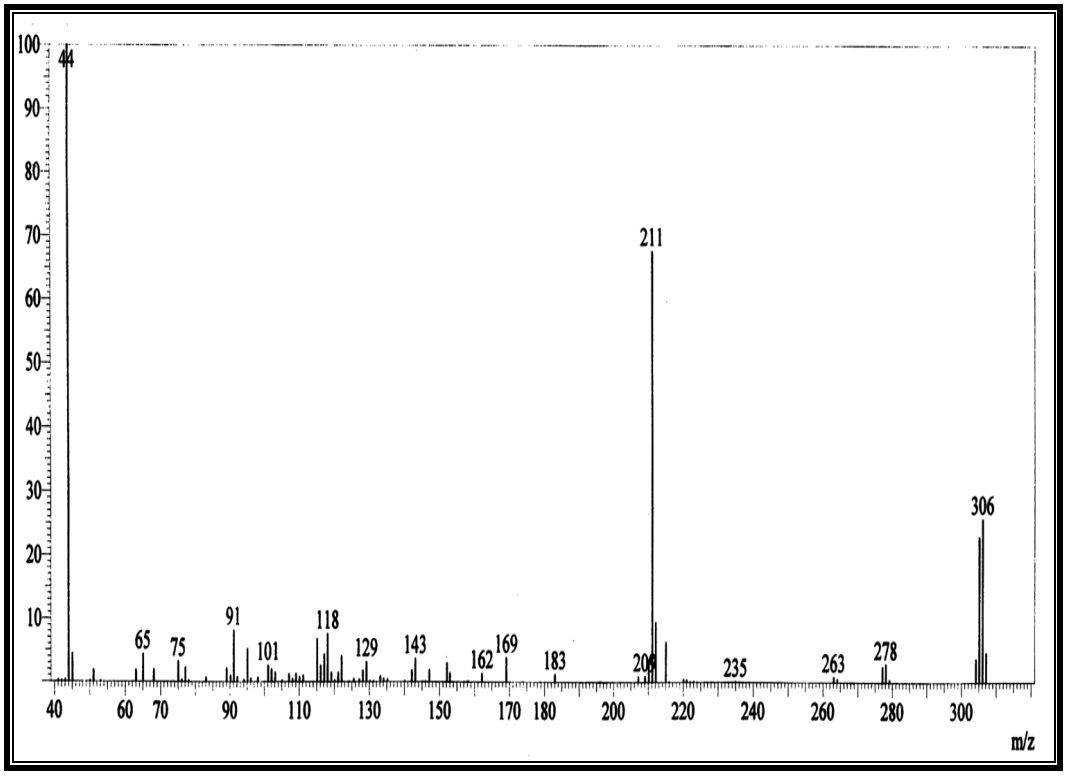
Expanded ^{1}H NMR spectrum of BHD-306



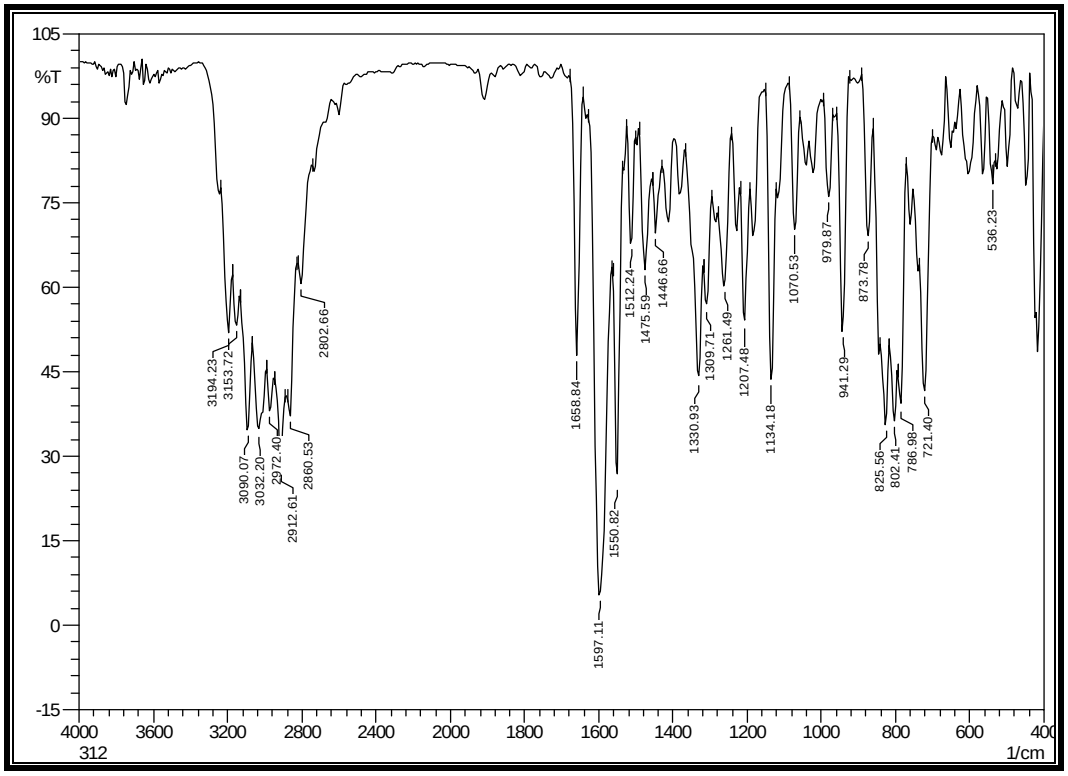
Expanded ^{1}H NMR spectrum of BHD-306



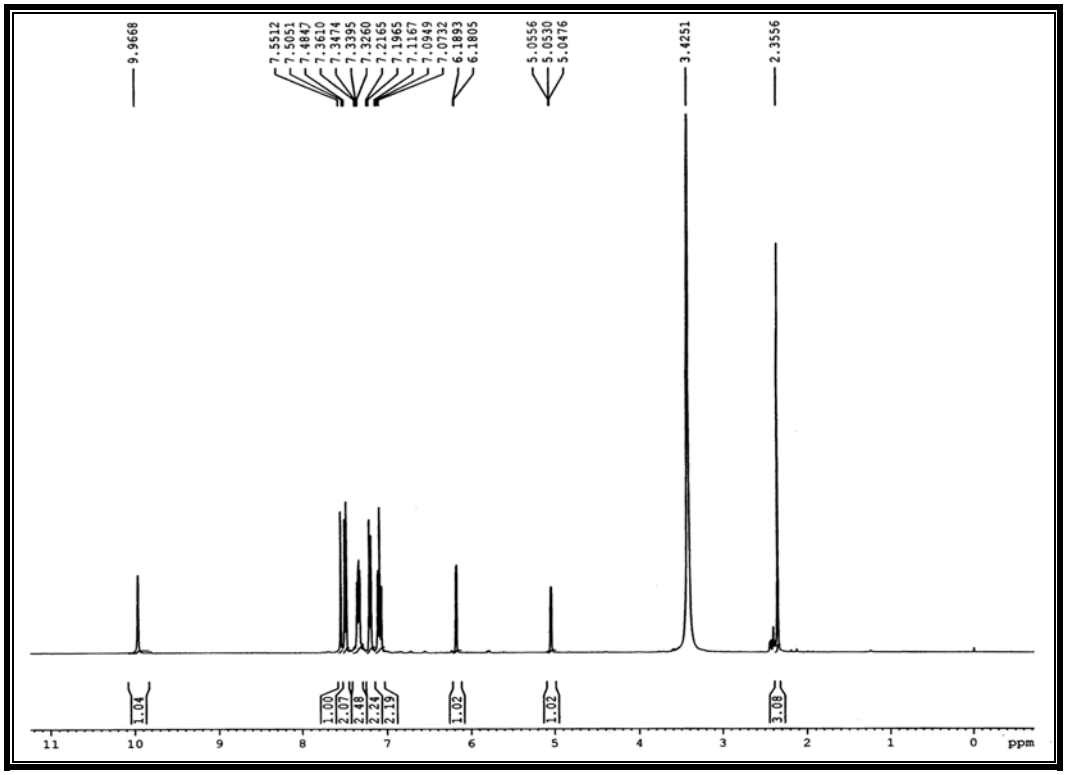
Mass spectrum of BHD-312



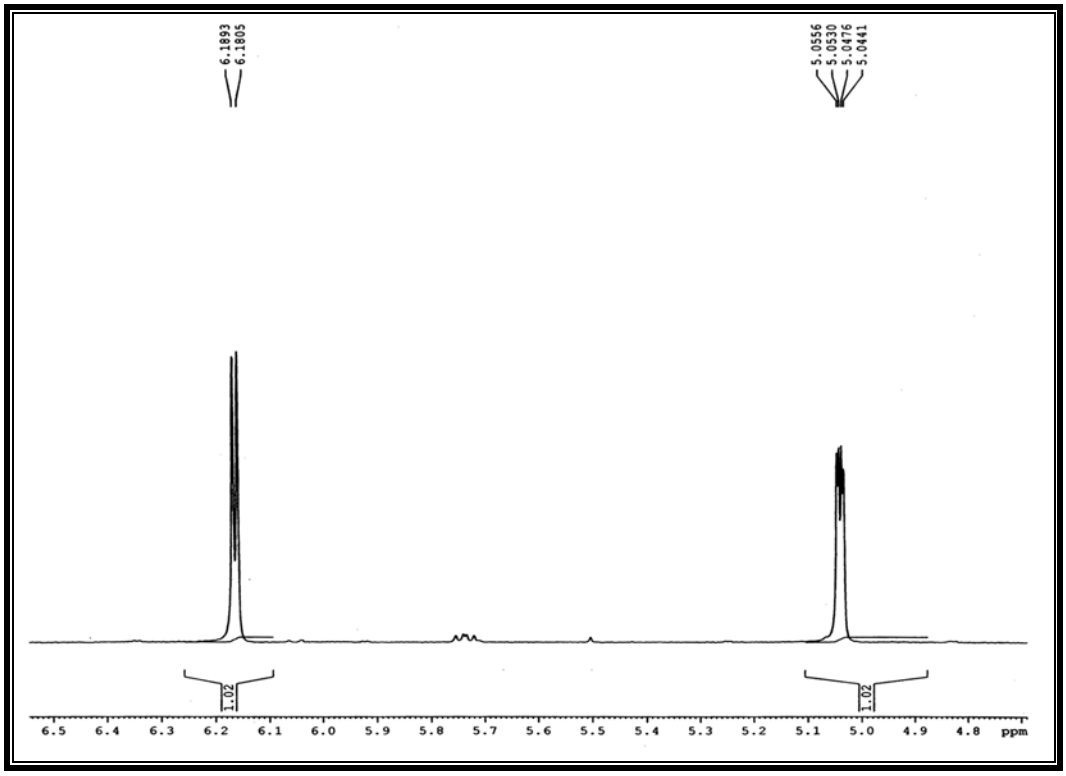
IR spectrum of BHD-312



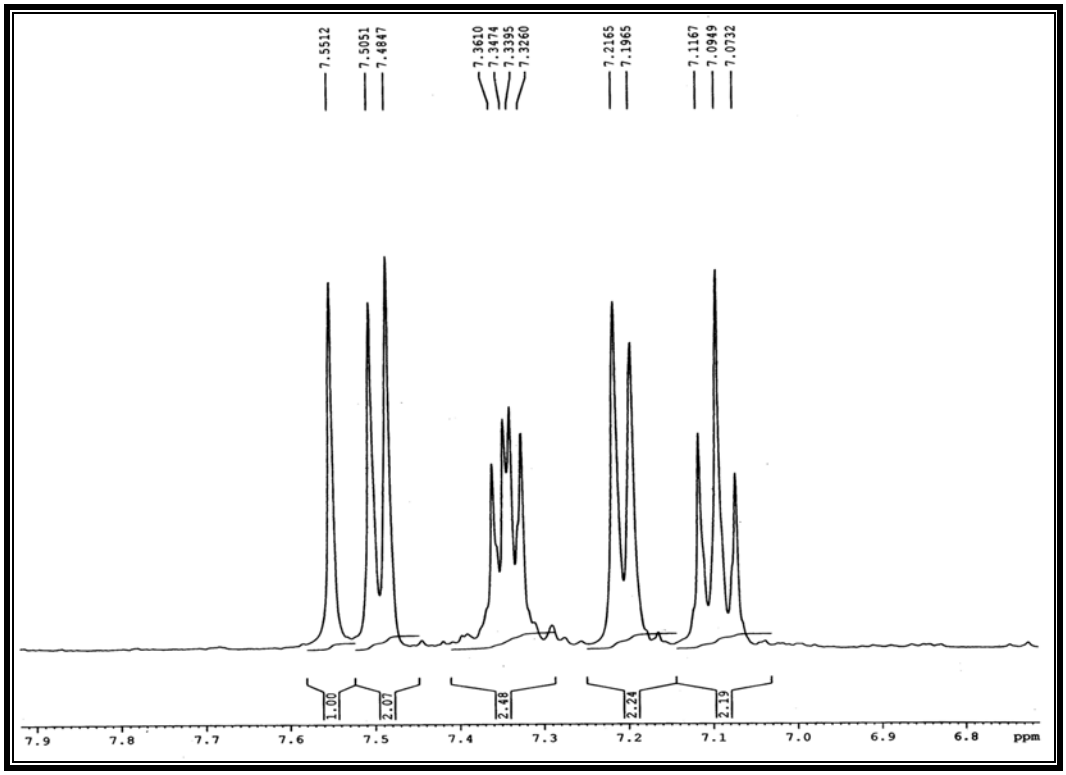
^{1}H NMR spectrum of BHD-312



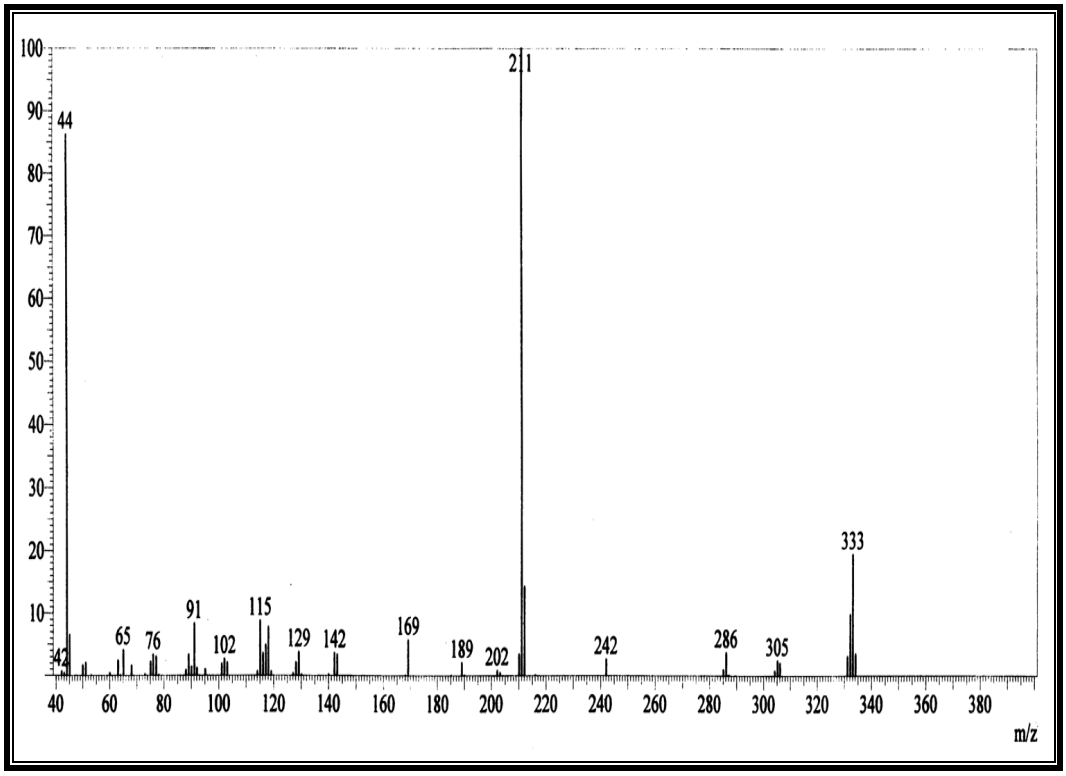
Expanded ^{1}H NMR spectrum of BHD-312



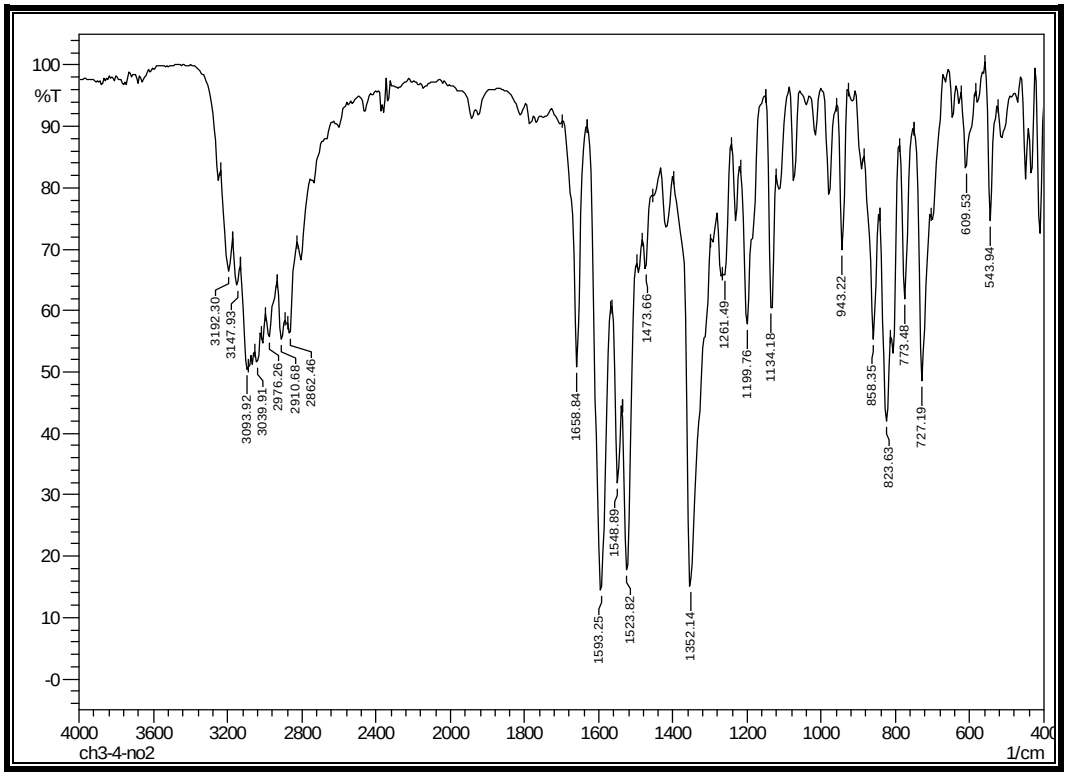
Expanded ^{1}H NMR spectrum of BHD-312



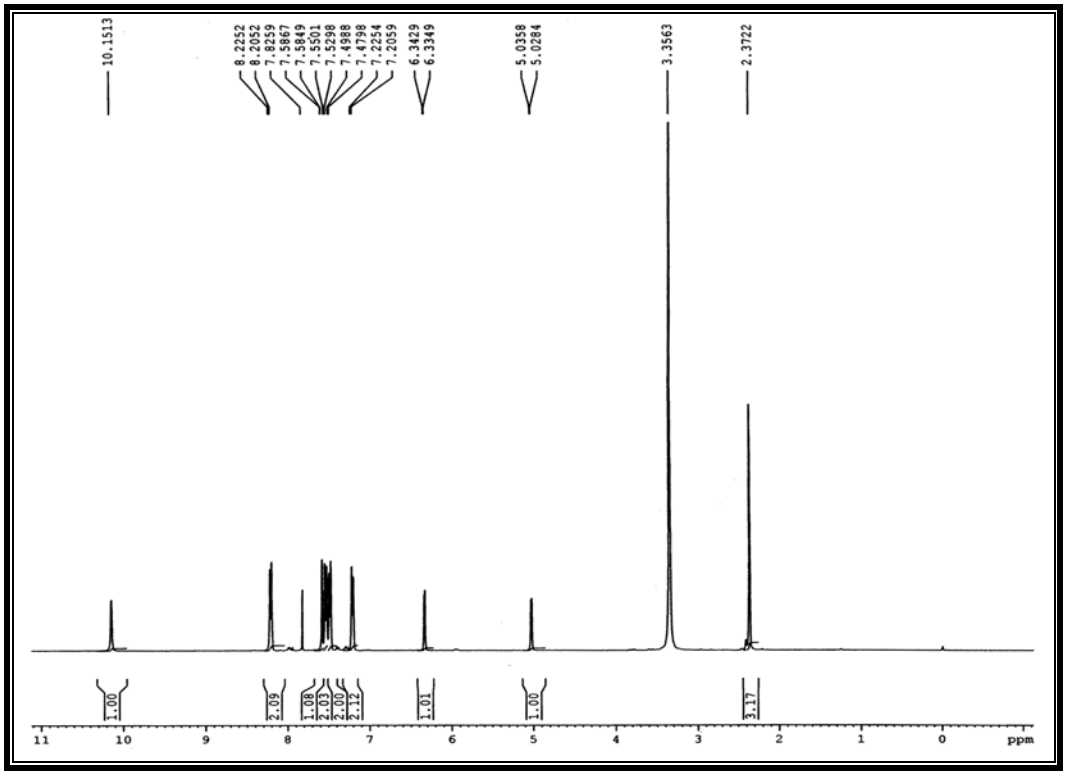
Mass spectrum of BHD-314



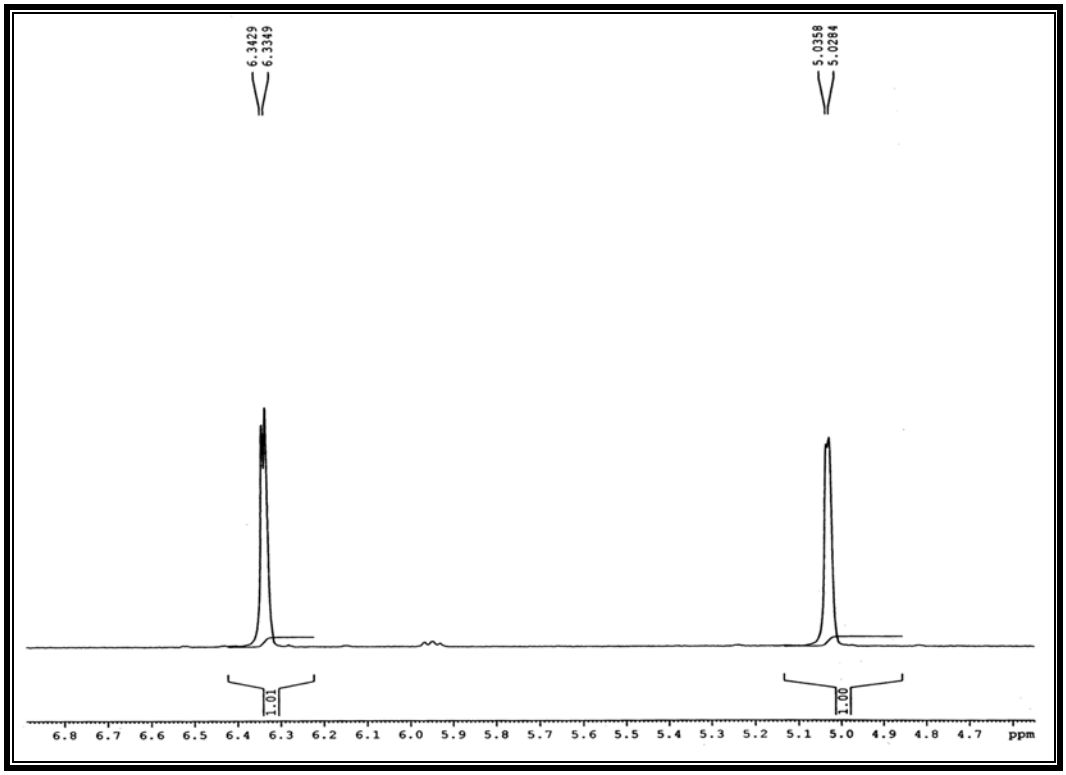
IR spectrum of BHD-314



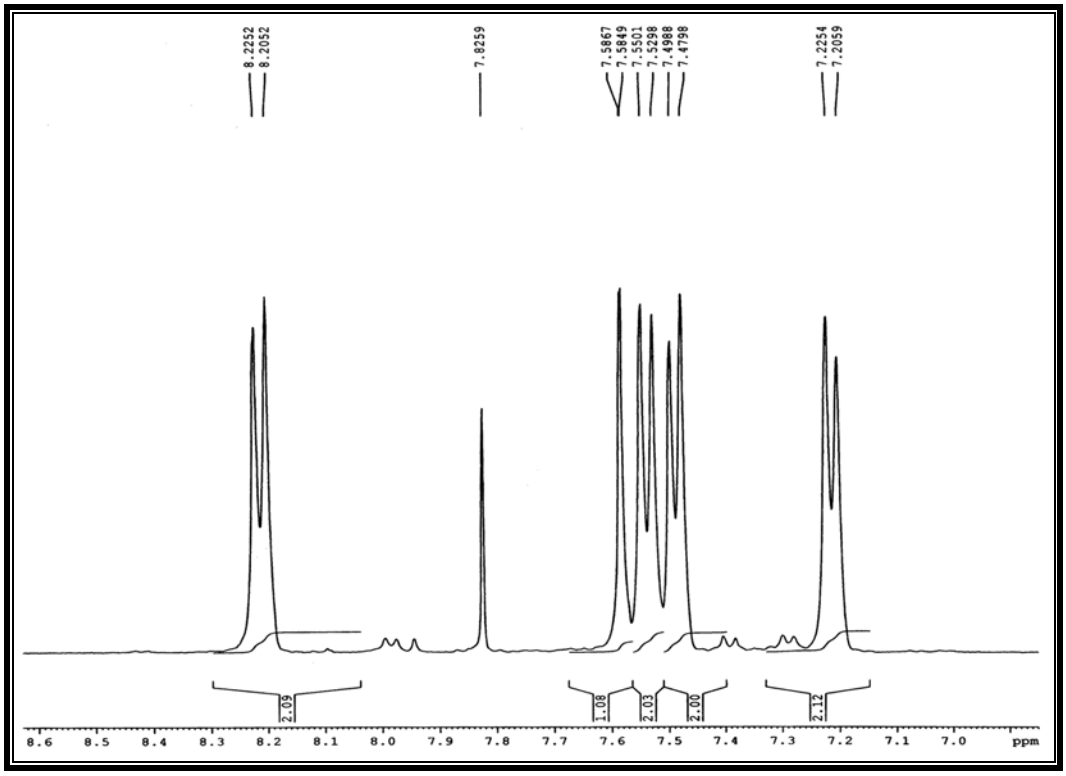
^{1}H NMR spectrum of BHD-314



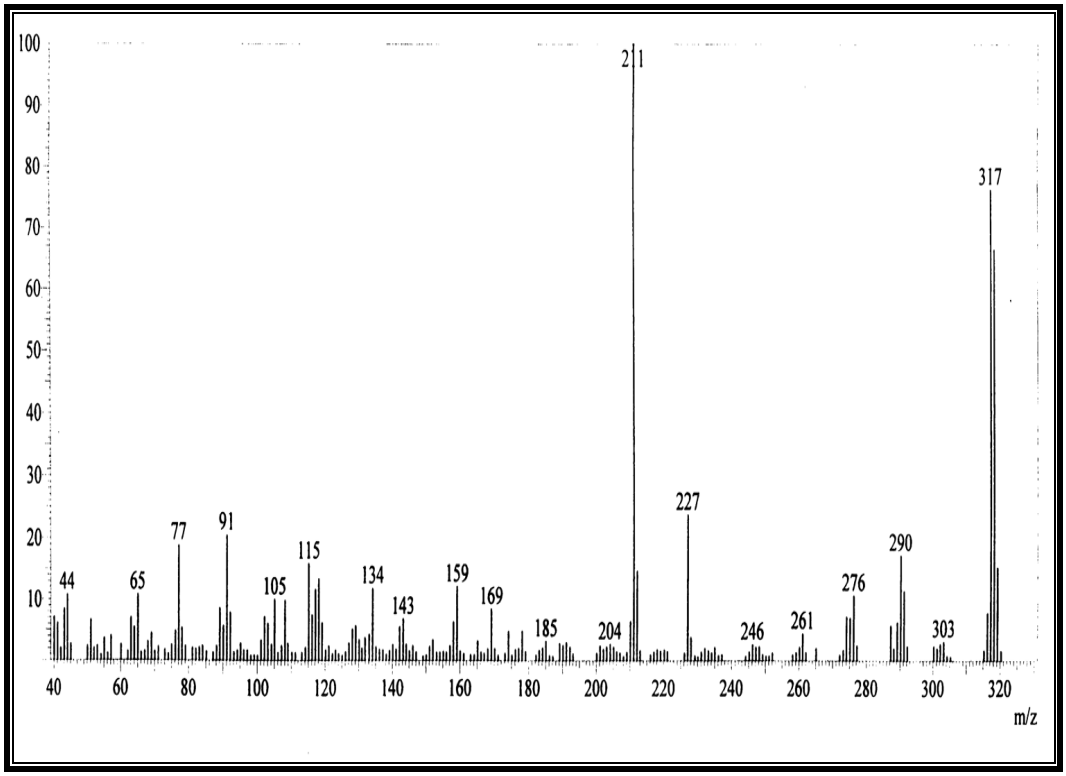
Expanded ^{1}H NMR spectrum of BHD-314



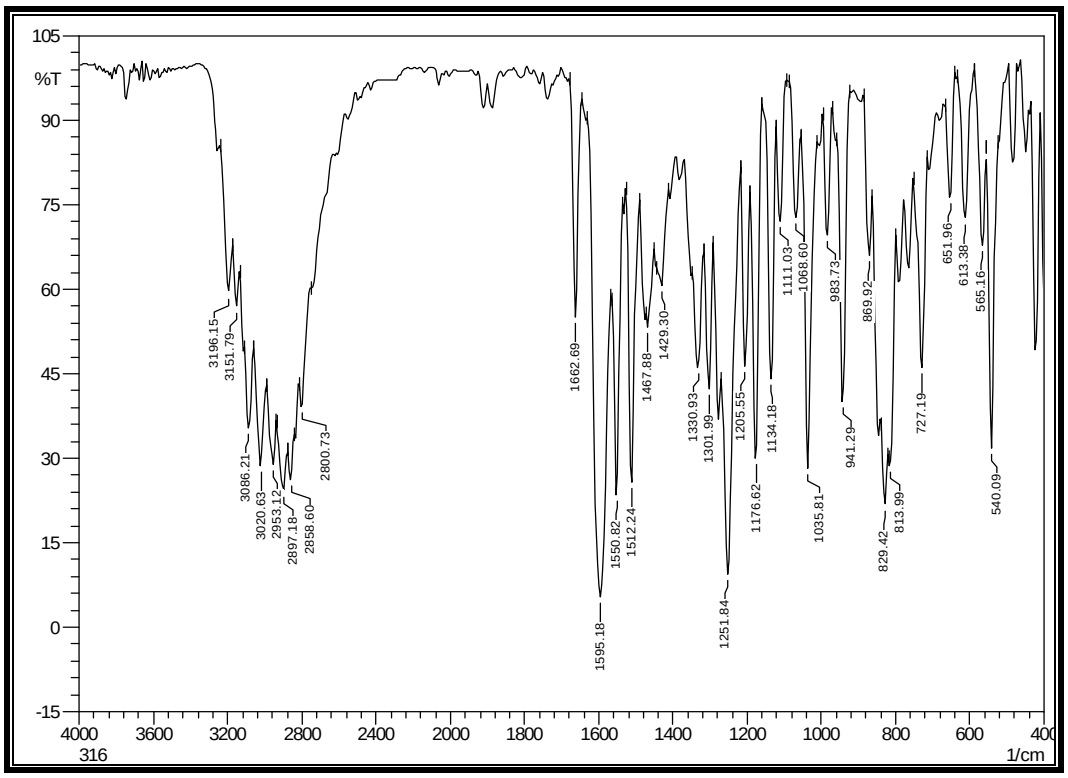
Expanded ^{1}H NMR spectrum of BHD-314



Mass spectrum of BHD-316



IR spectrum of BHD-316



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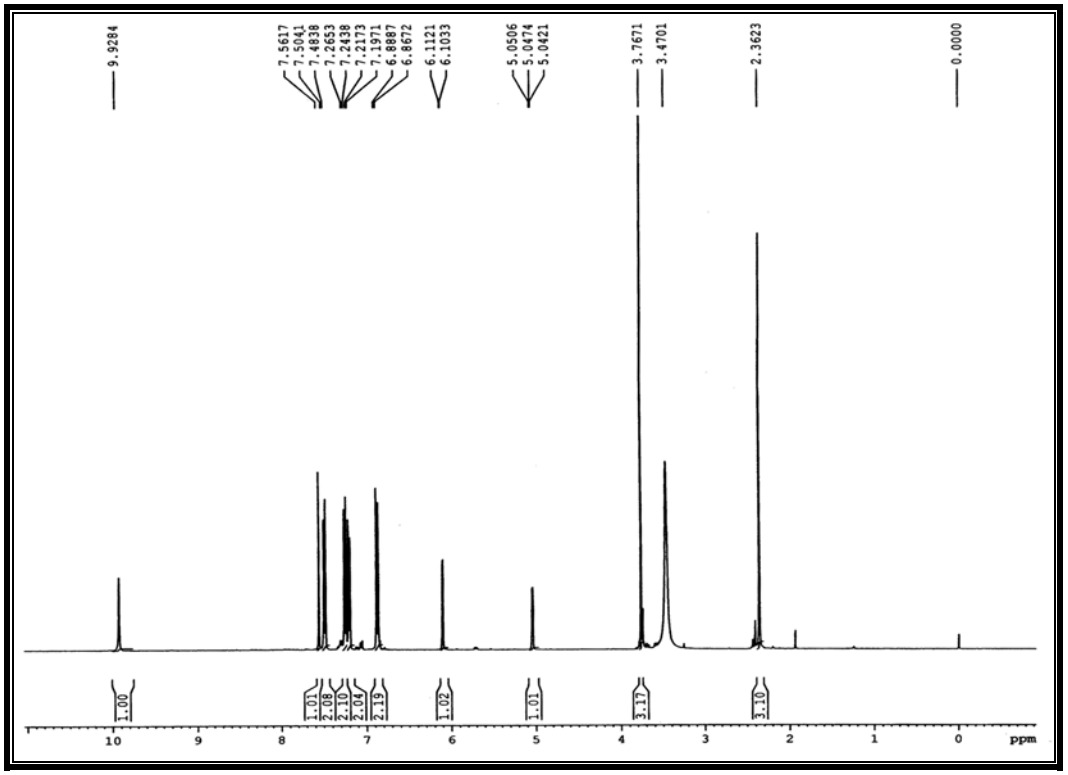
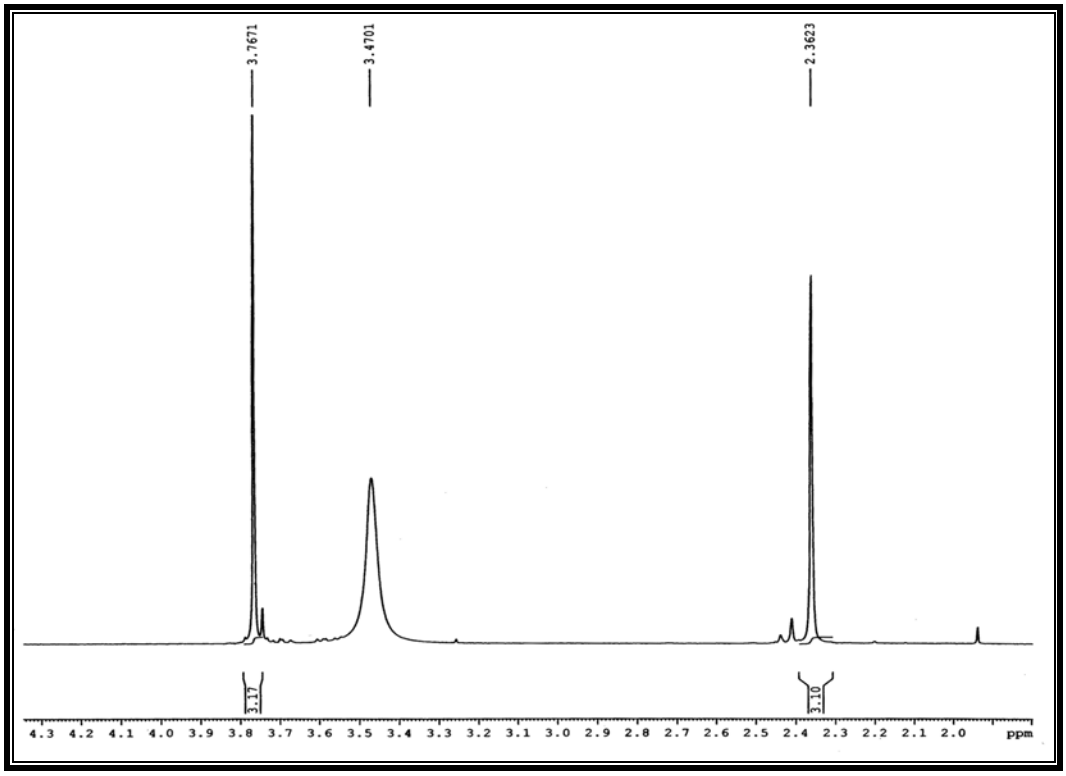


Expanded ^{1}H NMR spectrum of BHD-316



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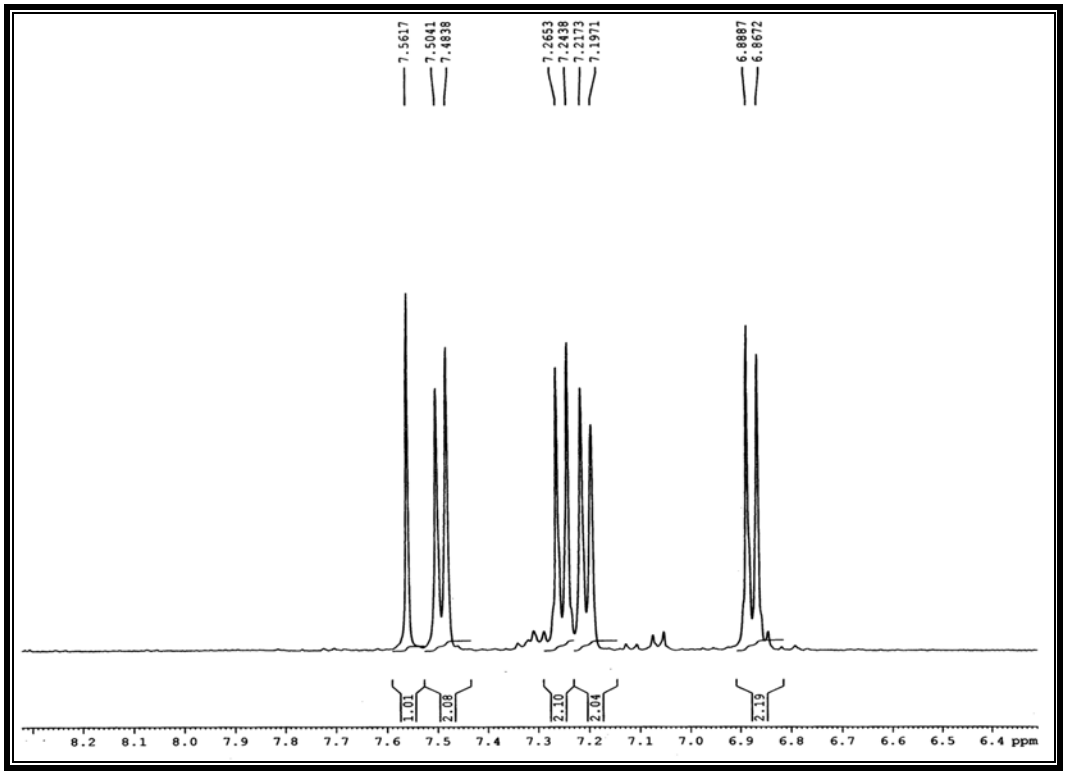
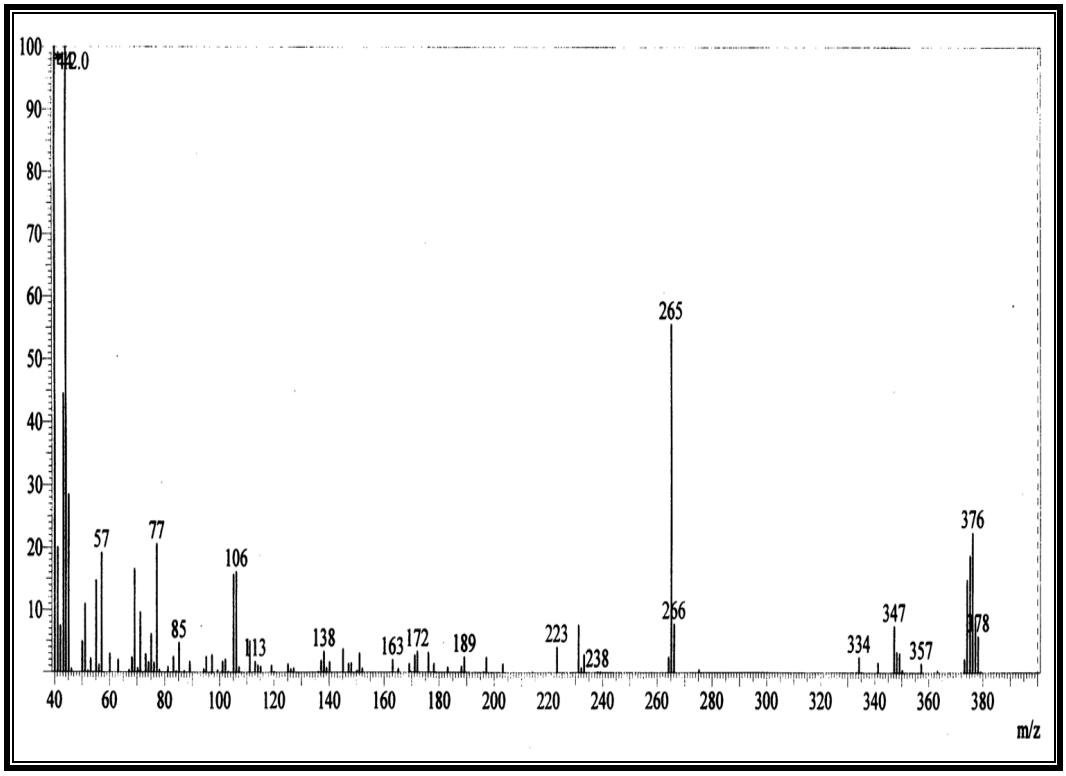
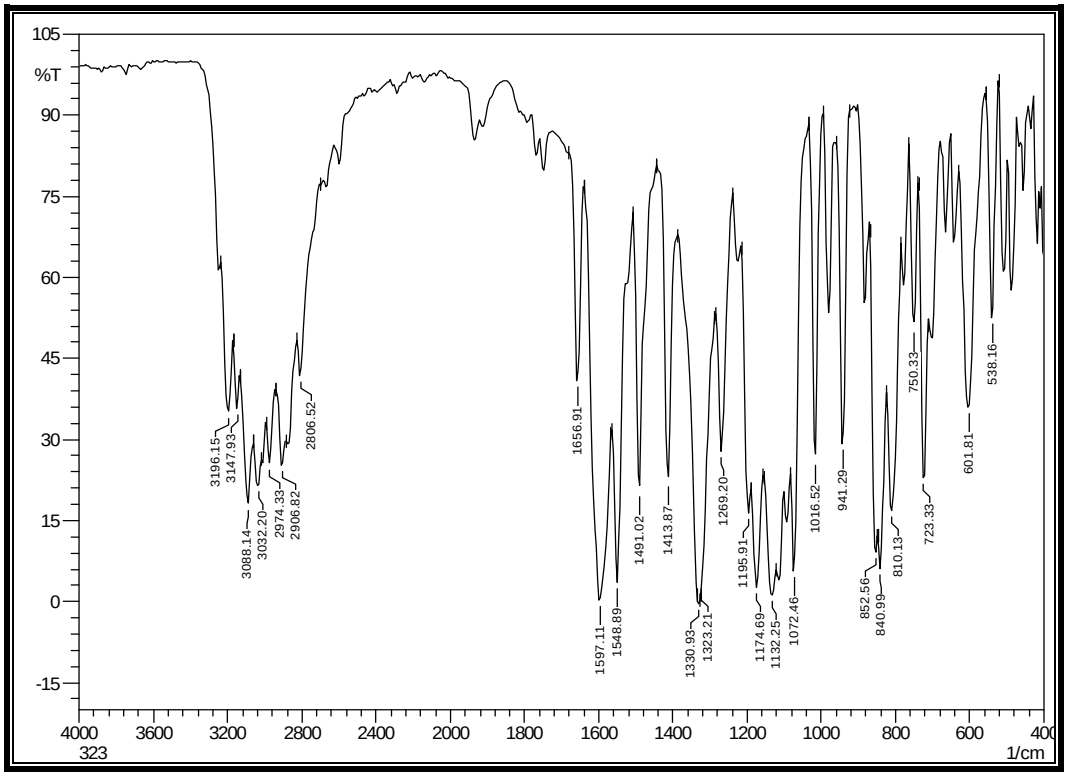


Mass spectrum of BHD-323



IR spectrum of BHD-323



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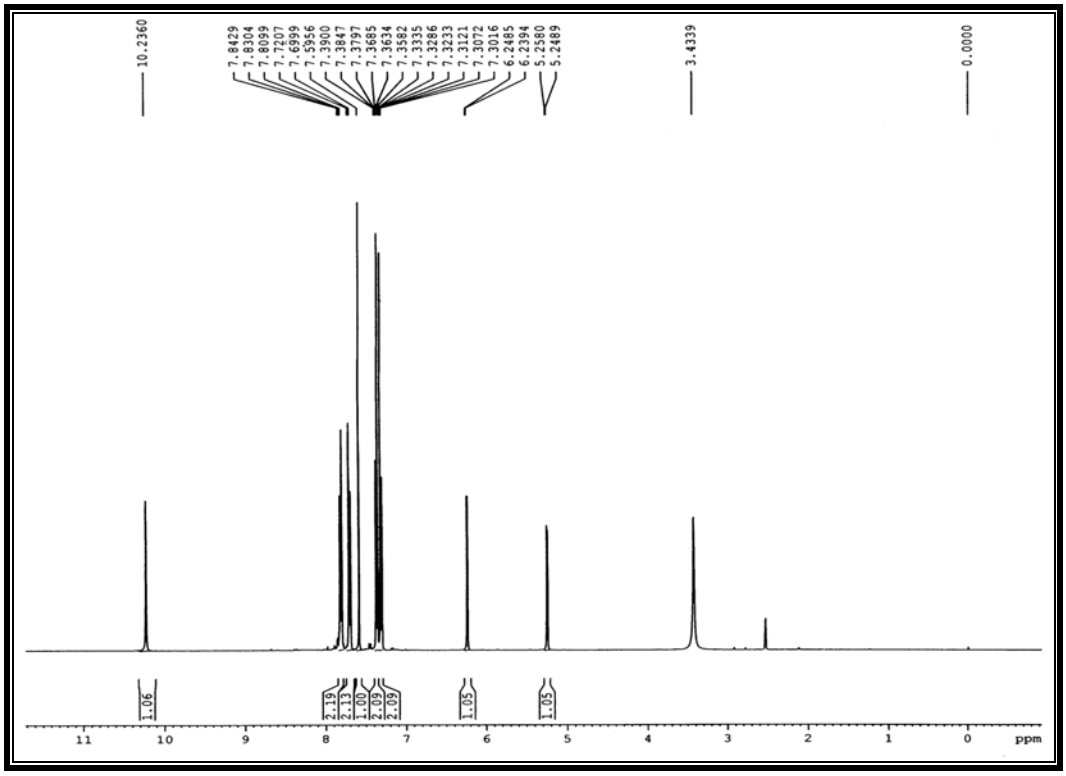
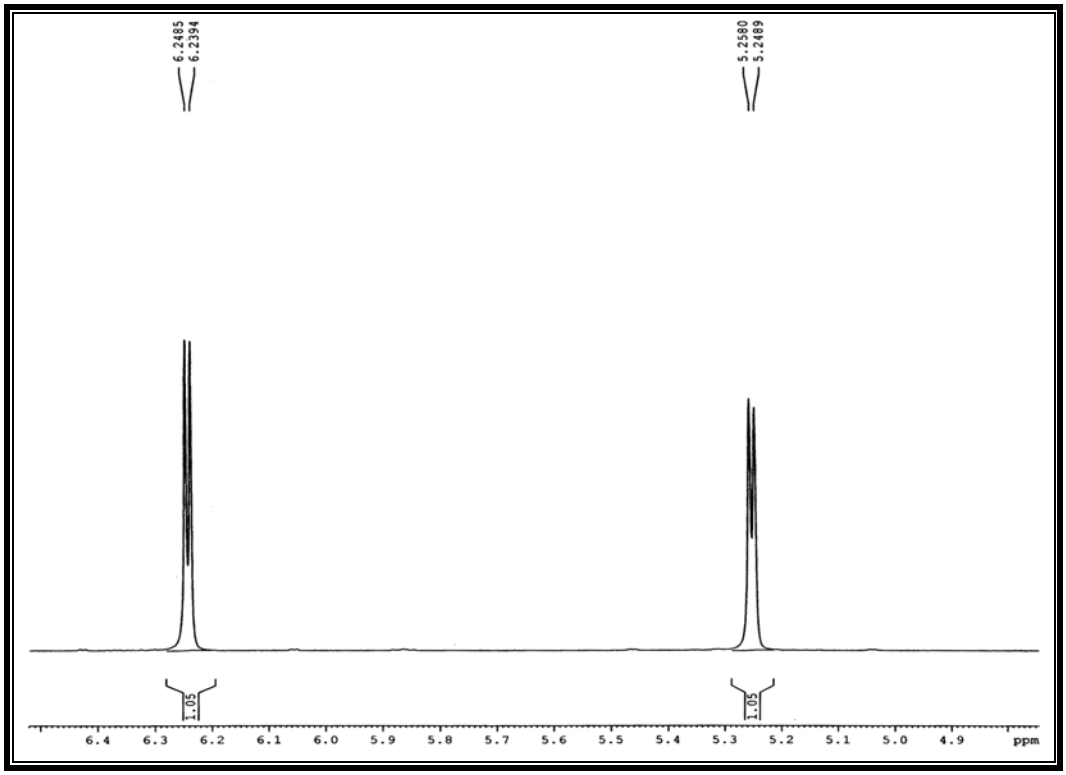
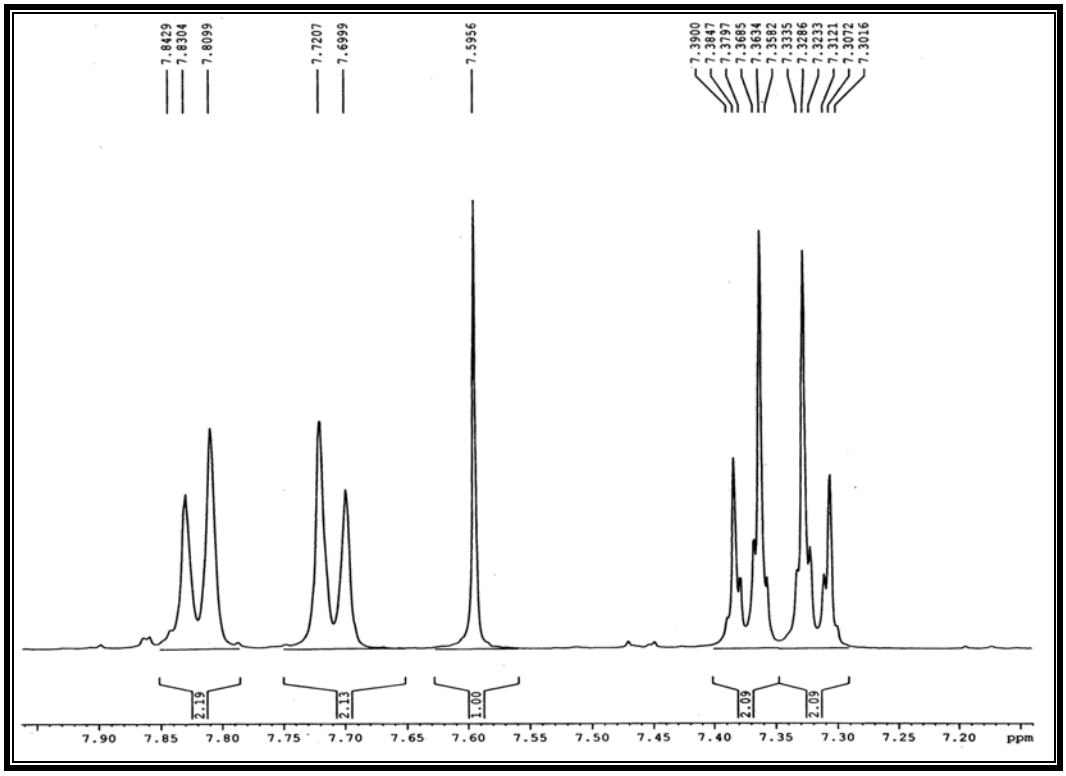


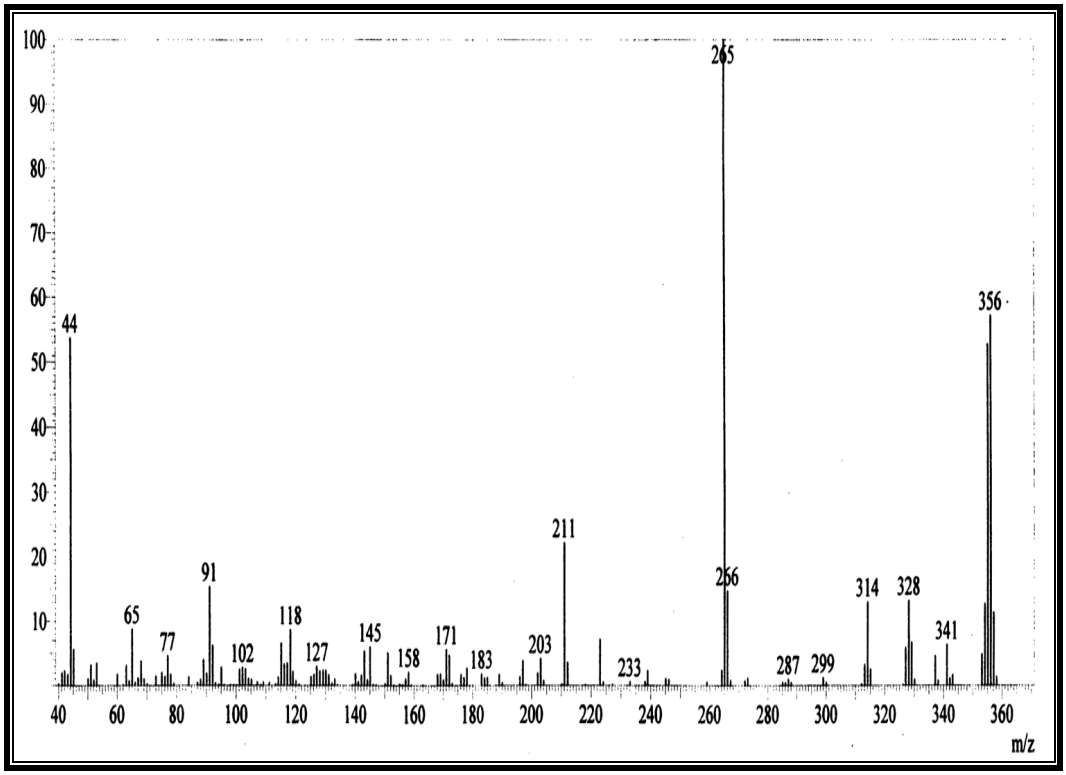
Expanded ^{1}H NMR spectrum of BHD-323



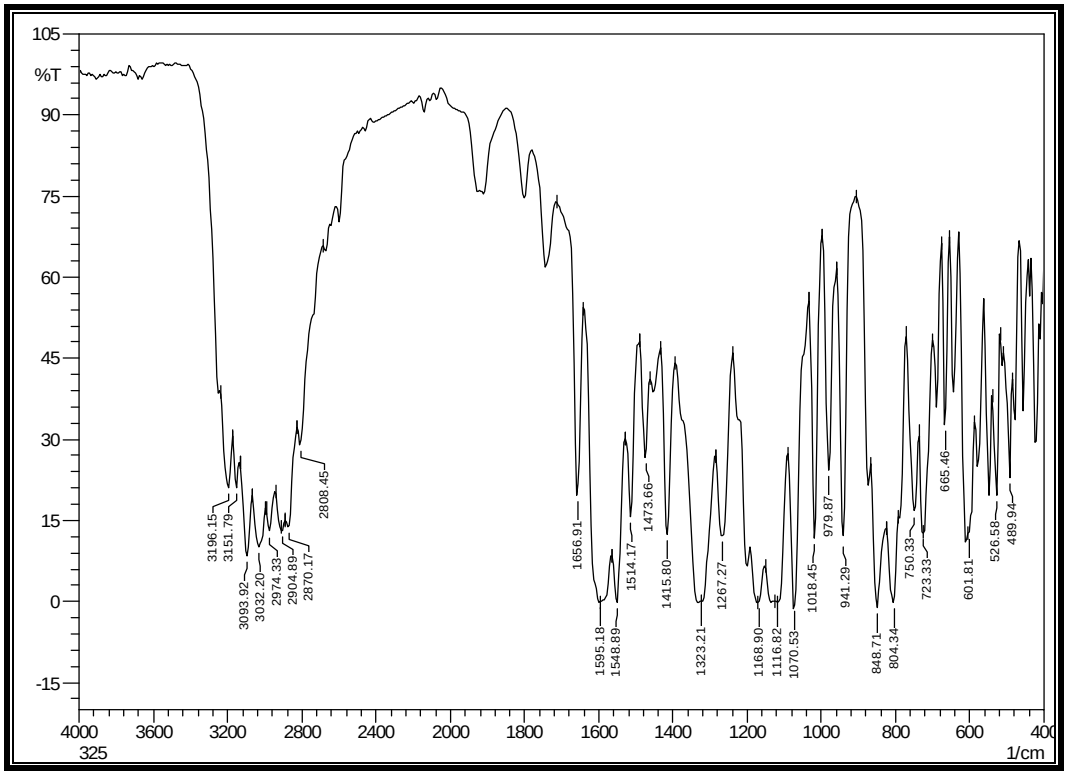
Expanded ^{1}H NMR spectrum of BHD-323



Mass spectrum of BHD-325



IR spectrum of BHD-325



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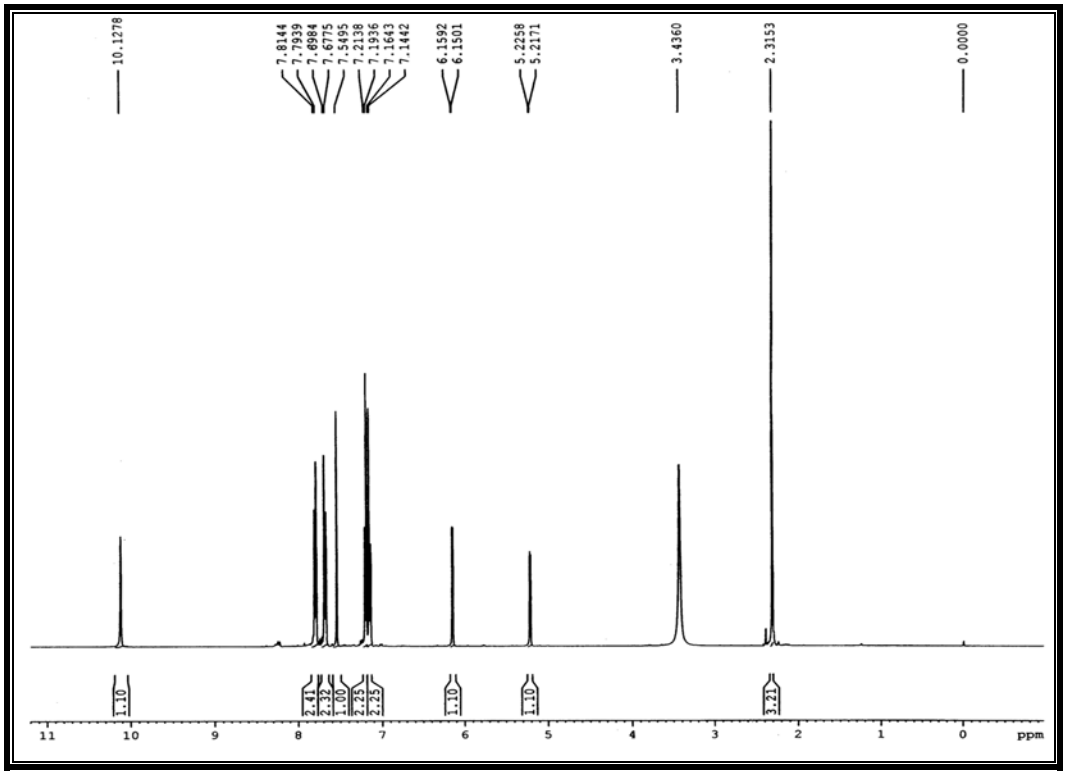
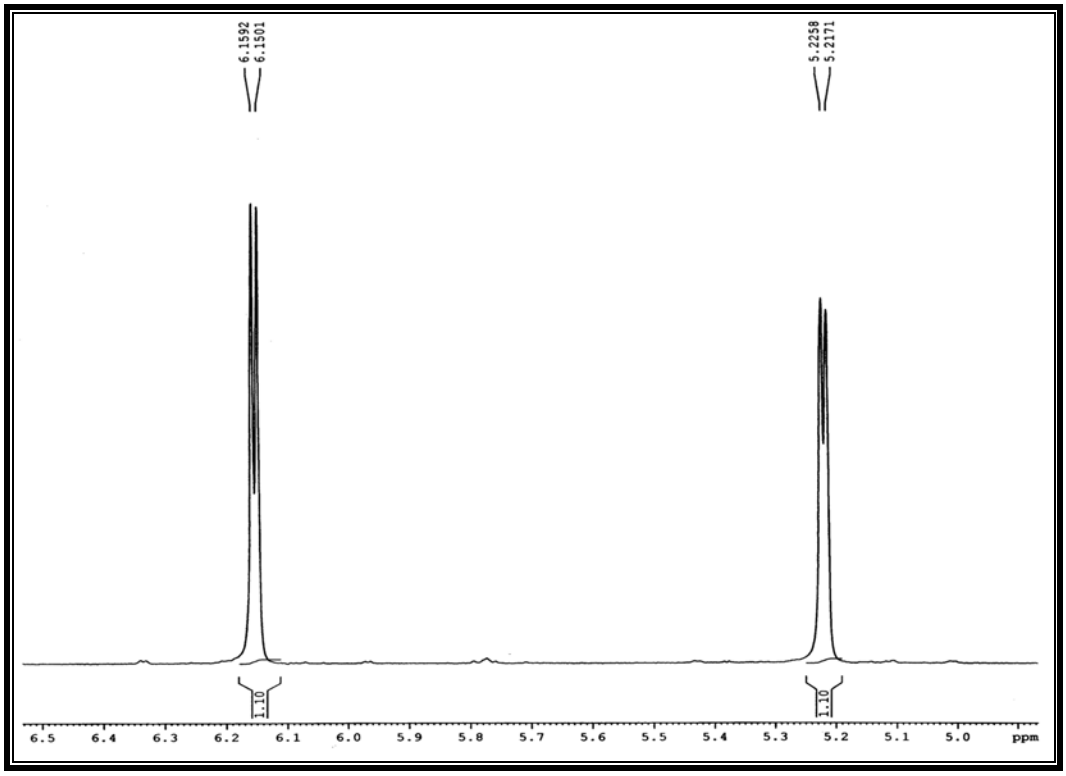
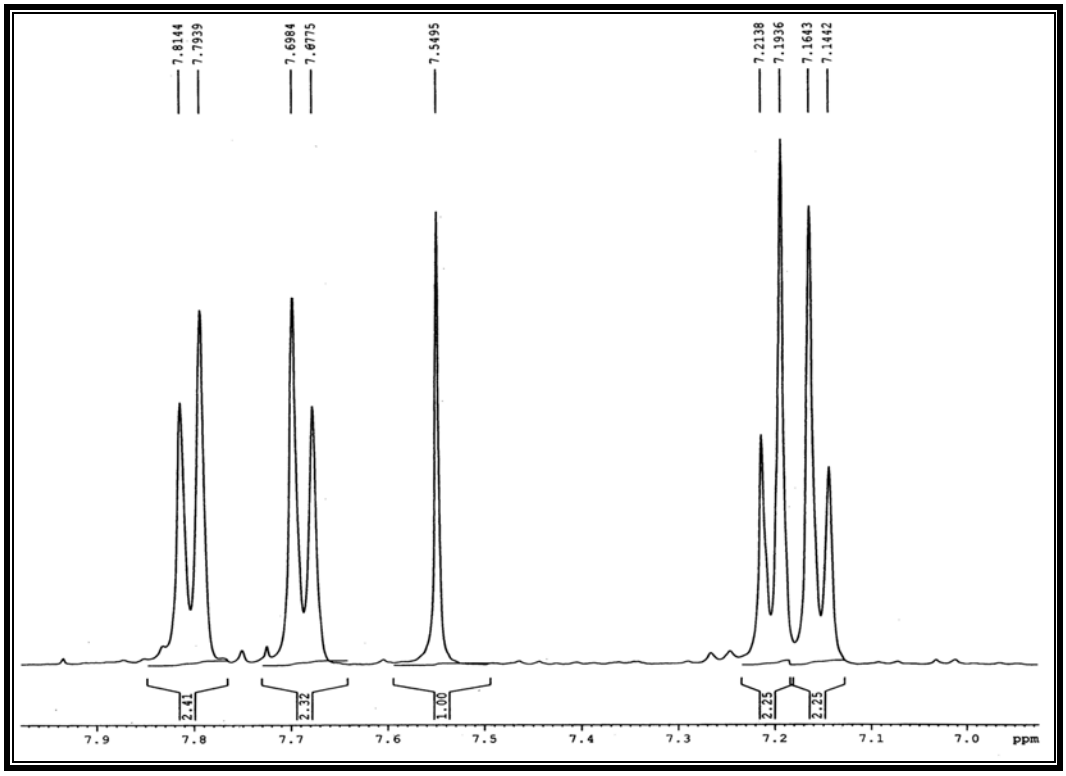


Expanded ^{1}H NMR spectrum of BHD-325



Expanded ^{1}H NMR spectrum of BHD-325



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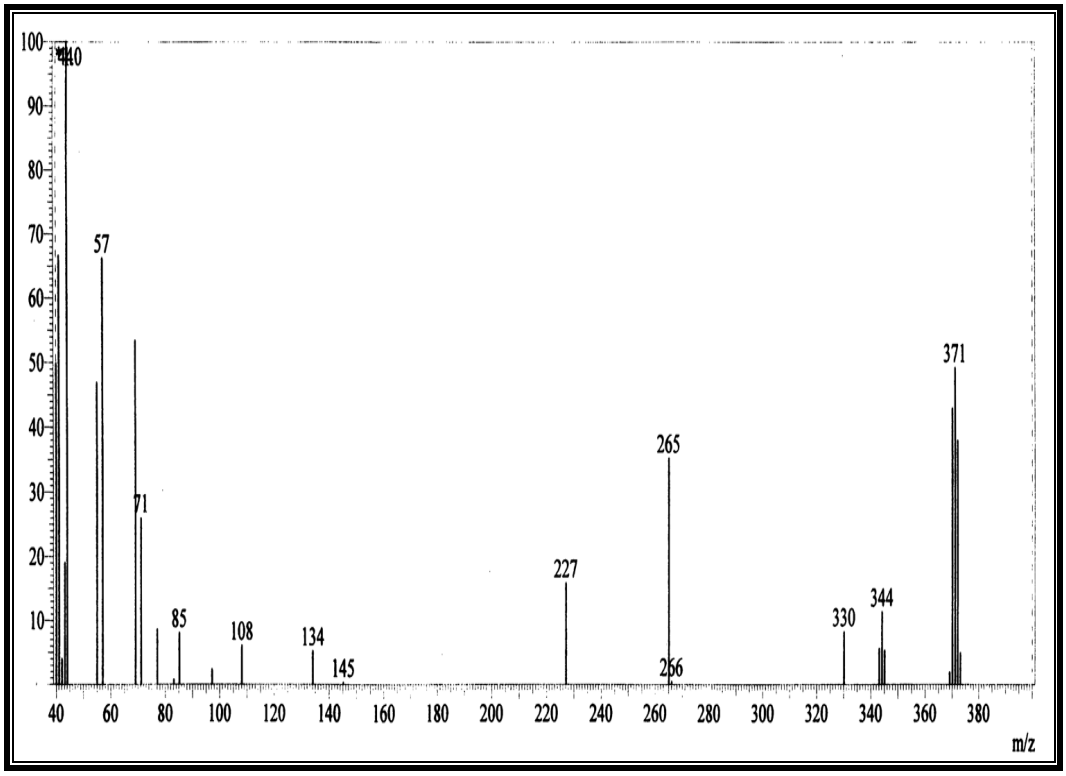

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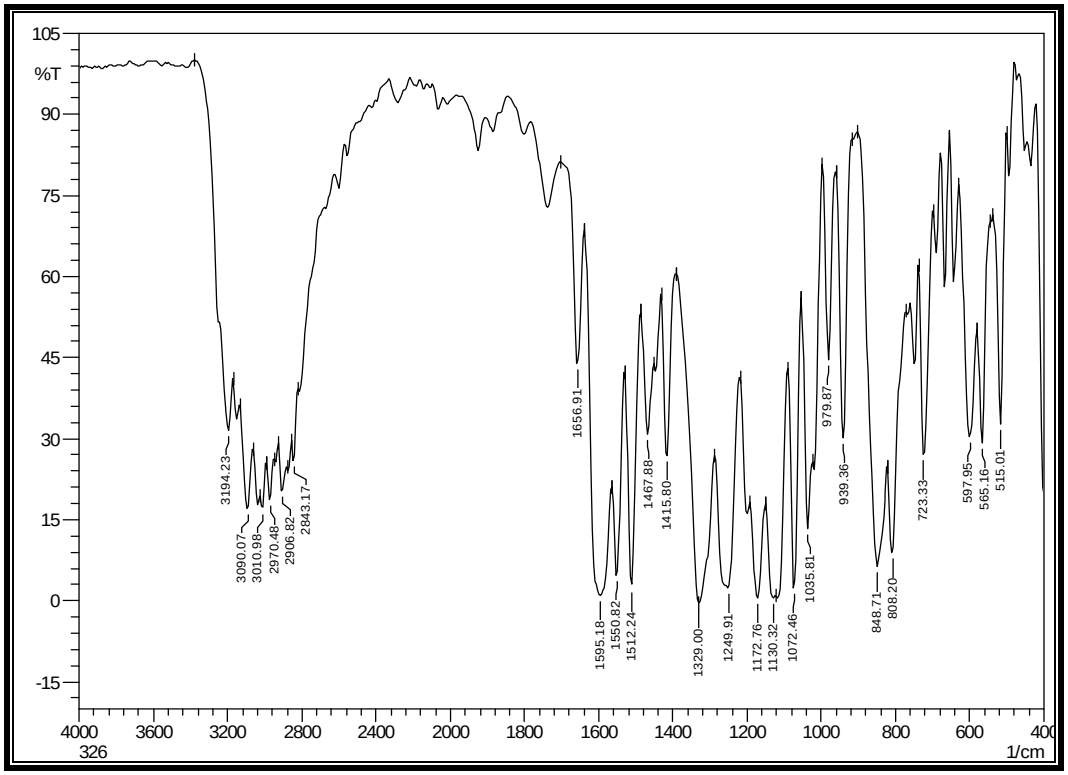

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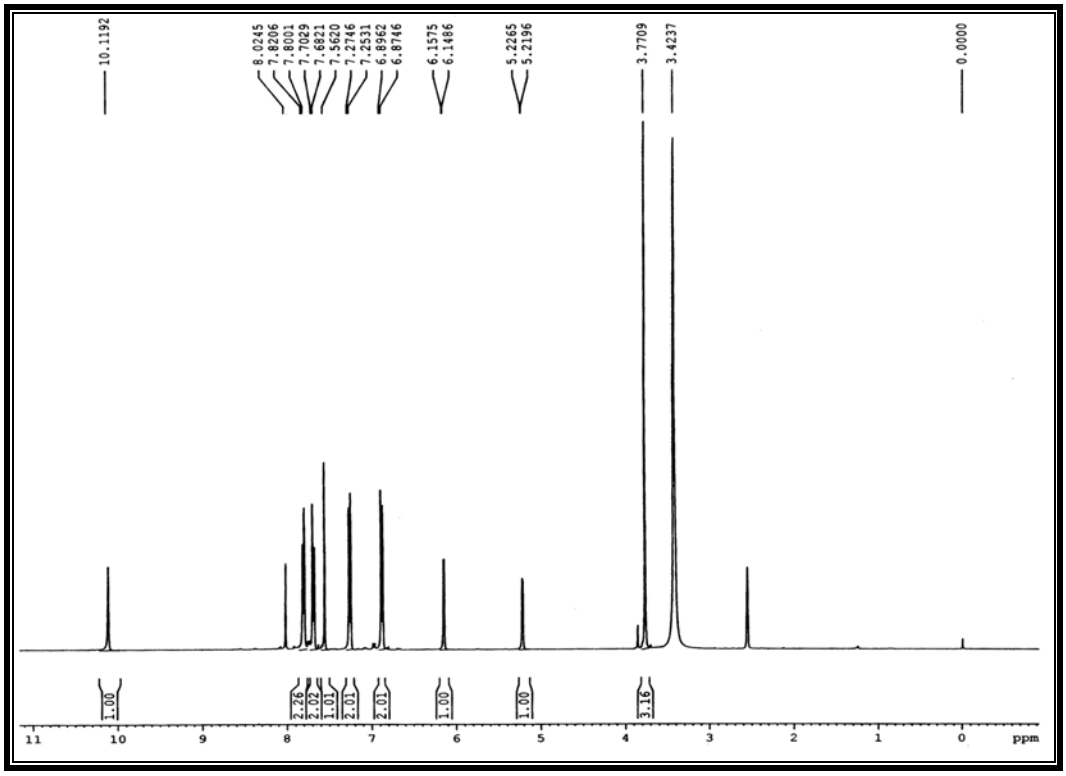

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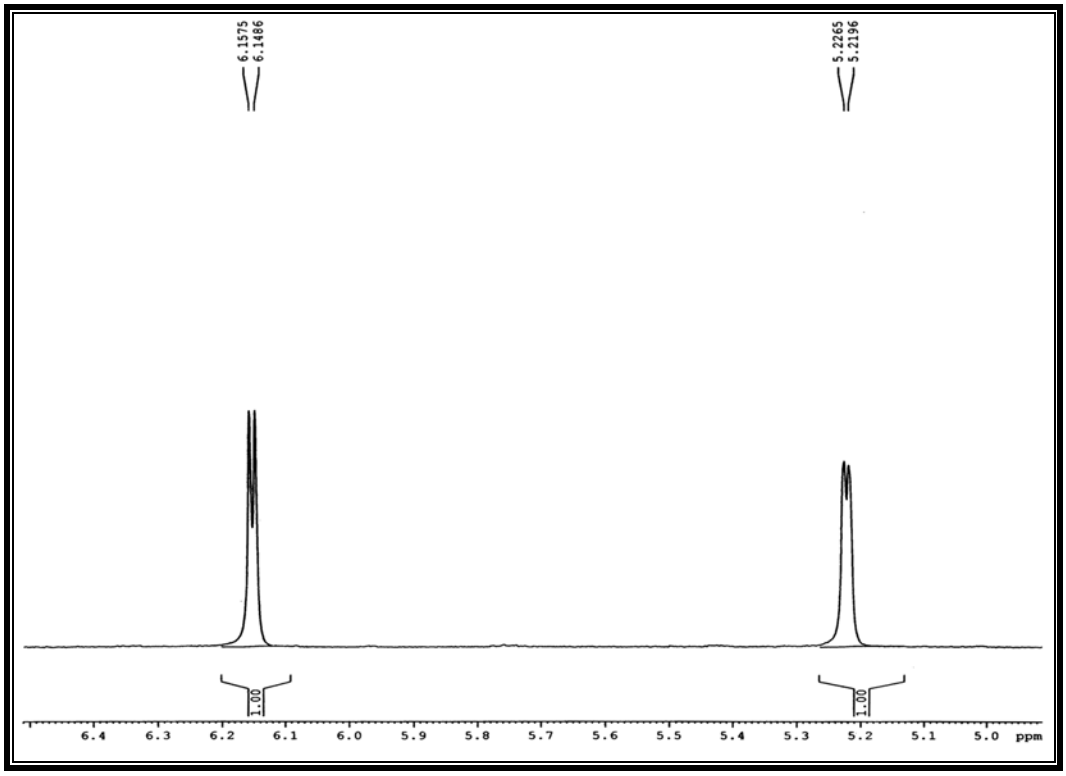

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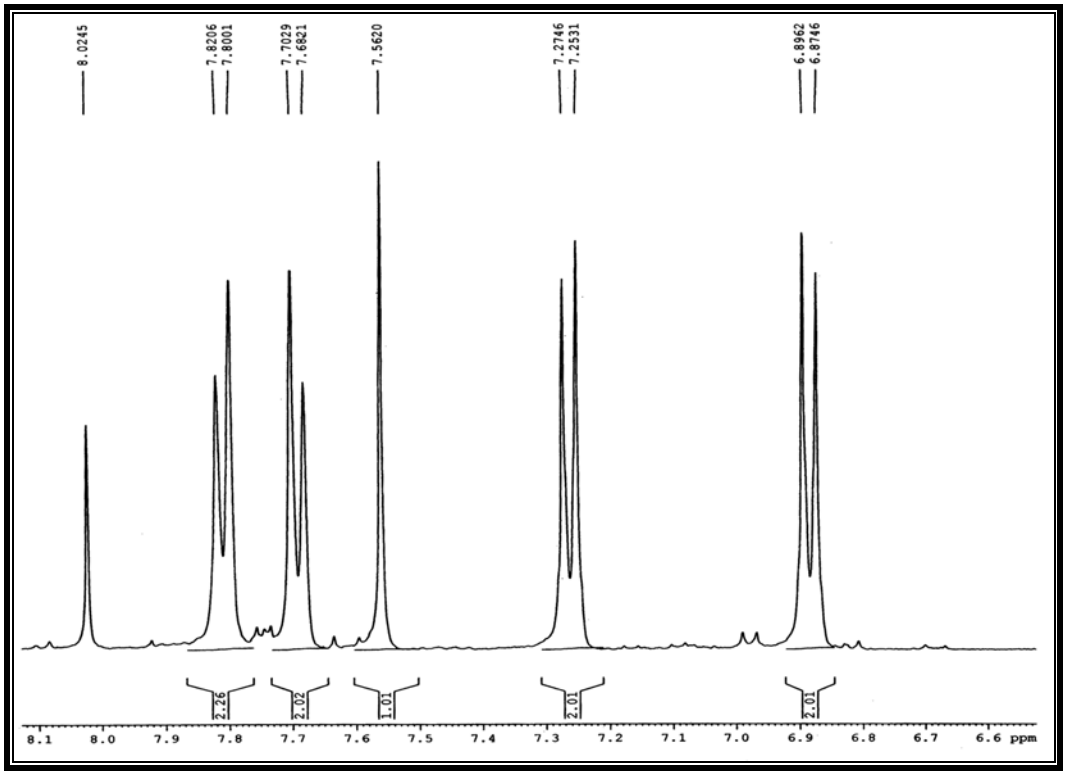
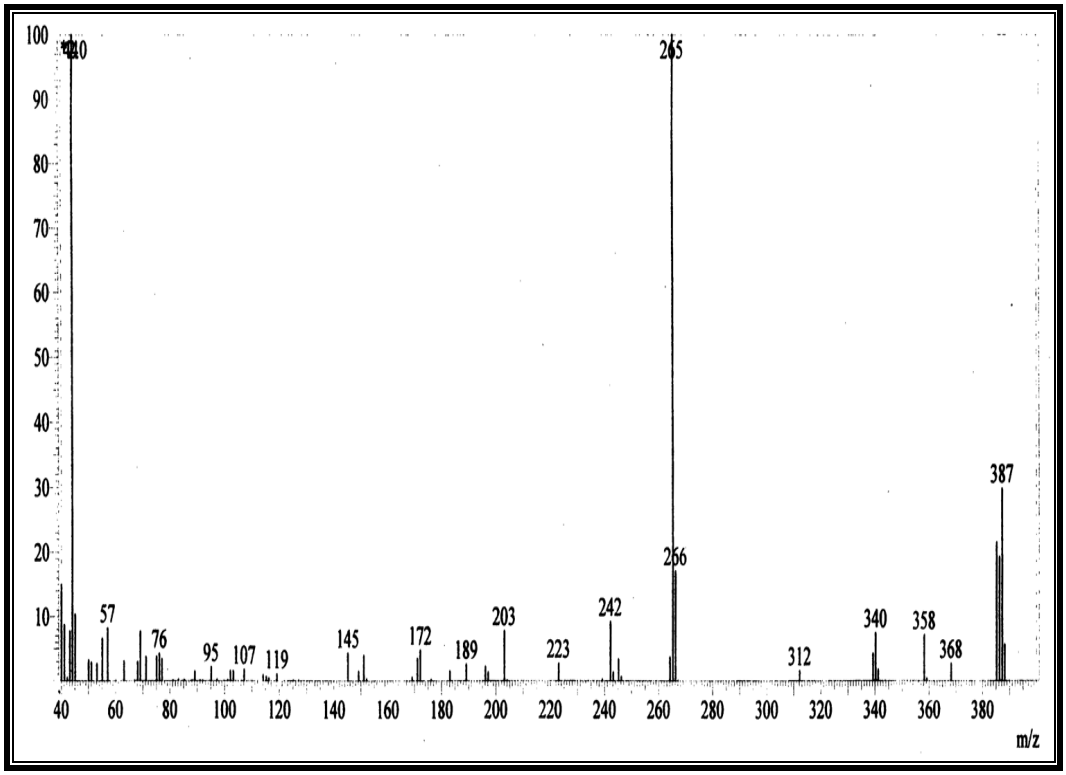
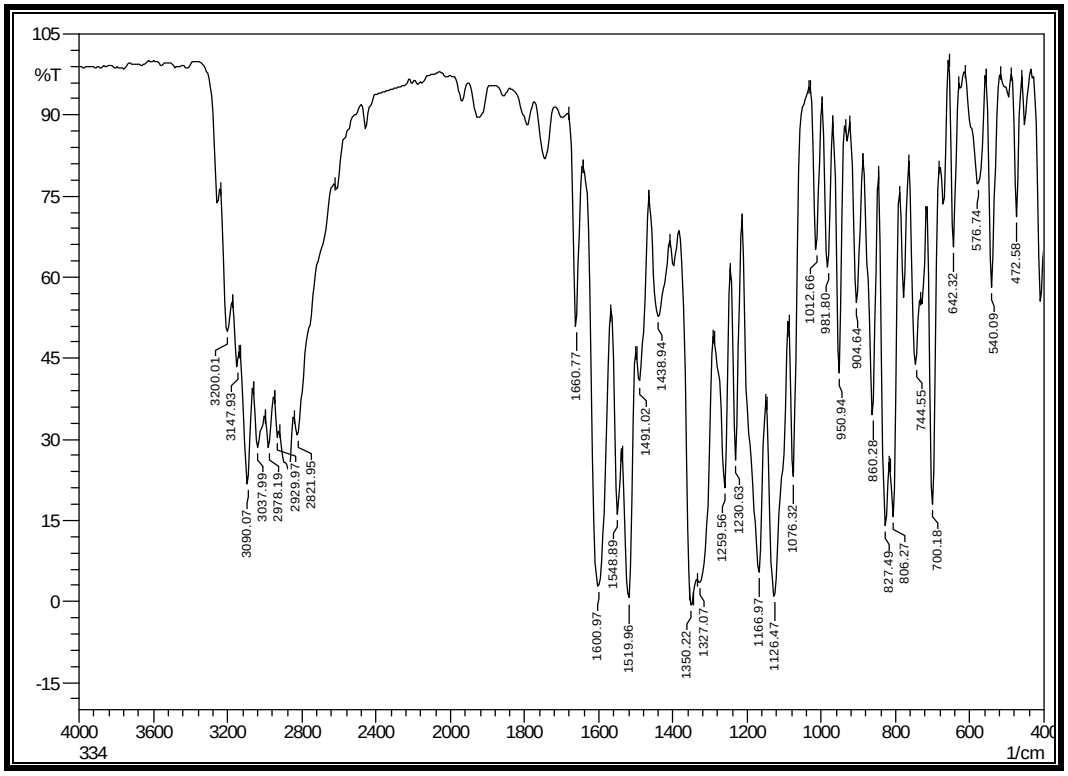


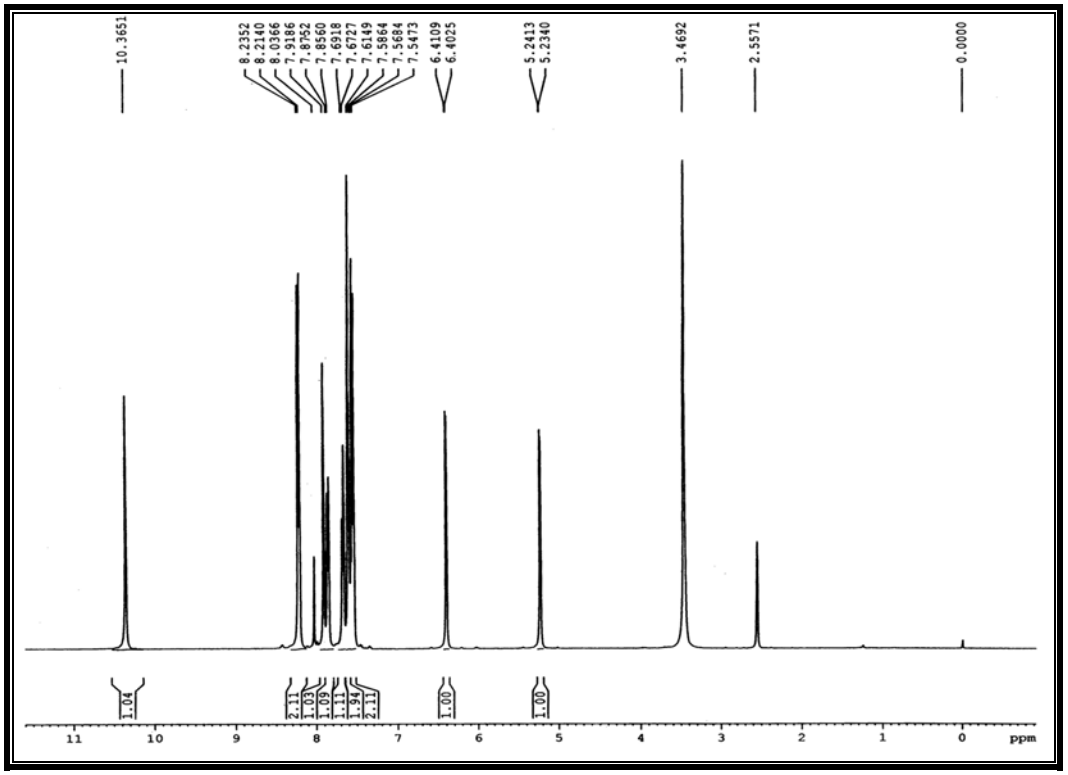
Mass spectrum of BHD-334



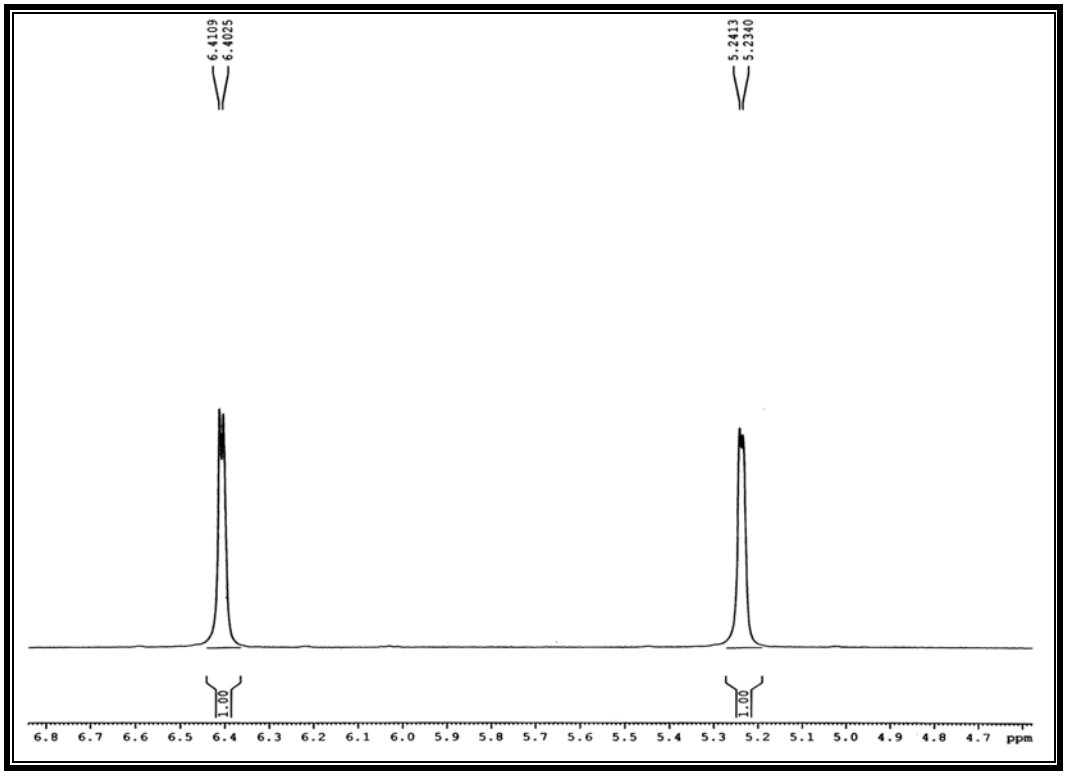
IR spectrum of BHD-334



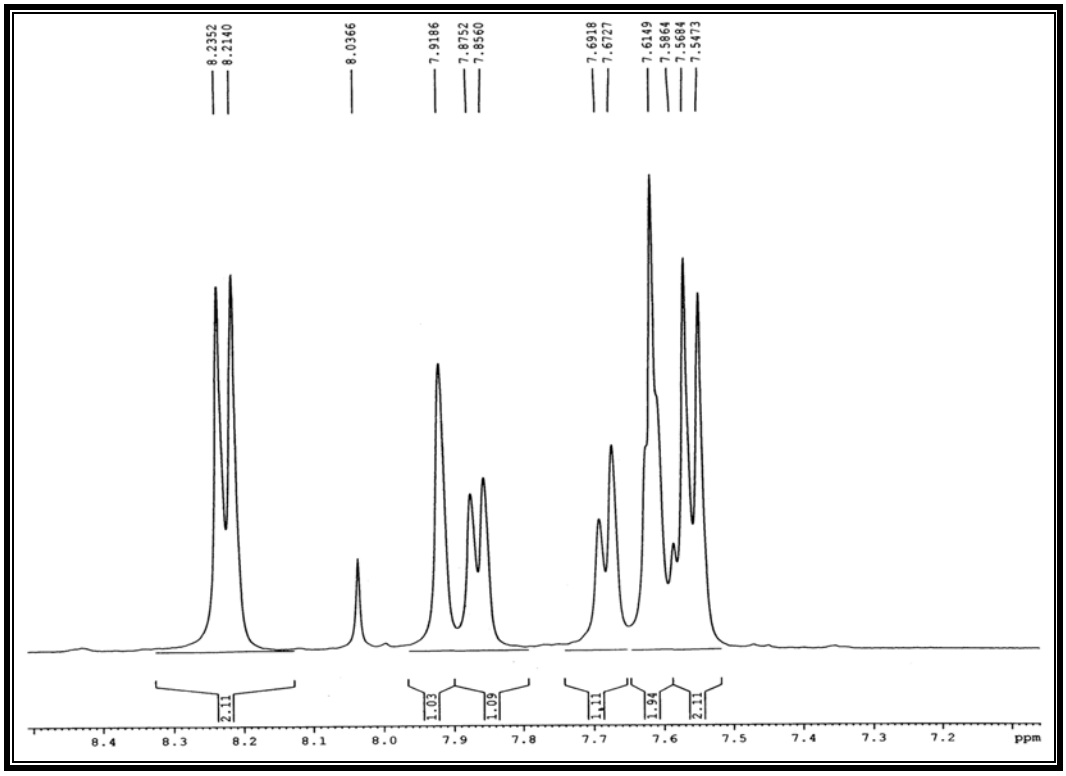
^{1}H NMR spectrum of BHD-334



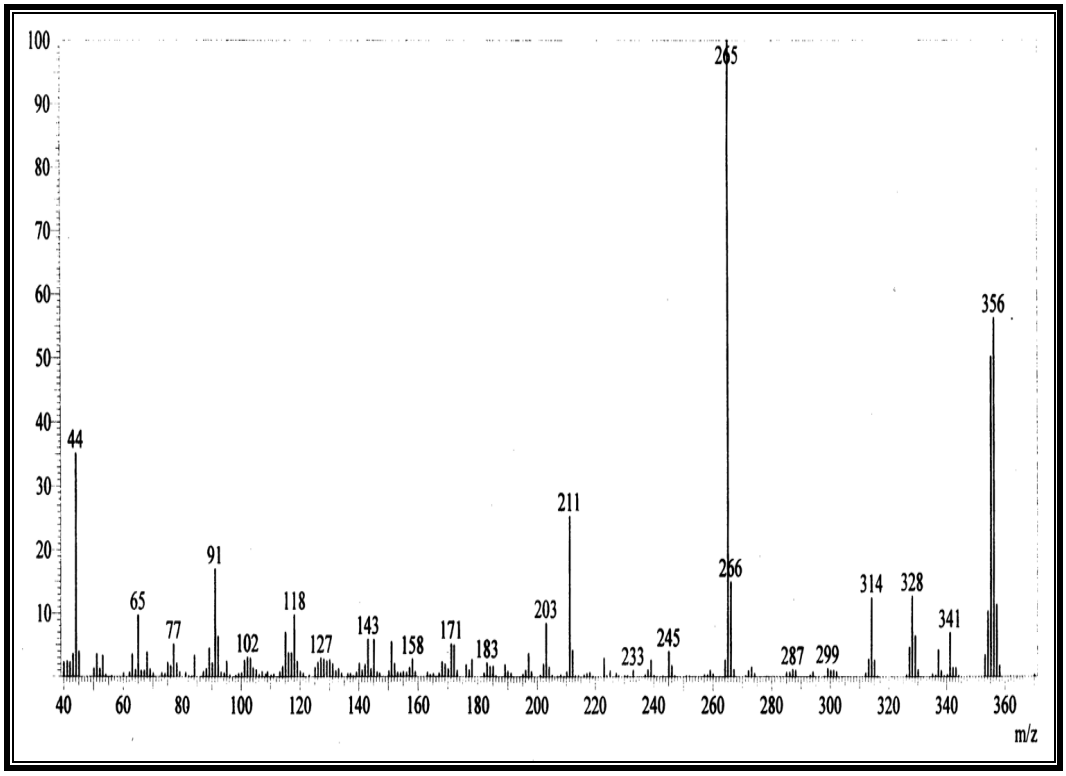
Expanded ^{1}H NMR spectrum of BHD-334



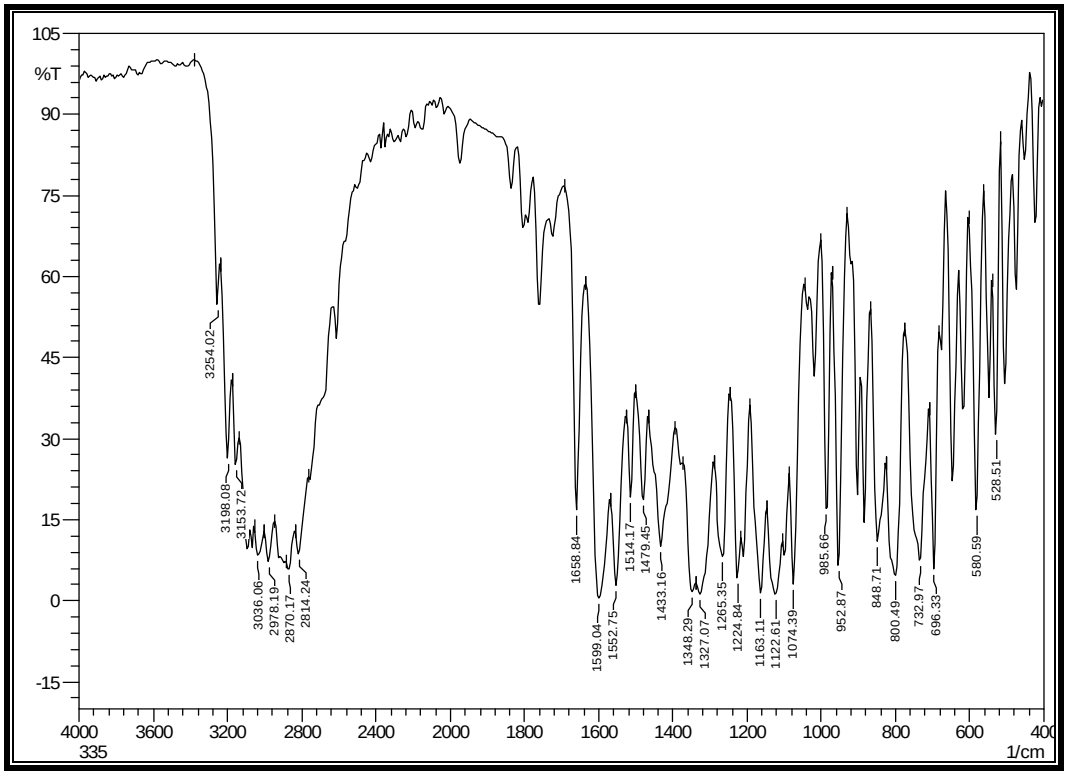
Expanded ^{1}H NMR spectrum of BHD-334



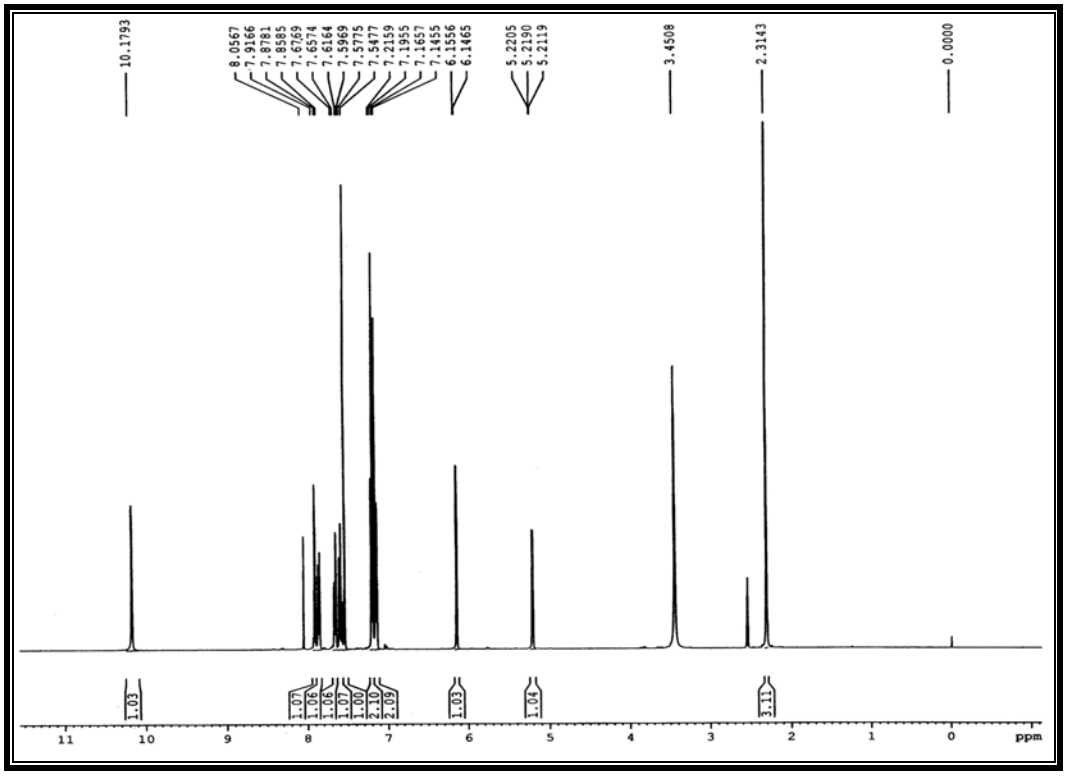
Mass spectrum of BHD-335



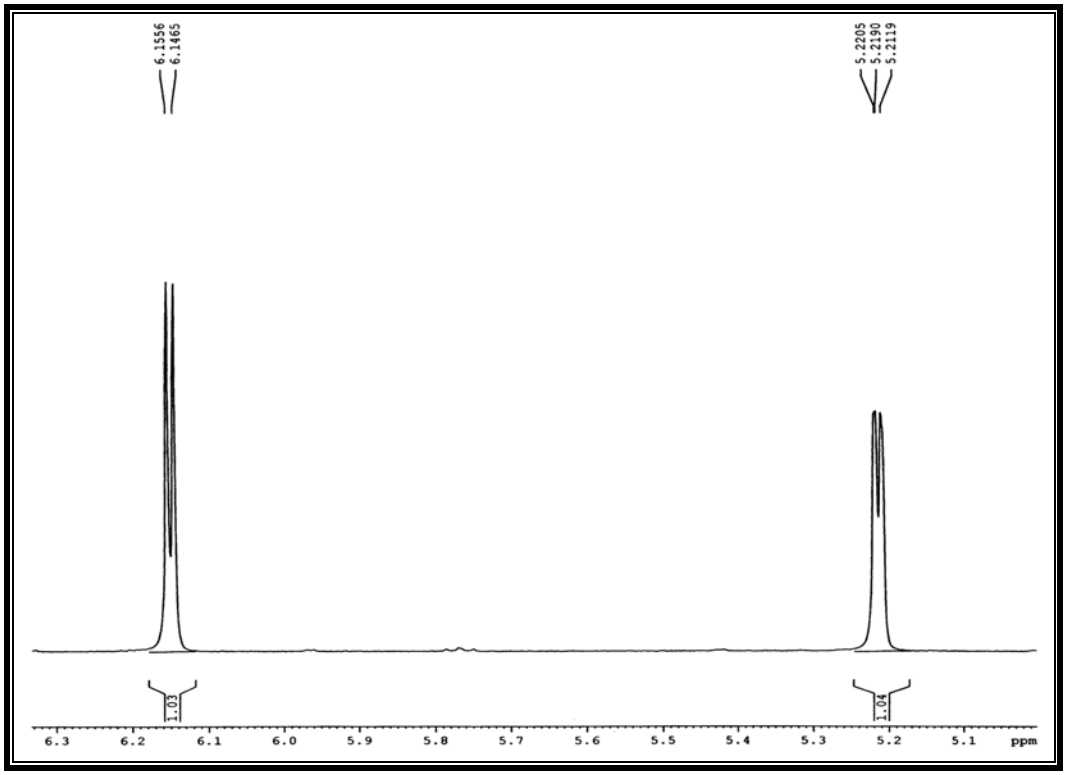
IR spectrum of BHD-335



^{1}H NMR spectrum of BHD-335



Expanded ^{1}H NMR spectrum of BHD-335



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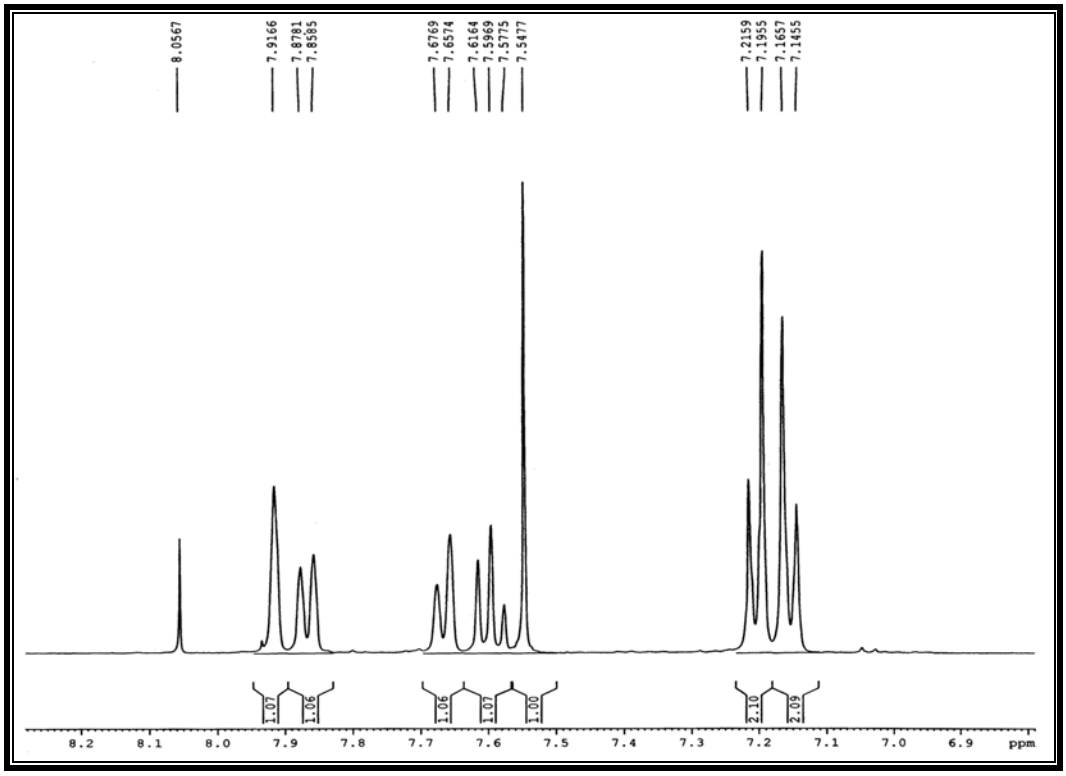
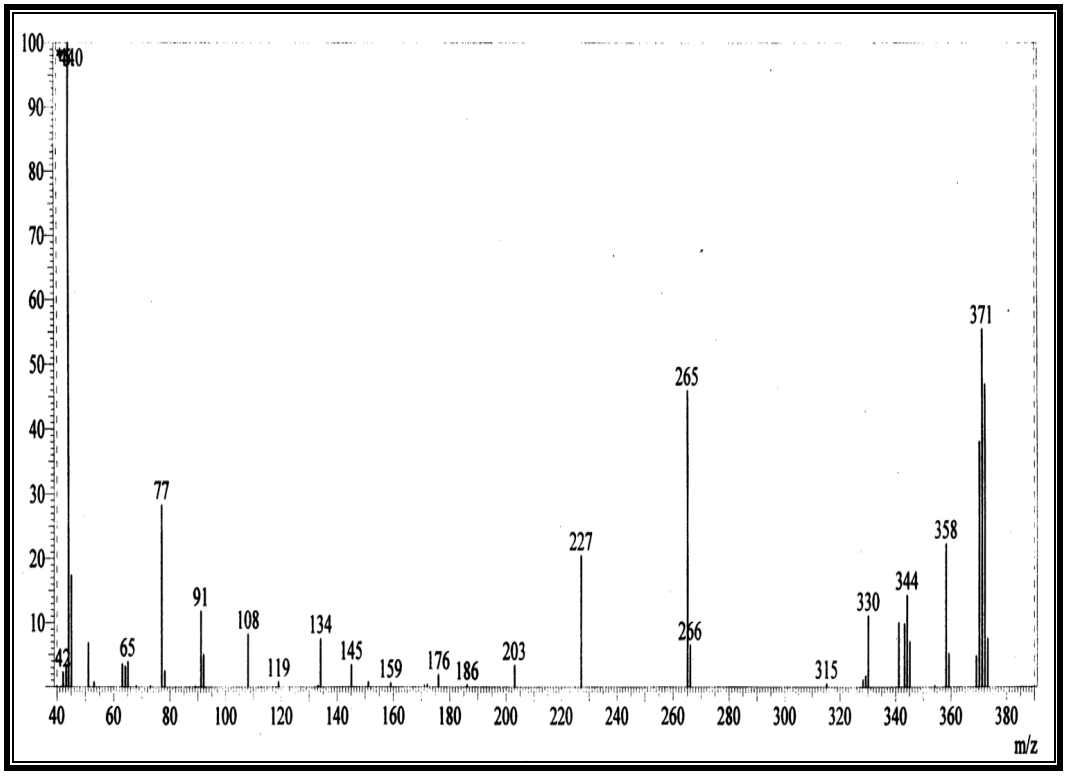
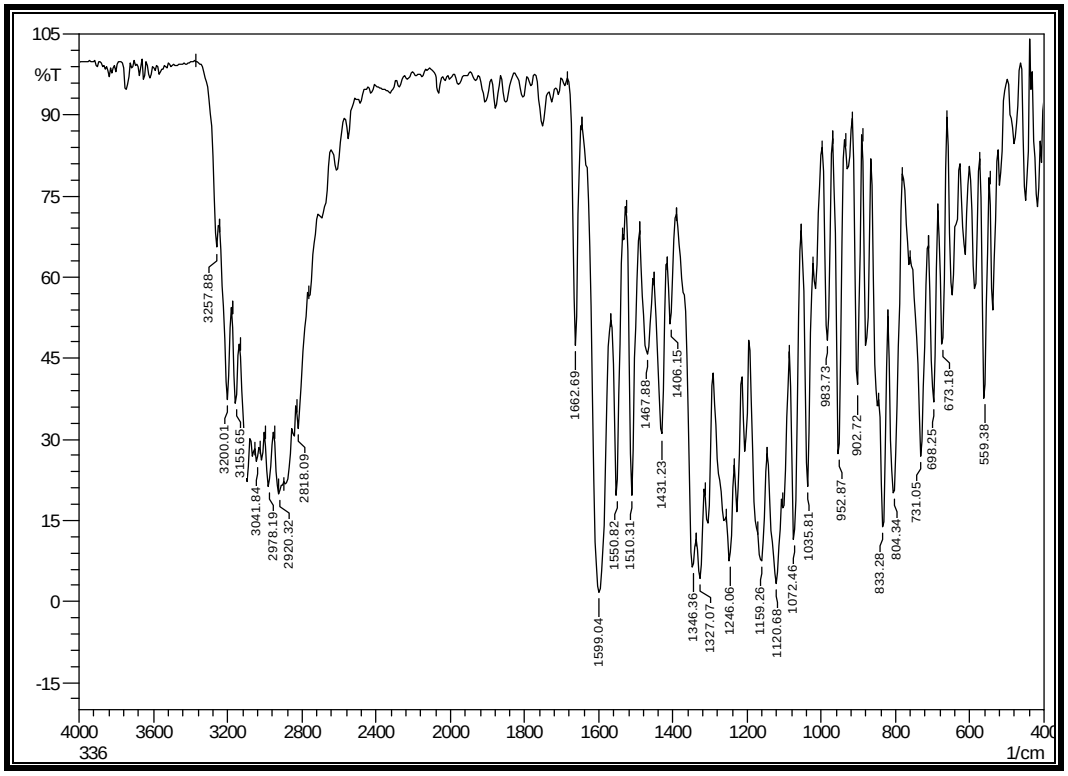


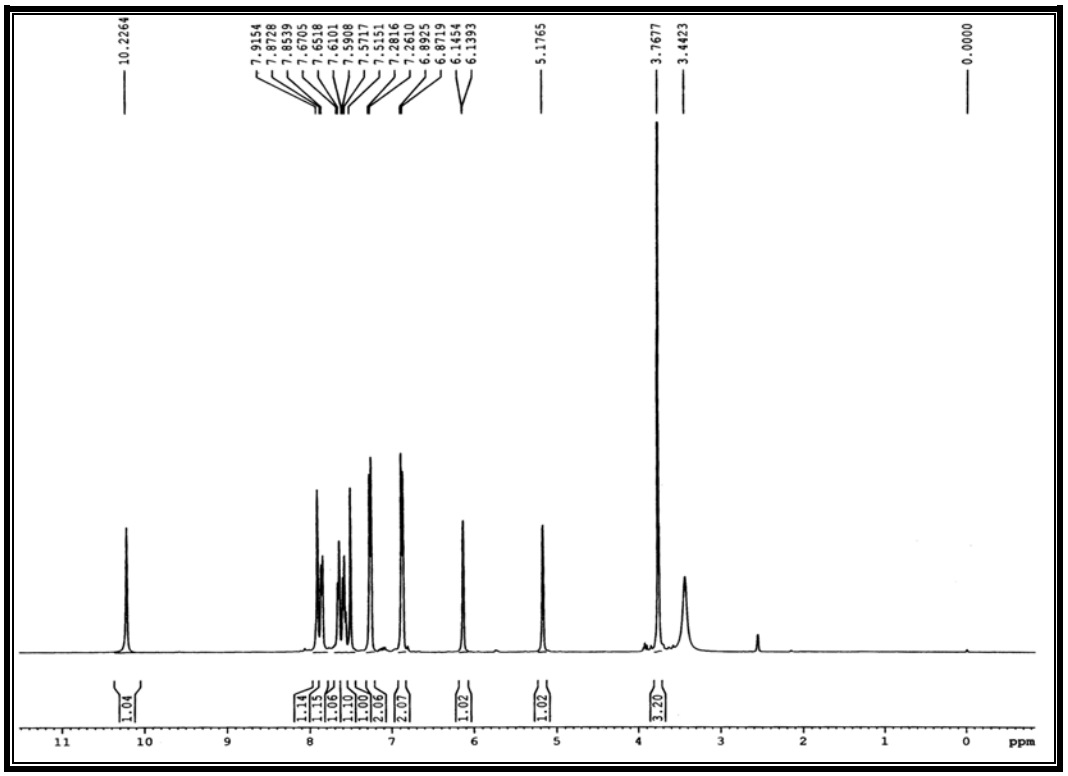
Mass spectrum of BHD-336



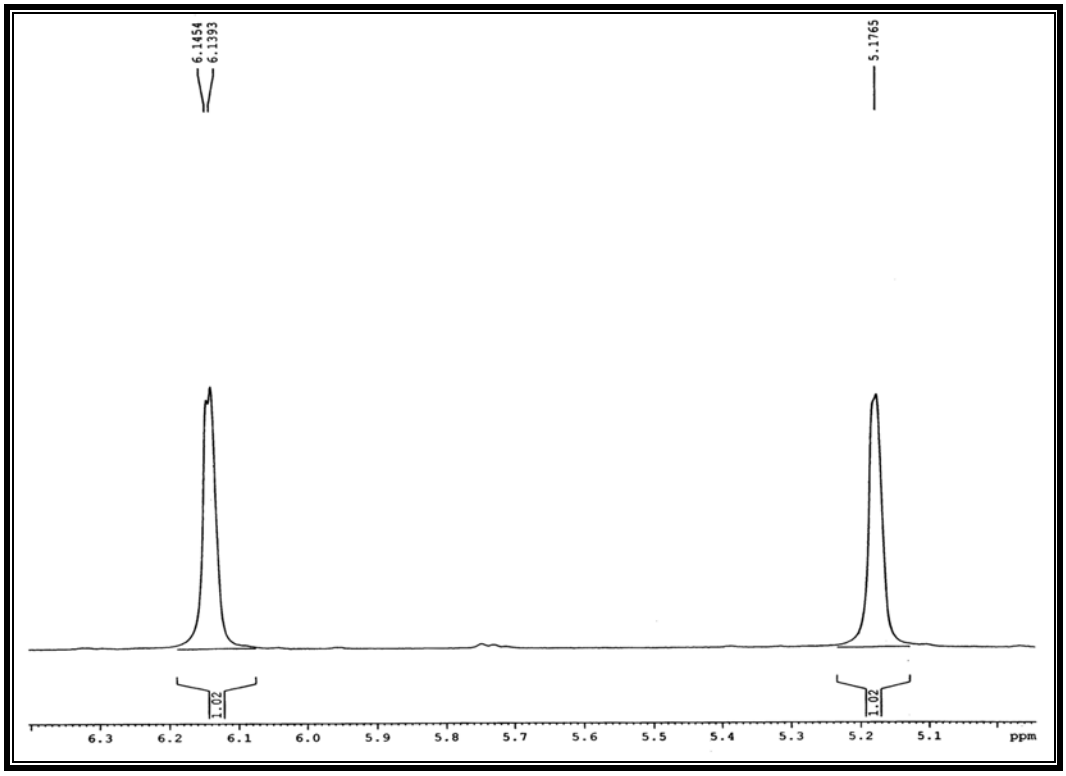
IR spectrum of BHD-336



^{1}H NMR spectrum of BHD-336



Expanded ^{1}H NMR spectrum of BHD-336



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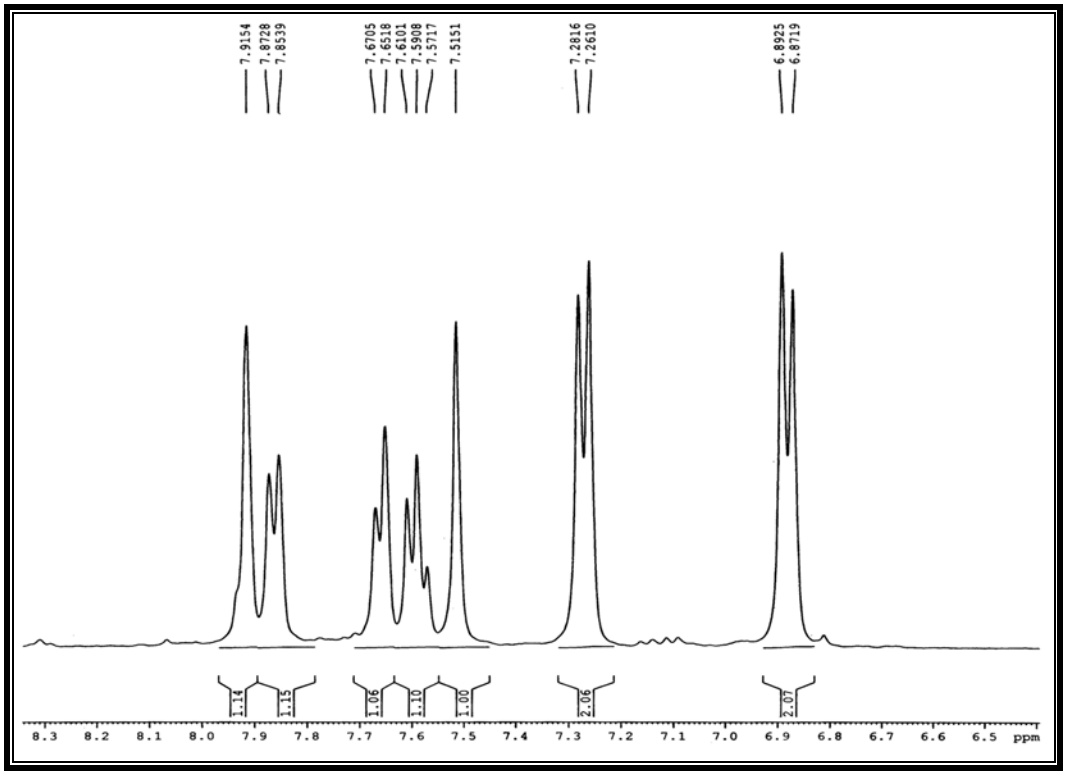

4.9 Biological evaluation

4.9.1 Antimicrobial evaluation

All the synthesized compounds (BHD-301 to 340) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [129, 130] 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 greseofulvin 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 [129]. 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 200 μg mL^{-1}, 100 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1}, and 6.25 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 BHD-301 to 340

Code	Minimal 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.***
BHD-301	200	100	100	100	250	1000	250
BHD-302	500	500	250	250	250	200	200
BHD-303	500	200	100	250	500	500	>1000
BHD-304	500	500	250	500	500	>1000	1000
BHD-305	250	62.5	250	500	1000	500	500
BHD-306	100	200	62.5	200	500	200	>1000
BHD-307	250	250	250	500	1000	500	>1000
BHD-308	500	500	200	500	>1000	>1000	>1000
BHD-309	100	200	500	500	250	>1000	>1000
BHD-310	500	500	100	250	250	1000	250
BHD-311	500	62.5	250	250	250	200	200
BHD-312	100	250	100	250	500	500	>1000
BHD-313	500	250	250	500	500	>1000	1000
BHD-314	500	500	250	500	>1000	>1000	>1000
BHD-315	500	250	100	100	500	>1000	250
BHD-316	200	500	250	500	1000	500	>1000
BHD-317	250	500	500	500	1000	500	500
BHD-318	250	500	500	500	250	>1000	>1000
BHD-319	500	500	1000	1000	500	1000	1000
BHD-320	200	100	100	500	500	1000	200
BHD-321	250	250	250	250	500	500	1000
BHD-322	100	500	500	1000	250	500	500
BHD-323	500	100	62.5	100	500	500	>1000
BHD-324	250	500	500	500	200	500	200
BHD-325	500	250	500	500	1000	1000	1000
BHD-326	500	100	500	250	1000	>1000	1000
BHD-327	100	62.5	62.5	100	250	100	500
BHD-328	500	250	200	500	500	1000	>1000
BHD-329	100	250	500	1000	1000	>1000	>1000
BHD-330	250	62.5	100	100	250	1000	1000
BHD-331	500	500	250	200	>1000	>1000	>1000
BHD-332	200	500	250	200	500	>1000	>1000
BHD-333	250	250	500	500	500	250	500
BHD-334	500	500	250	500	>100	>1000	>1000
BHD-335	500	500	250	500	250	>1000	>1000
BHD-336	250	200	200	100	250	500	250
BHD-337	200	500	250	1000	250	500	>1000
BHD-338	100	62.5	200	500	1000	>1000	500
BHD-339	500	250	500	250	>1000	>1000	1000
BHD-340	250	250	500	250	>1000	>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
Greseofulvin	-	-	-	-	500	100	100

4.9.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds BHD-301 to BHD-340 is currently under investigation and results are awaited.

4.10 References and notes

[1] Fischer, G. *Adv. Heterocycl. Chem.* **1993**, *57*, 81.

[2] Shaban, M.A.E.; Morgan, A.E.A. *Adv. Heterocycl. Chem.* **2000**, *77*, 345.

[3] Shaban, M.A.E.; Morgan, A.E.A. *Adv. Heterocycl. Chem.* **2000**, *73*, 131.

[4] Shaban, M.A.E.; Morgan, A.E.A. *Adv. Heterocycl. Chem.* **2000**, *75*, 243.

[5] Zhang, N.; Ayral-Kaloustian, S.; Nguyen, T.; Afragola, J.; Hernandez, R.; Lucas, J.; Gibbons, J.; Beyer, C. *J. Med. Chem*. **2007**, *50*, 319.

[6] Havlicek, L.; Fuksova, K.; Krystof, V.; Orsag, M.; Vojtesek, B.; Strnad, M. *Bioorg. Med. Chem*. **2005**, *13*, 5399.

[7] Fraley M. E., Hoffman W. F., Rubino R. S. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 2767.

[8] Chen, Q.; Zhu, X. L.; Jiang, L. L.; Liu, Z. M.; Yang, G. F. *Eur. J. Med. Chem.* **2008**, *43*, 595.

[9] Shigeko, U.; Shinya, T.; Takako, M.; Tomiichiro, O. *Brain Research* **2002**, *946*, 298

[10] Fairfield, B. J.; Andrew, C.; Allan, J. WO2004108136, **2004**.

[11] Peng, H.; Kumaravel, G.; Yao, G.; Sha, L.; Wang, J.; Van Vlijmen, H.; Bohnert, T.; Huang, C.; Vu, C. B.; Ensinger, C. L.; Chang, H.; Engber, T. M.; Whalley, E. T.; Petter, R. C. *J. Med. Chem.* **2004**, *47*, 6218.

[12] Ram, V.; Srivastava, P.; Singh, S. K.; Kandpal, M.; Tekwani, B. L. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1087.

[13] Beyer, C. F.; Zhang, N.; Hernandez, R.; Vitale, D.; Lucas, J.; Nguyen, T.; Discafani, C.; Ayral-Kaloustian, S.; Gibbons, J. J. *Cancer Res.* **2008,** *68*, 2292.

[14] Chen, Q.; Zhu, X.; Jiang, L.; Liu, Z.; Yang, G. *Eur. J. Med. Chem.* **2008**, *43*, 595.

[15] Li, H.; Tatlock, J.; Linton, A.; Gonzalez, J.; Jewell, T.; Patel, L.; Ludlum, S.; Drowns, M.; Rahavendran, S. V.; Skor, H.; Hunter, R.; Shi, S. T.; Herlihy, K. J.; Parge, H.; Hickey, M.; Yu, X.; Chau, F.; Nonomiya, J.; Lewis, C. *J. Med. Chem.* **2009,** *52,* 1255.

[16] Chen, C.; Lv, L.; Ji, F.; Chen, Q.; Xu, H.; Niu, C.; Xi, Z.; Yang, G. *Bioorg. Med. Chem*. **2009**, *17*, 3011.

[17] Richardson, C. M.; Williamson, D. S.; Parratt, M. J.; Borgognoni, J.; Cansfield, A. D.; Dokurno, P.; Francis, G. L.; Howes, R.; Moore, J. D.; Murray, J. B.; Robertson, A.; Surgenor, A. E.; Torrance, C. J. *Bioorg*. *Med*. *Chem. Lett*. **2006**, *16*, 1353.

[18] Shaaban, M. R.; Saleh, T. S.; Mayhoub, A. S.; Mansour, A.; Farag, A. M. *Bioorg*. *Med*. *Chem*. **2008**, *16*, 6344.

[19] Xin, Z.; Cun-long, Z.; Lei, H.; Qi, L.; Jiu-liang, W.; Xiao-li, S.; Ping, G. *CHEM. RES. CHINESE UNIVERSITIES* **2009**, *25*, 474.

[20] Liekfeld, H. *Pharmazeut. Ztg.* **1994***, 139*, 34.

[21] Leadbeater, N. *Chemistry World* **2004**, *1*, 38.

[22] Adam, D. *Nature* **2003**, *421*, 571.

[23] Kingston, H. M.; Haswell S. J. *Microwave-Enhanced Chemistry: Fundamentals, Sample preparation and Applications*, American Chemical Society: Washington DC, Eds. **1997**.

[24] Giberson, R. T.; Demaree R. S. (Eds.) *Microwave Techniques and Protocols*, Humana Press: Totowa, New Jersey, **2001**.

[25] Prentice, W. E. *Therapeutic Modalities for Physical Therapists*, McGraw-Hill: New York, **2002**.

[26] Gedye, R.; Smith, F.; Westaway, K.; Ali, H.; Baldisera, L.; Laberge, L.; Rousell, J. *Tetrahedron Lett.* **1986**, *27*, 279.

[27] Giguere, R. J.; Bray, T. L.; Duncan, S. M.; Majetich, G. *Tetrahedron Lett.* **1986**, *27*, 4945.

[28] (a) Loupy, A.; Petit, A.; Hamelin, J.; Texier-Boullet, F.; Jacquault, P.; Mathk, D. *Synthesis* **1998**, *34*, 1213; (b) Varma, R. S. *Green Chem.* **1999**, 43; (c) Kidawi, M. *Pure Appl. Chem.* **2001**, *73*, 147; (d) Varma, R. S. *Pure Appl. Chem.* **2001**, 73, 193; (e) Varma, R. S. *Tetrahedron* **2002**, *58*, 1235; (f) Varma, R. S. *Advances in Green Chemistry: Chemical Syntheses Using Microwave Irradiation*, Kavitha Printers: Bangalore, **2002**.

[29] (a) Bose, A. K.; Banik, B. K.; Lavlinskaia, N.; Jayaraman, M.; Manhas, M. S. *Chemtech* **1997**, 27, 18; (b) Bose, A. K.; Manhas, M. S.; Ganguly, S. N.; Sharma, A. H.; Banik, B. K. *Synthesis* **2002**, 1578.

[30] (a) Strauss, C. R.; Trainor, R. W. *Aust. J. Chem.* **1995**, *48*, 1665; (b) Strauss, C. R. *Aust. J. Chem.* **1999**, 52, 8; (c) Strauss, C. R. *Microwaves in Organic Synthesis* (Ed.: A. Loupy), Wiley-VCH: Weinheim, **2002**, 35.

[31] (a) Perreux, L.; Loupy, A. *Tetrahedron* **2001**, *57*, 9199; (b) Kuhnert, N. *Angew. Chem. Int. Ed.* **2002**, *41*, 1863; (c) Strauss, C. R. *Angew. Chem. Int. Ed.* **2002**, *41*, 3589.

[32] Kappe, C. O. *Angew. Chem. Int. Ed.* **2004**, *43*, 6250.

[33] Danks, T. N. *Tetrahedron Lett.* **1999**, *40*, 3957.

[34] Selvi, S.; Perumal, P. T. *J. Heterocycl. Chem.* **2002**, *39*, 1129.

[35] Usyatinsky, A. Ya.; Khmelnitsky, Y. L. *Tetrahedron Lett.* **2000**, *41*, 5031.

[36] Marrero-Terrero, A. L.; Loupy, A. *Synlett* **1996**, 245.

[37] Bentiss, F.; Lagrenée, M.; Barbry, D. *Tetrahedron Lett.* **2000**, *41*, 1539.

[38] Alterman, M.; Hallberg, A. *J. Org. Chem.* **2000**, *65*, 7984.

[39] Hayes, B. L. *Microwave Synthesis: Chemistry at the Speed of Light*, CEM Publishing: Matthews, NC, **2002**.

[40] Kaddar, H.; Hamelin, J.; Benhaoua, H. *J. Chem. Res.* **1999**, 718.

[41] (a) Chandra Sheker Reddy, A.; Shanthan Rao, P.; Venkataratnam, R. V. *Tetrahedron* **1997**, *53*, 5847. (b) Narsaiah, B.; Sivaprasad, A.; Venkataratnam, R. V. *J. Fluorine Chem.* **1994**, *66*, 47.

[42] Sridar, V. *Indian J. Chem.* **1997**, *36B*, 86.

[43] Molina, A.; Vaquero, J. J.; Garcia-Navio, J. L.; Alvarez-Builla, J. *Tetrahedron Lett.* **1993**, *34*, 2673.

[44] Öhberg, L.; Westman, J. *Synlett* **2001**, 1296.

[45] Quiroga, J.; Cisneros, C.; Insuasty, B.; Abonia, R.; Nogueras, M.; Sánchez, A. *Tetrahedron Lett.* **2001**, *42*, 5625.

[46] Avalos, M.; Babiano, R.; Cintas, P.; Clemente, F. R.; Jimenez, J. L.; Palacios, J. C.; Sanchez, J. B. *J. Org. Chem.* **1999**, *64*, 6297.

[47] Ranu, B. C.; Hajra, A.; Jana, U. *Tetrahedron Lett.* **2000**, *41*, 531.

[48] Eynde, J. J. V.; Hecq, N.; Kataeva, O.; Kappe, C. O. *Tetrahedron* **2001**, *57*, 1785.

[49] Ding, J.; Gu, H.; Wen, J.; Lin, C. *Synth. Commun.* **1994**, *24*, 301.

[50] Abenhaïm, D.; Díez-Barra, E.; de la Hoz, A.; Loupy, A.; Sánchez-Migallón, A. *Heterocycles* **1994**, *38*, 793.

[51] Villemin, D.; Gómez-Escalonilla, M. J.; Saint-Clair, J. F. *Tetrahedron Lett.* **2001**, *42*, 635.

[52] Combs, A. P.; Saubern, S.; Rafalski, M.; Lam, P. Y. S. *Tetrahedron Lett.* **1999**, *40*, 1623.

[53] Van der Eycken, E.; Appukkuttan, P.; De Borggraeve, W.; Dehaen, W.; Dallinger, D.; Kappe, C. O. *J. Org. Chem.* **2002**, *67*, 7904.

[54] Katritzky, A. R.; Singh, S. K. *J. Org. Chem.* **2002**, *67*, 9077.

[55] (a) Häbich, D.; Barth, W.; Rösner, M. *Heterocycles* **1989**, *29*, 2083. (b) Mearman, R. C.; Newall, C. E.; Tonge, A. P. *J. Antibiot.* **1984**, *37,* 885. (c) Olesen, P. H.; Nielsen, F. E.; Pedersen, E. B.; Becher, J. *J. Heterocycl. Chem.* **1984**, *21*, 1603.

[56] Dupau, P.; Epple, R.; Thomas, A. A.; Fokin, V. V.; Sharpless, K. B. *Adv. Synth. Catal*. **2002**, *344*, 421.

[57] Yamashkin, S. A.; Kucherenko, N. Y.; Yurovskaya, M. A. *Chem. Heterocycl. Compd.* **1997**, *33*, 499.

[58] Fischer, G. *Adv. Heterocycl. Chem.* **1993**, *57*, 81.

[59] Krasovsky, A. L.; Moiseev, A. M.; Nenajdenko, V. G.; Balenkova, E. S. *Synthesis* **2002**, 901.

[60] Hammouda, M. H.; Etman, E.; M. Metwally, A. *J. Serb. Chem. Soc.* **1992**, *57*, 165.

[61] Al-Schiekh, M. A.; El-Din, A. M. S.; Hafez, E. A.; Elnagdi, M. H. *J. Chem. Res.* **2004**, 174.

[62] Kuznetsova, O. A.; Filyakova, V. I.; Pashkevich, K. I.; Ulomskii, E. N.; Plekhanov, P. V.; Rusinov, G. L.; Kodess, M. I.; Rusinov, V. L. *Russ. Chem. Bull.* **2003**, *52*.

[63] Lipunova, G. N.; Nosova, E. V.; Kodess, M. I.; Charushin, V. N.; Rozin, Y. A.; Chasovskikh, O. M. *Russ. J. Org. Chem.* **2001**, *37*, 570.

[64] Hassaneen, H. M.; Abdallah, T. A.; Abdelhadi, H. A.; Hassaneen, H. M. E.; Pagni, R. M. *Heteroat. Chem.* **2003**, *14*, 491.

[65] Al-Zaydi, K. M.; Borik, R. M.; Elnagdi, M. H. *Molecules* **2003**, *8*, 910.

[66] Kanno, H.; Yamaguchi, H.; Ichikawa, Y.; Isoda S. *Chem. Pharm. Bull.* **1991**, *39*, 1099.

[67] Pelaez, W.; Gafarova, I. T.; Yranzo, G. I. *ARKIVOC* **2003**, *10*, 262.

[68] Su, J.; Ye, C.; Zheng, N. CP1537853, **2004**.

[69] Ebasˇek, P. Cˇ; Bevk, D.; Pirc, S.; Stanovnik, B.; Svete, J. *J. Comb. Chem.* **2006**, *8*, 95.

[70] Al-Zaydi, K. M.; Al-Shiekh, M. A. A.; Hafez, E. A. *J. Chem. Res.* **2000**, 13.

[71] Dawood, K. M.; Farag, A. M.; Kandeel, Z. E. *J. Chem. Res.* **1999**, 88.

[72] Al-Afaleq, E. I. *Synth. Commun.* **2000**, *30*, 1985.

[73] Hassaneen, H. M.; Abdallah, T. A.; Abdelhadi, H. A.; Hassaneen, H. M. E.; Pagni, R. M. *Heteroat. Chem.* **2003**, *14*, 491.

[74] Yang, G.; Lu, R.; Fei, X.; Yang, H. *Chin. J. Chem.* **2000**, *18*, 435.

[75] Granik, V. G.; Makarov, V. A.; Parkanyi, C. *Adv. Heterocycl. Chem.* **1998**, *72*, 283.

[76] Stanovnik, B. *Progr. Heterocycl. Chem.* **1993**, *5*, 34.

[77] Tominaga, Y. *Trends Heterocycl. Chem.* **1991**, *2*, 43.

[78] Gonzalez, J.; Jewell, T. M.; Li, H.; Linton, A.; Tatlock, J. H. WO018725, **2006**.

[79] Vartanyan, M. M.; Eliseev, O. L.; Solov'eva, T. Y.; Petukhov, V. A. *Russ. Chem. Bull.* **1993**, *42*, 1921.

[80] Bishop, B. C.; Marley, H.; Preston, P. N.; Wright, S. H. B. *J. Chem. Soc. Perkin Trans. 1* **1999**, 1527.

[81] Liu, Z.; Yang, G.; Xu, H.; Xiang, J. *Huazhong Shifan Daxue Xuebao Zirankexueban* **2001**, *35*, 180.

[82] Smith, S. L.; Thompson, K. S. J.; Sargent, B. J.; Heal, D. J. *CNS Drug Rev.* **2001**, *7*, 146.

[83] Petrich, S. A.; Qian, Z.; Santiago, L. M.; Gupton, J. T. *Tetrahedron* **1994**, *50*, 12113.

[84] Al-Saleh, B.; Makhseed, S.; Hassaneen, H. M. E.; Elnagdi, M. H. *Synthesis* **2006**, 59.

[85] Petrich, S. A.; Qian, Z.; Santiago, L. M.; Gupton, J. T. *Heterocycles* **1995**, *40*, 729.

[86] Elgemeie, G. H.; Fathy, N. M.; Farrag, D. A. *Egypt. J. Pharm. Sci.* **1998**, *38*, 351.

[87] Costales, M. J.; Kleschick, W. A.; Gerwick, B. C. *ACS Symp. Ser.* **1992**, *504*, 26.

[88] Kofman, T. P.; Kartseva, G. Y. *Russ. J. Org. Chem.* **2000**, *36*, 866.

[89] Krasovsky, A. L.; Moiseev, A. M.; Nenajdenko, V. G.; Balenkova, E. S. *Synthesis* **2002**, 901.

[90] Al-Schiekh, M. A.; El-Din, A. M. S.; Hafez, E. A.; Elnagdi, M. H. *J. Chem. Res.* **2004**, 174.

[91] Selby, T. P.; Andrea, T. A.; Denes, L. R.; Finkelstein, B. L.; Fuesler, T. P.; Smith, B. K. *ACS Symp. Ser.* **1992**, *504*, 91.

[92] Otero, I.; Feist, H.; Michalik, D.; Michalik, M.; Quincoces, J.; Peseke, K. *Z. Naturforsch.* **2005**, *60B*, 1175.

[93] Shiota, T.; Yamamori, T.; Sakai, K.; Kiyokawa, M.; Honma, T.; Ogawa, M.; Hayashi, K.; Ishizuka, N.; Matsumura, K.; Hara, M.; Fujimoto, M.; Kawabata, T.; Nakajima, S. *Chem. Pharm. Bull.* **1999**, *47*, 928.

[94] Su, J.; Ye, C.; Zheng, N. CP1, 537, 853, **2004**.

[95] Kolar, P.; Tisˇler, M. *J. Heterocycl. Chem.* **1993**, *30*, 1253.

[96] Bayomi, S. M.; Abdelal, A. M.; El Ashry, S. M.; Ghoneim, O. A. M. *Boll. Chim. Farm.* **1999**, *138*, 227.

[97] Rusinov, V. L.; Chupakhin, O. N.; *''Nitroazini'', Nauka, Sib. Otd. Novosibirsk* **1991**.

[98] Takano, M. EP947516, **1999**.

[99] Elnagdi, M. H.; Khalafalla, A. K.; Kandeel, Z. E.; Farag, A. M.; Negm, A. M.; Rassian, M. A. M. *Aswan Sci. Technol. Bull.* **1994**, *15*, 71.

[100] Lipson, V. V.; Desenko, S. M.; Orlov, V. D.; Shishkin, O. V.; Shirobokova, M. G.; Chernenko, V. N.; Zinov'eva, L. I. *Chem. Heterocycl. Compd.* **2000**, *36*, 1329.

[101] Al-Khamees, H. A.; Al-Deeb, O. A.; Bayomi, S. M. *Indian J. Heterocycl. Chem.* **1993**, *2*, 237.

[102] Shankar, R. B.; Pews, R. G. *J. Heterocycl. Chem.* **1993**, *30*, 169.

[103] Selby, T. P.; Andrea, T. A.; Denes, L. R.; Finkelstein, B. L.; Fuesler, T. P.; Smith, B. K. *ACS Symp. Ser.* **1992**, *504*, 91.

[104] Kato, F.; Kimura, H.; Omatsu, M.; Yamamoto, K.; Kazuhiro, M.; Miyamoto, R. WO040485. **2002**.

[105] Ram, V. J.; Srivastava, P. S.; Singh, K.; Kandpal, M.; Tekwani, B. L. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1087.

[106] Srivastava, R. P.; Kumar, V. V.; Bhatia, S.; Sharma, S. *Indian J. Chem., Sect. B* **1995**, *34*, 209.

[107] Abd El-Latif, F. M.; Khalil, M. A.; Helmy, I.; Solieman, H. A. *Heterocycl. Commun.* **2001**, *7*, 485.

[108] Al-Afaleq, E. I. *J. Saudi Chem.* **2002**, *6*, 59.

[109] Kandeel, Z. E. *J. Chem. Res.* **1995**, 290.

[110] Hammouda, M. H.; Etman, E.; Metwally, M. A. *J. Serb. Chem. Soc.* **1992**, *57*, 165.

[111] Shaban, M. A. E. *Adv. Heterocycl. Chem.* **1998**, *70*, 163.

[112] Mustazza, C.; del Giudice, M. R.; Borioni, A.; Gatta, F. *J. Heterocycl. Chem.* **2001**, *38*, 1119.

[113] Masuda, A.; Satoh, Y.; Akiyama, Y.; Saiga, K.; Toyoda, E. WO108729, **2004**.

[114] Al-Mousawi, S.; John, E.; Al-Kandery, N. *J. Heterocycl. Chem.* **2004**, *41*, 381.

[115] Dawood, K. M.; Farag, A. M.; Ragab, E. A. *J. Chin. Chem. Soc.* **2004**, *51*, 853.

[116] Kuznetsova, O. A.; Filyakova, V. I.; Pashkevich, K. I.; Ulomskii, E. N.; Plekhanov, P. V.; Rusinov, G. L.; Kodess, M. I.; Rusinov, V. L. *Russ. Chem. Bull.* **2003**, *52*, 1190.

[117] Shikhaliev, K. S.; Kryl'skii, D. V.; Yu. Potapov, A.; Yu. Krysin, M. *Russ. Chem. Bull.* **2005**, *54*, 2903.

[118] Lipunova, G. N.; Nosova, E. V.; Laeva, A. A.; Kodess, M. I.; Charushin, V. N. *Russ. J. Org. Chem.* **2005**, *41*, 1071.

[119] Reiter, J.; Pongo´, L.; Ko¨ vesdi, I.; Pallagi, I. *J. Heterocycl. Chem.* **1995**, *32*, 407.

[120] Hassan, A. A.; Mohamed, N. K.; Aly, A. A.; Mourad, A. E. *Pharmazie* **1997**, *52*, 23.

[121] Hammouda, M.; Metwally, M. A.; Abou-Zeid, Z. M.; Zimaity, T. *Indian J. Chem. Sect. B*, **1993**, *32*, 440.

[122] Hammouda, M.; Etman, H. E.; Metwally, M. A. *J. Serb. Chem. Soc.* **1992**, *57*, 165.

[123] Elotmani, B.; El-Mahi, M.; Essassi, E. M. C. R. *Chim*. **2002,** *5*, 517.

[124] Kepe, V.; Kocˇevar, M.; Polanc, S. *Heterocycles* **1993**, *35*, 955.

[125] Kandeel, Z. E.; Farag, A. M.; Negm, A. M.; Khalafalla, A. K.; Rassian, M. A. M.; Elnagdi, M. H. *J. Chem. Res.* **1994**, 416.

[126] Elnagdi, M. H.; Khalafalla, A. K.; Kandeel, Z. E.; Farag, A. M.; Negm, A. M.; Rassian, M. A. M. *Aswan Sci. Technol. Bull.* **1994**, *15*, 71.

[127] El Ashry, E. S. H.; El Kilany, Y.; Rashed, N.; Mousaad, A.; Assafir, H. *Z. Naturforsch.* **1998**, *53B*, 1203.

[128] Kishida, M.; Natsume, F.; Kawaguchi, S. *Jpn. Kokai* **2004**, *107*, 228.

[129] National Committee for Clinical and Laboratory Standards, Method for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically Approved Standard, fourth ed. NCCLS, Villanova, Italy, **1997**, Document M 100-S7. S100-S157.

[130] (a) Isenberg, D. H. Essential Procedure for Clinical Microbiology, *American Society for Microbiology*, Washington, **1998**; (b) Zgoda, J. R.; Porter, J. R. *Pharm. Biol.* **2001**, *39*, 221.

Summary

The work presented in the Thesis entitled “Synthesis of Heterocyclic Compounds of Therapeutic 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.

In Chapter 2, synthesis of forty novel 3-cyano-2-pyridone derivatives are reported, which draw a special attention for their wide spectrum biological activities along with their importance and utility as intermediates in preparing variety of heterocyclic compounds. The synthesis was achieved by the reaction of an aromatic aldehydes, 2-cyano-*N*-(substituted)acetamides and malononitrile by using methanol as a solvent and piperidine as a catalyst.

Chapter 3 describes the applications of multicomponent one-pot synthesis and brief review of the reported synthetic strategies for the synthesis of pyranopyrazole derivatives. Pyranopyrazoles have been the subject of intense research due to the interesting pharmacological activities found for several of their derivatives. Chapter 3 includes synthesis of thirty novel pyrano[2,3-*c*]pyrazoles, which has been synthesized by one-pot three-component cyclocondensation reaction of aromatic aldehydes, malononitrile and substituted pyrazolin-5-ones in the presence of piperidine as catalyst.

Chapter 4 describes applications of microwaves in heterocyclic ring formation. Recently, 1,2,4-triazolo[1,5-*a*]pyrimidines have aroused increasing from the standpoint of biological activity, due to their diverse pharmacological activities. It includes synthesis of forty novel 1,2,4-triazolo[1,5-*a*]pyrimidines and brief review of the reported synthetic strategies. Forty 1,2,4-triazolo[1,5-*a*]pyrimidines were synthesized by one-pot, microwave-assisted condensation reaction of aromatic aldehyde, corresponding acetophenone and 5-amino-1,2,4-triazole using glacial acetic acid as a solvent. Thus, a new green chemistry approach was developed 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*, 2010, 20(20), 6100-6102.
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 Dodiya and Viresh H. Shah. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2010, 25(6), 893-899.
3. 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. *Bioorganic & Medicinal Chemistry Letters*, Submitted, Under Review.
4. 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.
5. Synthesis and anti-tubercular evaluation of some novel pyrazolo[3,4-*d*]pyrimidine derivatives, by Amit R. Trivedi, Arif B. Siddiqui, **Bipin H. Dholariya** and Viresh H. Shah. *Medicinal Chemistry Research*, Submitted, Under Review.
6. Synthesis and biological evaluation of some novel 1,4-dihydropyridines as potential antitubercular agents, by Amit Trivedi, Dipti Dodiya, **Bipin Dholariya**, Vipul Kataria, Vimal Bhuva, Viresh shah. *Chemical Biology & Drug Design*, Submitted, Under Review.

Conferences/Seminars participated

1. "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).
2. "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).
3. "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).
4. "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).
5. 15^{th} ISCB International Conference on "Bridging gapes in Discovery and Development: Chemical and Biological Sciences for Affordable health, Wellness and Sustainability" jointly organized by Indian Society of Chemists and Biologists, Lucknow and Department of Chemistry, Saurashtra University, Rajkot (February 4-7, 2011).

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