"Synthesis and pharmacological Study of some New Chemical Entities"

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IN
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BY

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UNDER THE GUIDANCE OF

PROF. ANAMIK SHAH

DEPARTMENT OF CHEMISTRY

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Namaste

I bow down and honor the place in you, in which the entire universe dwells.
I bow down and honor the place in you, which is of love, of truth, of light and of peace.
When you are in that place inside you and I am in that place inside me, ***we are one.***

Dedicated With Lots of Love, Respect and Gratitude to…

Mummy, Pappa, Anshul and Vijeta.

-Nilay

Statement under *O. Ph. D. 7* of Saurashtra University

The work included in the thesis is done by me under the supervision of Prof. Anamik K. Shah and the contribution made thereof is my own work.

Date:

Place: **Nilay U. Pandya**

Certificate

This is to certify that the present work submitted for the Ph.D. Degree of Saurashtra University by Mr. Nilay U. Pandya has been the result of work carried out under my supervision and is a good contribution in the field of organic, heterocyclic and synthetic medicinal chemistry.

Date:

Place: Prof. Anamik K. Shah

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A MICROWAVE ASSISTED SYNTHESIS OF Thiazolidinones like 3-(2-Methylindoline-1-yl)-2-substituted phenylthiazolidin-4-ones using thioglycolic acid

GENERAL REMARKS

1. Melting points were recorded by open capillary method and are uncorrected.
2. Infrared spectra were recorded on Shimadzu FT IR-8400 (Diffuse reflectance attachment) using KBr. Spectra were calibrated against the polystyrene absorption at 1610 cm^{-1}.
3. ^{1}H & ^{13}C NMR spectra were recorded on Bruker Avance II 400 spectrometer. Making a solution of samples in DMSO d_6 and $CDCl_3$ solvents using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned, and are given in the δ scale. The standard abbreviations s, d, t, q, m, dd, dt, br s refer to singlet, doublet, triplet, quartet, multiplet, doublet of a doublet, doublet of a triplet, AB quartet and broad singlet respectively.
4. Mass spectra were recorded on Shimadzu GC MS-QP 2010 spectrometer operating at 70 eV using direct injection probe technique.
5. Analytical thin layer chromatography (TLC) was performed on Merck precoated silica gel-G F_{254} aluminium plates. Visualization of the spots on TLC plates was achieved either by exposure to iodine vapor or UV light.
6. The chemicals used for the synthesis of intermediates and end products were purchased from Spectrochem, Sisco Research Laboratories (SRL), Thomas Baker, Sd fine chemicals, Loba chemie and SU-Lab.
7. All the reactions were carried out in Samsung MW83Y Microwave Oven which was locally modified for carrying out chemical reactions
8. All evaporation of solvents was carried out under reduced pressure on Heidolph LABOROTA-400-efficient.
9. % Yield reported are isolated yields of material judged homogeneous by TLC and before recrystallization.
10. The structures and names of all compounds given in the experimental section and in physical data table were generated using ChemBio Draw Ultra 10.0.
11. Elemental analysis was carried out on Vario EL Carlo Erba 1108.

ABBREVIATIONS

AcOH	Acetic Acid
AMOS	Aqua Mediated Organic Synthesis
AIDS	Acquired Immuno Deficiency Syndrome
Ar	Aromatic
ARC	Aids Related Complex
Av.	Average
Bmim	1-Butyl-3-methylimidazolium hexafluorophosphate
BP	Boiling Point
CoMSIA	Comparative Molecular Similarity Index Analysis
CoMFA	Comparative Molecular Field Analysis
cAMP	Cyclic Adenosine Mono Phosphate
CuCN	Copper Cyanide
CMV	Cyto Megalo Virus
CNS	Central Nervous System
CC_{50}	Cytotoxic Concentration
$CCID_{50}$	Cell Culture Infective Dose
Conc.	Concentrated
CPE	Cyto Pathogenic Effect
CRH	Corticotropic Releasing Hormone
3D-QSAR	N,N-dicyclohexyl carbodiimide
DCC	N,N'-Dicyclohexylcarbodiimide
DHP	Dihydropyridine
DMAP Catalyst	4-Dimethylaminopyridine
DMF	Di Methyl Formamide
DMSO	Dimethyl Sulfoxide
D.M.	Demineralized Water
DNA	Deoxy Ribo Nucleic Acid
Equiv	Equivalent
Exp No	Experiment No
FSH	Follicle Stimulating Hormone
FT-IR	Fourier Transform Infrared

GC -MS	Gas Chromatography Mass Spectra
GNRH	Gonadotropin Releasing Harmone
HMBC	Heteronuclear Multiple Bond Coherence
HMQC	Heteronuclear Multiple Quantum Coherence
GFP	Green Fluorescent Protein
HIV	Human Immunodeficiency Virus
HBTU	2-(1H-benzotriazo-1-yl)-1,1,3,3-tetramethyl uranium hexafluorophospate
Hr.	Hour
HTLV 1	Human T-Lymphotropic Virus type 1
HSV	Herpes Simplex Virus
HTS	High Throughput Screening
Hz	Hertz
IC_{50}	Inhibitory Concentration
IR	Infra Red
IV	Intra Venus
KOH	Potassium Hydroxide
K_2CO_3	Potassium Carbonate
MAOS	Microwave-Assisted Organic Synthesis
Max. Prot.	Maximum Protection
MDR	Multi Drug Resistance
MF	Molecular Formula
MHz	Mega Hurtz
MIN	Minutes
m	Meta
mM	Mili moles.
MP	Melting Point
MS	Mass Spectra
MW	Microwave
MW	Mili Watt
MW	Molecular Weight
MWI	Micro Wave Irradiation
NOE	Nuclear Overhauser Effect

NaCN	Sodium Cyanide
$NaHCO_3$	Sodium Bicarbonate
NaOH	Sodium Hydroxide
NCEs	New Chemical Entities
nm	Nano Meter
NMP	N-Methyl Pyrrolidone
NMR	Nuclear Magnetic Resonance
o	Ortho
OVC	Organic Volatile Compound
OD	Optical Density
p	Para
$POCl_3$	Phosphorous Oxychloride
PhMe	Toluene
ppm	Parts Per Million
PR	Pulse Rate
Rf	Retention Factor
RH	Relative Humidity
RNA	Ribo Nucleic Acid
RT	Reverse Transcriptase
R.T.	Room Temperature
SAR	Structure Activity Relationship
SD	Standard Deviation
SI	Selectivity Index
TB	Tuberculosis Basilus
TBAB	Tetra-*n*-butylammonium bromide
TFA	Trifluoroacetic acid
THF	Tetrahydro Furan
TLC	Thin Layer Chromatography
TMS	Tetra Methyl Silane
TZD	Thiazolidinone
UV	Ultra Violet
VH Reagent	Vilsmeier–Haack Reagent
w/v	Weight/Volume

CHAPTER – 1

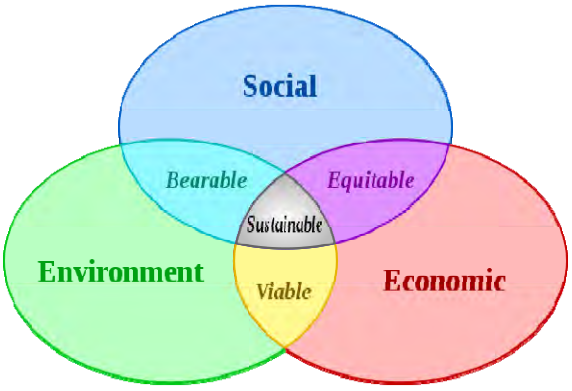
General Introduction

1.1 GREEN CHEMISTRY: *SCIENCE FOR "SUSTAINABILITY"*

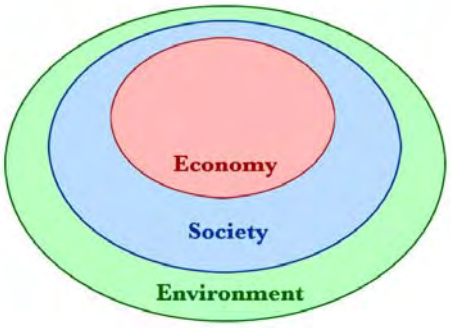
It is important to understand the word *"Sustainability"* before discussing the general concepts regarding *"GREEN CHEMISTRY."* Sustainability is just one word and yet there exists over 300 definitions [1].

The word sustainability is derived from the Latin *sustinere* (*tenere*, to hold; *sus*, up). Dictionaries provide more than ten meanings for *sustain* [2] [3], the main ones being to "maintain", "support", or "endure".[4][5] However, since the 1980s *sustainability* has been used more in the sense of human sustainability on planet Earth and this has resulted in the most widely quoted definition of sustainability and sustainable development, that of the Brundtland Commission of the United Nations: ***"sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs."***[6]

It is usually noted that this requires the reconciliation of environmental, social and economic demands - the "three pillars" of sustainability.[7] This view has been expressed as an illustration using three overlapping ellipses indicating that the three pillars of sustainability are not mutually exclusive and can be mutually reinforcing.[8]



Definitions of sustainability often refer to the "three pillars" of social, environmental and economic sustainability



Another representation of sustainability showing that how both economy and society are constrained by environmental limits.

The UN definition is not universally accepted and has undergone various interpretations.[9][10][11] What sustainability is, what its goals should be, and how these goals are to be achieved is all open to interpretation.[12] For many environmentalists the idea of sustainable development is an oxymoron as development seems to entail environmental degradation.[13][14]. A deeper understanding would make us appreciate that the economy is a subsystem of human society, which is itself a subsystem of the biosphere, and a gain in one sector is a loss from another[15], illustrated as three concentric circles above.

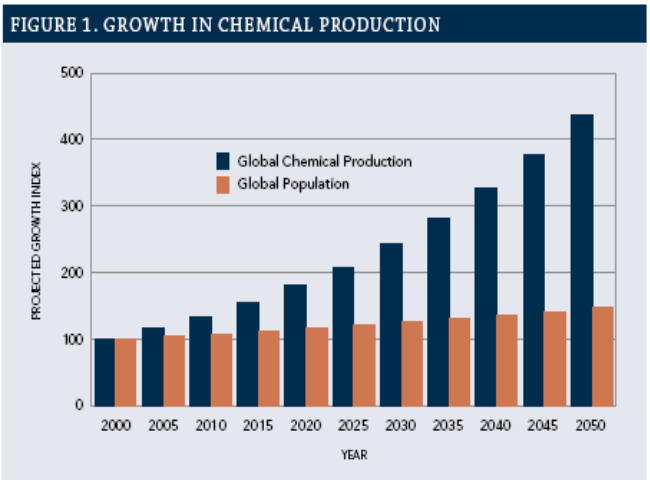
A universally-accepted definition of sustainability is elusive because it is expected to achieve many things. On the one hand it needs to be factual and scientific, a clear statement of a specific “destination”. The simple definition "sustainability is improving the quality of human life while living within the carrying capacity of supporting eco-systems",[16] though vague, conveys the idea of sustainability having quantifiable limits. But sustainability is also a call to action, a task in progress or “journey”[17] and therefore a political process, so some definitions set out common goals and values. The Earth Charter [18] speaks of “a sustainable global society founded on respect for nature, universal human rights, economic justice, and a culture of peace.”

Industries utilizing chemistry and chemical engineering have been major contributors to worldwide economic development over the past century, and yet the chemical industry is often taken to task for many serious environmental problems. The bad public image of modern chemistry has resulted in an alarming decrease in the number of talented high-school students who choose to pursue advanced studies and careers in the field. As evident from the statistics given below the use of chemicals is not going to be out of demand in the near future. Not only is environment in danger but as mentioned above the human species is also intricately intertwined with the environment and hence the pollution has had an adverse effect on the human beings as well. Hence, chemists, biochemists, and chemical engineers should therefore do all that we can to change the negative image of the chemical industry. Public awareness should be created regarding the chemical community’s positive and invaluable contributions to the continuous improvement of the quality of everyday life. Given below are 2 statistics revealing these bitter facts.

Table 1. Selected examples of toxic substances found in umbilical cord blood, breast milk and adult tissues.

Contaminants	Examples of sources	How people are exposed
Volatile Organic Compounds		
Naphthalene	Vehicle exhaust, deodorizers, paints, glues	Outdoor and indoor air, drinking water, workplaces
Perchloroethylene	Dry cleaning solvent, degreasing products	Treated clothing, proximity to dry cleaners, workplaces
Benzene	Gasoline, glues, detergents, vehicle exhaust	Outdoor air, workplaces
Agricultural Products		
Organophosphates	Pesticides, flea & tick pet products	Food, proximity to agriculture, field work, indoor air
Atrazine	Herbicide	Food, water, proximity to agriculture, field work
Persistent Organic Pollutants		
Polybrominated diphenyl ethers (PBDEs)	Flame retardants in furniture and electronics	Food, indoor air and dust
Dioxins & Furans	Byproduct of waste incineration, paper mills, Manufacturing	Food, outdoor air, drinking water
PFOA/PFOS	Non-stick and stain-resistant coatings	Consumer products, food, water, workplaces
Plastics Components		
Phthalates	Cosmetics, detergents, household cleaners, vinyl materials, lacquers	Cosmetics, detergents, household cleaners, vinyl materials, lacquers
Bisphenol A	Hard plastic containers, canned food linings	Food, water
Heavy Metals		
Cadmium	Batteries, fertilizer production, waste incineration, plastics, metal coatings	Food, air, water, workplaces
Lead	Paint, electronics, batteries, fossil fuels	Toys, food, soil, drinking water, workplaces

FIGURE 1. GROWTH IN CHEMICAL PRODUCTION



Growth in chemical production outpaces population growth. Global chemical production is expected to grow 3% per year, while global population will grow 0.77% per year. On this trajectory, chemical production will increase 330% by 2050, compared to a 47% increase in population, relative to year 2000. Source: Organization for Economic Cooperation and Development 2001; American Chemistry Council 2003; United Nations 2004

As the natural resources are used up in the world, chemists and biotechnologists are being asked to come up with innovative ways in which renewable resources can be used to replace nonrenewable ones. But there will continue to be a demand for some non-renewable resources. If we wish to make materials that use fewer resources today, we should try to minimize the amount of raw material that is incorporated in the object.

The science of chemistry is central to addressing the problems facing the environment. Through the utilization of various sub disciplines of chemistry and molecular sciences, there is an increasing appreciation that the emerging area of green chemistry is need in the design and attainment of sustainable development. A central driving force in this increasing awareness is that **Green Chemistry accomplishes** both **economic** and **environmental** goals, simultaneously through the **use of sound, fundamental scientific principles**.

The term "**Green Chemistry**", as adopted by the IUPAC Working Party on Synthetic Pathways and Processes in Green Chemistry, **is defined as**: ***"The invention, design, and application of chemical products and processes to reduce or to eliminate the use and generation of hazardous substances".***

The concept of "design" in the definition is an essential element in requiring the conscious and deliberative use of a set of criteria, principles, and methodologies in the practice of green chemistry. Because green chemistry is intentionally designed, it is definitionally impossible to do green chemistry by accident. The phrase the "use or generation" implies the requirement of life-cycle considerations. Green chemistry can be utilized anywhere in the life cycle, from feedstock origins to beyond end of useful life. The term "hazardous" is used in its broadest context including physical (e.g., explosion, flammability), toxicological (e.g., carcinogenic, mutagenic), and global (e.g., ozone depletion, climate change).

The term green chemistry[19],[20] describes an area of research arising from scientific discoveries about pollution and from public perception, in much the same way as the identification and understanding of a deadly disease stimulating the call for a cure. This term, which was coined at the Environmental Protection Agency (EPA) by Paul Anastas, represents the assumption that chemical processes that carry

environmental negatives can be replaced with less polluting or non-polluting alternatives. Green chemistry is the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products, associated with a particular synthesis or process. Thus chemists can greatly reduce risk to human health and the environment.

1.2 A BRIEF HISTORY

In the United States, the Pollution Prevention Act of 1990[21] established source reduction as the highest priority in solving environmental problems. Passage of this act signaled a move away from the "command and control" response to environmental issues and toward pollution prevention as a more effective strategy that focused on preventing waste from being formed in the first place. Shortly after the passage of the Pollution Prevention Act, it was recognized that a variety of disciplines needed to be involved in source reduction. This recognition extended to chemists, the designers of molecular structures and transformations. In 1991, the Office of Pollution Prevention and Toxics in the U.S. Environmental Protection Agency launched the first research initiative of the Green Chemistry Program, the Alternative Synthetic Pathways research solicitation. [22] Foundational work in chemistry and engineering at the National Science Foundation's program on Environmentally Benign Syntheses and Processes was launched in 1992, and formed a partnership with EPA through a Memorandum of Understanding that same year. In 1993, the EPA program officially adopted the name "U.S. Green Chemistry Program". Since its inception, the U.S. Green Chemistry Program has served as a focal point for major activities within the United States, such as the Presidential Green Chemistry Challenge Awards and the annual Green Chemistry and Engineering Conference.

In the first half of the 1990s, both Italy [23] and the United Kingdom [24] launched major initiatives in green chemistry. Several researchers in the U.K. established research and education programs in green chemistry. In Italy, a multi university consortium (INCA) featured research on green chemistry as one of its central themes. During the last half of the decade, Japan organized the Green and Sustainable Chemistry Network (GSCN), [25] with an emphasis on promoting research and development on green and sustainable chemistry. The first books, papers, and

symposia on the subject of green chemistry were introduced in the 1990s. The inaugural edition of the journal *Green Chemistry*, sponsored by the Royal Society of Chemistry, appeared in 1999. [26] Research groups in many countries quickly coalesced, and adoption by industry was evident but difficult to quantify. In 1995, the U.S. Presidential Green Chemistry Challenge Award was announced as a way of recognizing accomplishments by industry, academia, and government in green chemistry. The five awards first given in 1996, along with the numerous nominations for the award, provided a first, if understated, measure of adoption of green chemistry. Japan, Italy, the U.K., Australia, and other nations have adopted green chemistry awards for the purpose of highlighting the environmental and economic accomplishments of green chemistry.

Green chemistry, an approach to the synthesis, processing and use of chemicals that reduce risks to humans and the environment, covers the following areas:

- Application of innovative technology to established industrial processes.
- Development of environmentally improved routes to important products.
- Design of new green chemicals and materials.
- Use of sustainable resources.
- Use of biotechnology alternatives.
- Methodologies and tools for evaluating environmental impact.

Green chemistry involves the design and redesign of chemical syntheses and chemical products to prevent pollution and thereby solve environmental problems. The 12 principles postulated for Green Chemistry are as follows:

1. Prevention

It is better to prevent waste than to treat or clean up waste after it has been created.

2. Atom Economy

Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.

3. Less Hazardous Chemical Syntheses

Wherever practicable, synthetic methods should be designed to use and

generate substances that possess little or no toxicity to human health and the environment.

4. Designing Safer Chemicals

Chemical products should be designed to effect their desired function while minimizing their toxicity.

5. Safer Solvents and Auxiliaries

The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.

6. Design for Energy Efficiency

Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.

7. Use of Renewable Feedstocks

A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.

8. Reduce Derivatives

Unnecessary derivatization (use of blocking groups, protection/ deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.

9. Catalysis

Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

10. Design for Degradation

Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

11. Real-time analysis for Pollution Prevention

Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.

12. Inherently Safer Chemistry for Accident Prevention

Substances and the form of a substance used in a chemical process should be

chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

Over the course of the past decade, green chemistry has demonstrated how fundamental scientific methodologies can protect human health and the environment in an economically beneficial manner. Significant progress is being made in several key research areas, such as catalysis, the design of safer chemicals and environmentally benign solvents, and the development of renewable feedstocks. Current and future chemists are being trained to design products and processes with an increased awareness for environmental impact. Outreach activities within the green chemistry community highlight the potential for chemistry to solve many of the global environmental challenges we now face. The origins and basis of green chemistry chart a course for achieving environmental and economic prosperity inherent in a sustainable world.

1.3 SOME ASPECTS OF ORGANIC SYNTHESIS - A GREEN CHEMISTRY PERSPECTIVE

Since its commencement in the last decade there were quite a few new technologies, new routes and new approaches that were developed in synthetic organic chemistry viz. Microwave Assisted Organic Synthesis, Aqua Mediated Organic Synthesis, Synthesis using different solvents like Ionic Liquids as well as some super critical solvents etc. which brought about a kind of revolution and also a shift in the mind set of common people as well as a shift of focus among the scientific fraternity.

As expected, green chemistry has grown into a significant internationally engaged focus area within chemistry. The importance of green chemistry was highlighted aptly in a cover story in *Chemical and Engineering News.* [27] Major Research, education, and outreach initiatives have been established around the globe. Major research programmes in all the major continents started focusing efforts around Principles of Green chemistry. The breadth of this research was very wide and incorporated areas such as polymers, solvents, catalysis, biobased / renewables, analytical method development, synthetic methodology development, and the design

of safer chemicals. Excellent research started being conducted within each of these areas that strived to incorporate one or more of the 12 Principles of Green Chemistry.

1.3.1 Polymers

The nature of the hazards that can be posed by polymers in their manufacture, use, and disposal has been widely recognized in recent years, as have the green chemistry methodologies that can be used to address these hazards.[28] Research on renewable feedstocks and biobased transformations, structural design, and design for degradability are all promising areas.[29] Carbon dioxide, for example, is a renewable feedstock that has been recovered from flue gas and, in its supercritical state, combined with pastes from fly ash to yield products such as roofing tiles and wallboard.[30-33] Polymers derived from carbohydrate feedstocks such as soy[34] and corn[35] are found in consumer products like automobiles and food packaging. Microbial fermentation has been used to convert glucose to a biodegradable polymer.[36]

1.3.2 Solvents

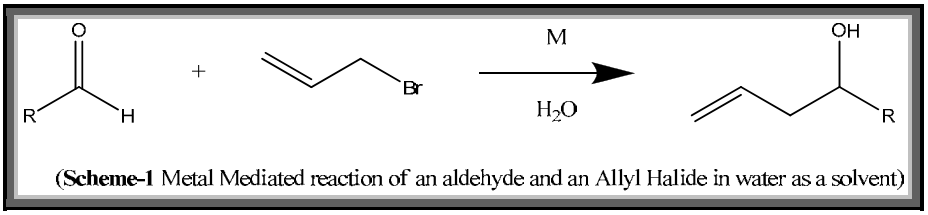
The design of environmentally benign solvents and solventless systems has been one of the most active areas of green chemistry over the past 10 years. Solvents are highly regulated and used in large quantities. Organic solvents pose a particular concern to the chemical industry because of the sheer volume used in synthesis, processing, and separations. Many are classified as volatile organic compounds (VOCs) or hazardous air pollutants (HAPs) and are flammable, toxic, or carcinogenic.

Breakthroughs in the use of supercritical fluids such as carbon dioxide have met with success in the research laboratory as well as commercially. Supercritical fluids offer a number of benefits, such as the potential to combine reaction and separation processes and the ability to tune the solvent through variations in temperature and pressure. In the supercritical fluids area, CO_2 has received the most attention [37-43] because its critical temperature and pressure (*T*c) 31.1 °C, *P*c) 74 bar) are more accessible than those of other solvents (water, for example, has *T*c) 374 °C and *P*c) 221 bar). CO_2 offers numerous advantages as a benign solvent: it is nontoxic, nonflammable, and inexpensive, and can be separated from the product by simple

depressurization. Applications of supercritical CO_2 are found in the dry cleaning industry, where CO_2 replaces perchloroethylene as a solvent;[44-45] in semiconductor manufacturing, where the low surface tension of supercritical CO_2 avoids the damage caused by water in conventional processing;[46] and in chemical processing.[47]

The use of supercritical CO_2 as a reaction medium in organic synthesis provides an excellent example of the evolution from fundamental academic research into a commercial process. In collaboration with Thomas Swan & Co. Ltd,[48] researchers at the University of Nottingham[49] developed synthetic methodologies in supercritical CO_2 that are being employed in a new supercritical fluid plant in the U.K., with a capacity up to 1000 tons per year. Conventional solvents are replaced with supercritical fluids in such key technologies as hydrogenation, Friedel- Crafts alkylations and acylations, hydroformylations, and etherification.

The use of water as a solvent in ways previously not realized has been an active area of research in green chemistry (Scheme 1).[50,51] A number of classic organic reactions, traditionally run in organic solvents, can be carried out in water with the proper design of catalysts and reaction conditions. Even variants of the Grignard reaction, notoriously sensitive to water, can be run in an aqueous solvent using a variety of metals, such as indium [52] and zinc. [53] The use of an obviously benign and inexpensive solvent like water could yield significant green chemistry benefits if challenges of energy and separations can be met.



(**Scheme-1** Metal Mediated reaction of an aldehyde and an Allyl Halide in water as a solvent)

Ionic liquids, a relatively new area of solvent investigation, are attractive because of their negligible vapor pressure and their use in polar systems to generate new chemistries [54-57]. A plethora of ionic liquids can be produced by varying the cations and anions, permitting the synthesis of ionic liquids tailored for specific applications. While questions of intrinsic hazard must still be answered for this class

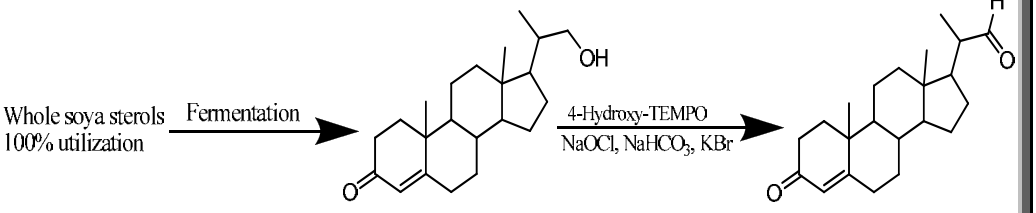
of solvents, the potential for the design of next generation ionic liquids holds significant promise for improved environmental benefits.

Fluorous solvent systems have demonstrated particular advantages in synthetic systems.[58-60] Fluorous systems are particularly appealing in fluorous biphasic catalysis in which the homogeneous catalyst and the product reside in separate phases, thereby eliminating the need for energy-intensive separations. In addition to efficiency, fluorous biphasic systems may reduce accident potential by eliminating the possibility of runaway exothermic reactions.

1.3.3 Catalysis

The area of catalysis is sometimes referred to as a “foundational pillar” of green chemistry. [61] Catalytic reactions [62-68] often reduce energy requirements and decrease separations due to increased selectivity; they may permit the use of renewable feedstocks or minimize the quantities of reagents needed. There is little doubt that the 2001 Nobel Prize-winning work of Sharpless, Noyori, and Knowles met many green chemistry goals. [69] Their research on catalytic asymmetric synthesis has been crucial in producing single enantiomer compounds, particularly for the pharmaceutical industry.

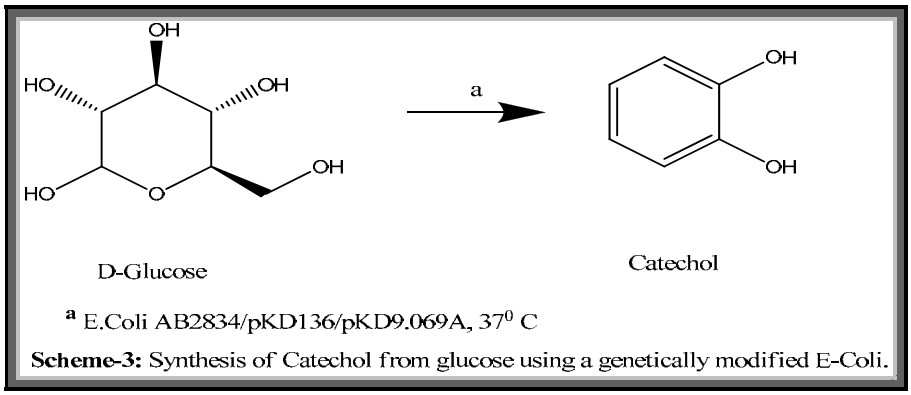
Catalysis often permits the use of less toxic reagents, as in the case of oxidations using hydrogen peroxide in place of traditional heavy metal catalysts. [70] Renewable resources, such as soya sterols [71] (Scheme 2) and glucose, [72] serve as feedstocks when catalytic methods are employed. Recently, water has been split into oxygen and hydrogen using a photocatalyst that absorbs light in the visible range. [73] While still at the research stage, this technology has the potential to provide an efficient source of hydrogen for use in fuel cells. Hydrogen fuel cells in cars would greatly reduce air pollution, as the oxidation product (water) is environmentally benign. [74] The application of catalysis to dematerialization, reduced toxicity systems, benign and renewable energy systems, and efficiency makes it a central focus area for green chemistry research.



Scheme 2. Synthesis of Bisnoraldehyde from Renewable Feedstock

1.3.4 Biobased / Renewables

The utilization of benign, renewable feedstocks is a needed component of addressing the global depletion of resources. More than 98% of all organic chemicals are derived from petroleum. [75] Achieving a sustainable chemical industry dictates switching from depleting finite sources to renewable feedstocks. Research in this area has focused on both the macro and molecular levels. The carbohydrate economy provides a rich source of feedstocks for synthesizing commodity [76] and specialty chemicals. For example, agricultural wastes have been converted into useful chemical intermediates such as levulinic acid,[77] alcohols, ketones, and carboxylic acids.[78] Shells from crabs and other sea life serve as a valuable and plentiful source of chitin, which can be processed into chitosan, a biopolymer with a wide range of potential applications that are being currently explored for use in the oil-drilling industry.[79] At the molecular level, genetic engineering produces valuable chemical products via nontraditional pathways. Glucose yields catechol and adipic acid [80] (Scheme 3) using genetically engineered *Escherichia coli.* Recombinant *Saccharomyces* yeasts convert both glucose and xylose, present in cellulosic biomass, into ethanol.[81] Carbon dioxide is also a renewable feedstock that has been incorporated into polymers.[82]

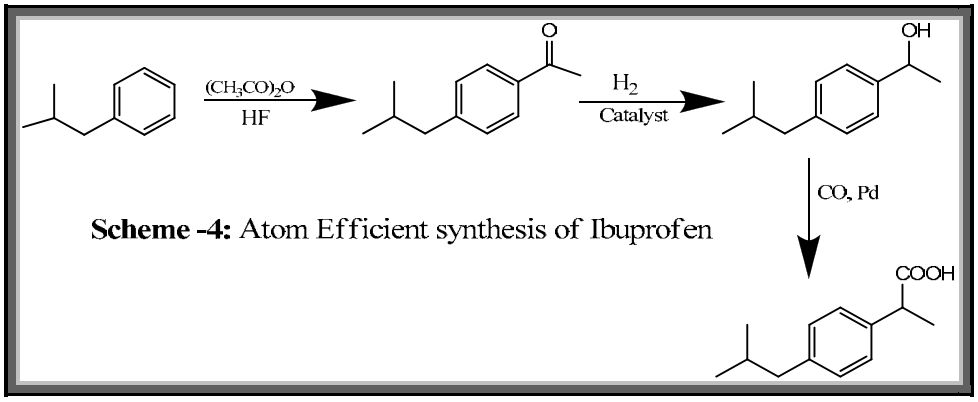


[a] E.Coli AB2834/pKD136/pKD9.069A, 37[0] C

Scheme-3: Synthesis of Catechol from glucose using a genetically modified E-Coli.

1.3.5 Synthetic Methodologies

Synthetic methodologies are being designed in both academia and industry that are more environmentally benign and more atom efficient.[83-85] New synthetic protocols have eliminated waste streams, improved worker safety, and increased yield in pharmaceutical processes (Scheme 4).[86-88] Polymer synthesis has been redesigned to eliminate the use of highly toxic reagents and organic solvents.[89] The utilization of biomimetic approaches,[90,91] cascading reactions,[92-93] and molecular self-assembly[94,95] represents some of the new chemistries being developed with green chemistry goals incorporated at the design stage.



Scheme -4: Atom Efficient synthesis of Ibuprofen

1.3.6 Analytical Methods

Analytical chemistry played a central role in the environmental movement by detecting, measuring, and monitoring environmental contaminants. As we move toward prevention and avoidance technology, analytical methods are being incorporated directly into processes in real time in an effort to minimize or eliminate

the generation of waste before it is formed.[96,97] Continuous process monitoring assists in optimizing the use of feedstocks and reagents while minimizing the formation of hazardous substances and unwanted byproducts. In addition, analytical methodologies have, themselves, historically used and generated hazardous substances and are being redesigned with green chemistry goals in mind by using benign mobile and stationary phases and placing greater emphasis on in situ analysis.

1.3.7 Design for safer chemicals

Design for reduced hazard is a green chemistry principle that is being achieved in classes of chemicals ranging from pesticides to surfactants, from polymers to dyes [98-100]. The principles of mechanistic toxicology allow for molecular design for reduced toxicity. Pesticides have been designed that are more selective and less persistent [101] than many traditional organic pesticides. Surfactants [102] and polymers, [103] have been developed to degrade in the environment at the end of their useful lifetime. Dyes without heavy metals [104] are finding applications in the textile industry. Understanding the physicochemical properties that underlie even global hazards allows for manipulation to reduce those hazards. The systematic development and application of design rules for reduced hazard is one of the most important challenges facing green chemistry.

In the upcoming chapters we will throw some light on Microwave Assisted Organic Synthesis (MAOS) as well as Aqua Mediated Organic Synthesis (AMOS) which are now an integral part of green chemistry cause they have numerous advantages over classical organic synthesis. The following headings would also serve as the introductions for the upcoming chapters regarding the synthesis of some new chemical entities in this thesis, so as to build up a solid foundation. More over the biological significance of some of the class of chemicals which have been synthesized would also be discussed ahead.

1.4 MICROWAVE ASSISTED ORGANIC SYNTHESIS (MAOS): A BRIEF REVIEW

From the kitchen to the laboratory, 'microwave chemistry' has come up as a boon in disguise for the eco friendly conscious chemists. As an integral part of Green Chemistry, the field of Microwave assisted organic synthesis (MAOS) has seen tremendous development in the recent years. The microwave mediated organic reactions [105,106] take place more rapidly, safely, and in an environmentally friendly manner, with high yields. Very little solvent and even the use of water as a solvent is a big advantage of microwave chemistry. In many cases, microwave-mediated reactions are carried out in dry media on solid support, i.e. without the use of solvent. Therefore the use of toxic and expensive organic solvents can be avoided. Such reactions not only reduce the amount of waste solvent generated, but also the products often need very little or no purification. These processes will hopefully be adapted by big industries as well, thereby contributing to the betterment of the environment.

Within two decades is should be possible to:

- Eliminate nearly 100% of emissions in polymer manufacturing and processing.
- Replace all solvents and acid-based catalysts that have adverse environmental effects with solids, or 'greener alternatives'.
- Achieve 30–40% reduction in waste.
- Reduce more than 50% quantity of plastics in landfills.

Heterogeneous organic reactions have proven useful to chemists in the laboratory as well as in the industrial context. These reactions are effected by the reagents immobilized on the porous solid supports and have advantages over the conventional solution phase reactions because of the good dispersion of active reagent sites, associated selectivity and easier work-up. The recyclability of some of these solid supports renders these processes into truly eco-friendly green protocols. Although the first description of surface-mediated chemistry dates back to 1924, [107] it was not until the late 1970s that the technique received genuine attention with the appearance of two reviews, [108] followed by a series of books and account articles [109].

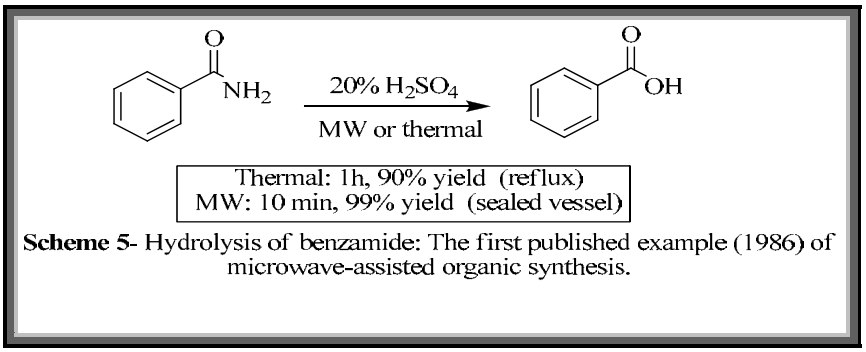
A related development that had a profound impact on these heterogeneous reactions was the use of microwave (MW) irradiation techniques for the acceleration of organic reactions. Since the appearance of the first article on the application of microwaves for chemical synthesis in polar solvents, [110] the approach has blossomed into a useful technique for a variety of applications in organic synthesis and functional group transformations. The focus has lately shifted to less cumbersome solvent-free methods wherein the neat reactants, often in the presence of mineral oxides or supported catalysts, undergo facile reactions to provide high yields of pure products thus eliminating or minimizing the use of organic solvents.

Microwave reactions involve selective absorption of MW energy by polar molecules, non-polar molecules being inert to MW dielectric loss. The initial experiments with microwave techniques centered on the use of high dielectric solvents such as dimethyl sulfoxide (DMSO) and dimethylformamide (DMF). The rate enhancements in such reactions are now believed to be due to rapid superheating of the polar solvents. However, in these solution-phase reactions, the development of high pressures and the use of specialized Teflon vessels and sealed containers are some of the limitations. During recent years, a practical dimension to the microwave heating protocols has been added by accomplishing reactions on solid supports under solvent-free conditions. In these reactions, the organic compounds adsorbed on the surface of inorganic oxides, such as alumina, silica and clay, or ‘doped’ supports absorb microwaves whereas the solid support does not absorb or restrict their transmission. The bulk temperature is relatively low in such solvent free reactions although higher localized temperatures may be reached during microwave irradiation. These solvent-free MW assisted reactions provide an opportunity to work with open vessels thus avoiding the risk of high pressure development and increasing the potential of such reactions to upscale.

1.4.1 A brief history of Microwave assisted organic synthesis

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. [111]



Scheme 5- 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 [118]. 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 [119], 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. 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 [120] – 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 [121]. 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 [122], 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

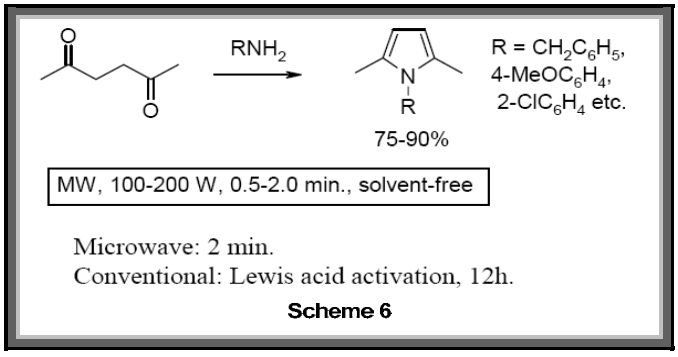
associated with the flammability of organic solvents in a microwave field and the lack of available dedicated microwave reactors allowing for adequate temperature and pressure control were major concerns. Important instrument innovations now allow for careful control of time, temperature and pressure 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. 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.

1.4.2 Applications of microwaves in heterocyclic ring formation

1.4.2.1 Five-membered heterocyclic rings

A. Pyrroles

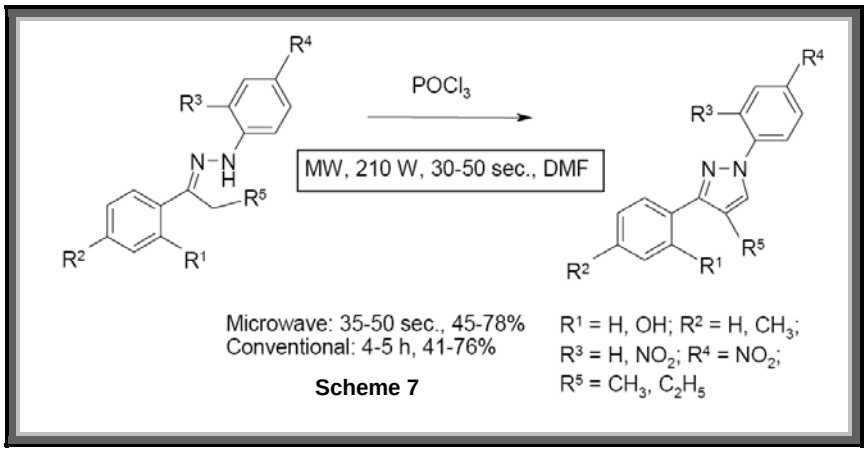
The classical Paal-Knorr cyclization of 1,4-diketones to give pyrroles is dramatically speeded- up under microwave irradiation and high yields are obtained as shown in scheme 6 [123].



Scheme 6

B. Pyrazoles

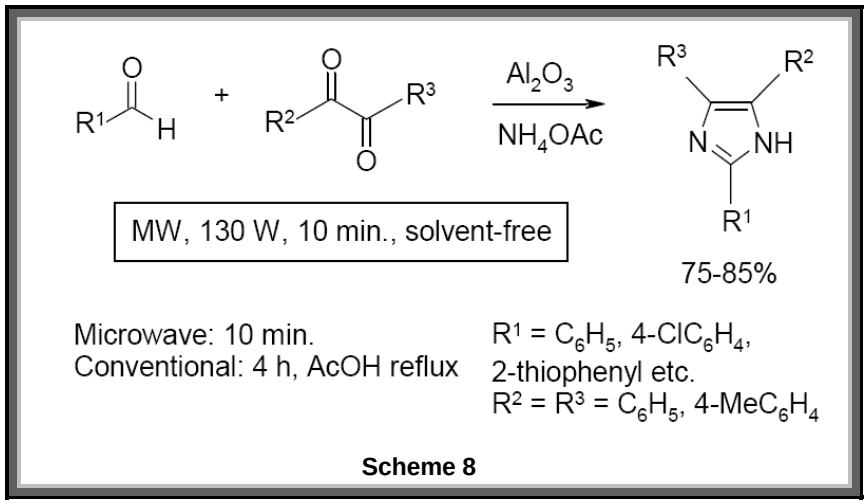
Another recent application of microwaves in cyclization is the preparation of pyrazoles from hydrazones using the Vilsmeier cyclization method by treatment with $POCl_3$ and DMF [124]. As shown in Scheme 7, once again the reaction is speeded-up by factors of several 100-fold.



Scheme 7

C. Imidazoles

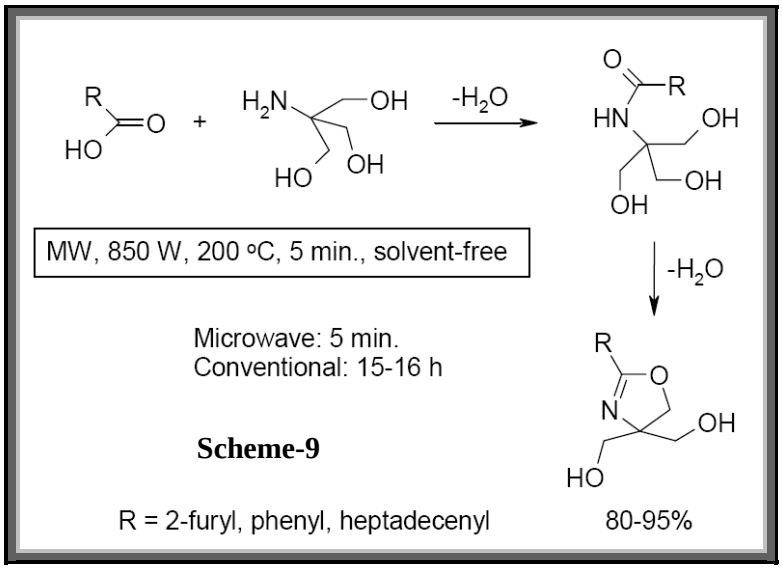
An important classical preparation of imidazoles is from an α-diketone, an aldehyde and ammonia. Here again, excellent yields can be obtained in reaction times of a few minutes as shown in Scheme 8 [125].



Scheme 8

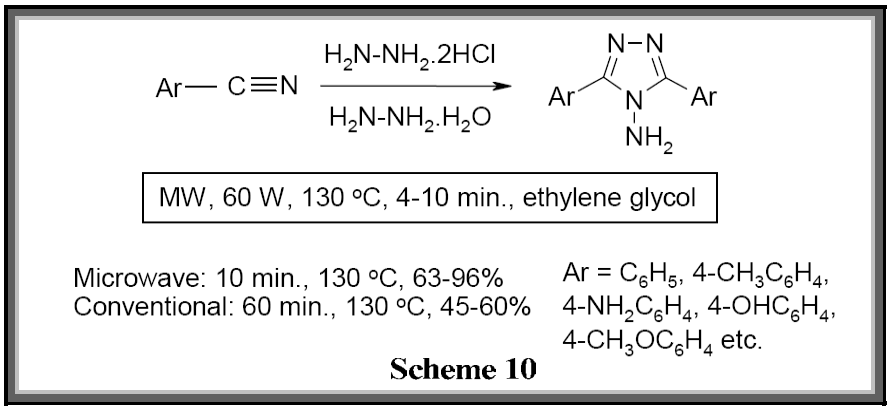
D. Oxazolines

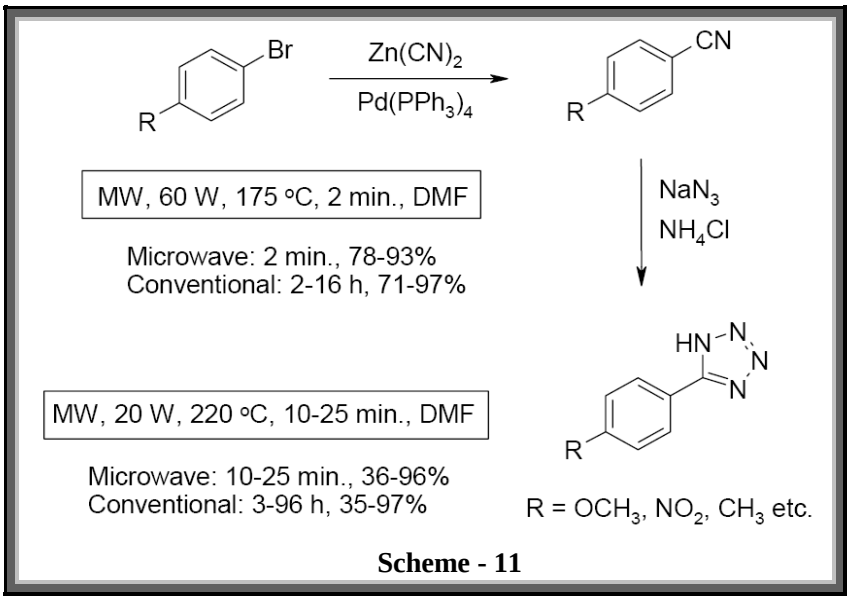
The example of Scheme 9, the preparation of oxazolines shows that partially saturated five-membered rings can also be prepared advantageously using microwaves [126].



E. Triazoles and Tetrazoles

Schemes 10 and 11 continue the overview of five-membered rings with illustrations of the advantageous preparation of 1,2,4–triazoles (Scheme 10) [127] and tetrazoles (Scheme 11) [128] using microwaves. Notice that in Scheme 6 the starting aryl cyanides are also made by a Pd-catalyzed but microwave-enhanced replacement of aryl bromides using zinc cyanide.

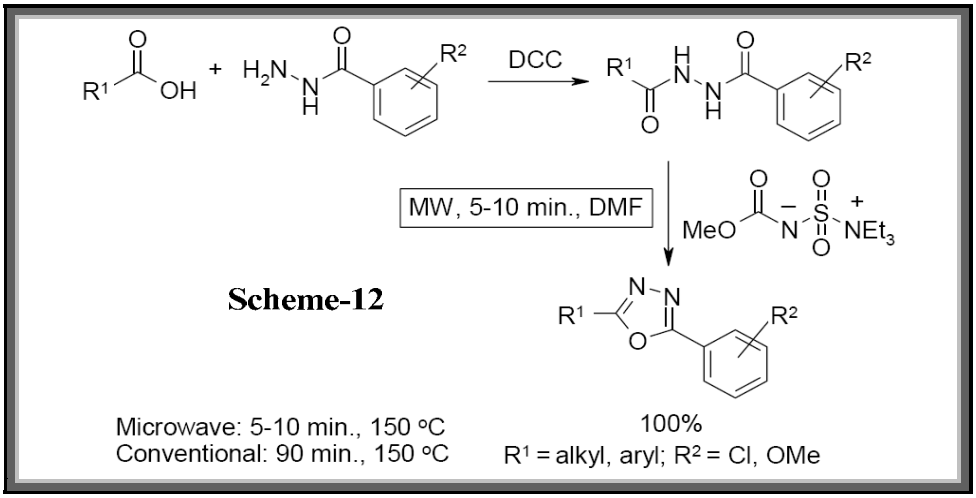




Scheme - 11

F. Oxadiazoles

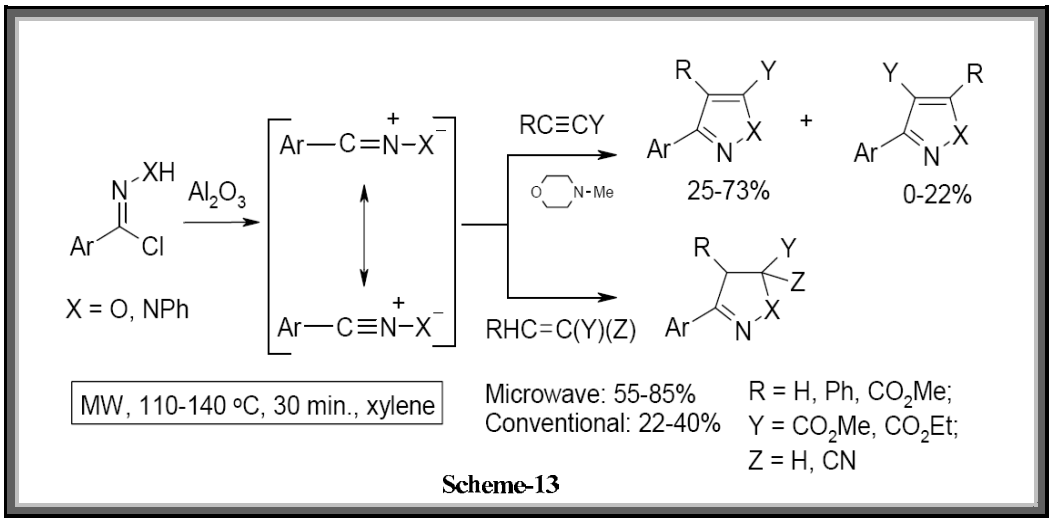
The dehydration of unsymmetrical diacylhydrazines (themselves prepared by a conventional Mitsunobu reaction) using Burgess's reagent is shown in Scheme 12 to give 1,3,4-oxadiazoles rapidly under microwave irradiation [129].



Scheme-12

G. 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 13; the resulting compounds are obtained in far high yield than under conventional conditions [130].

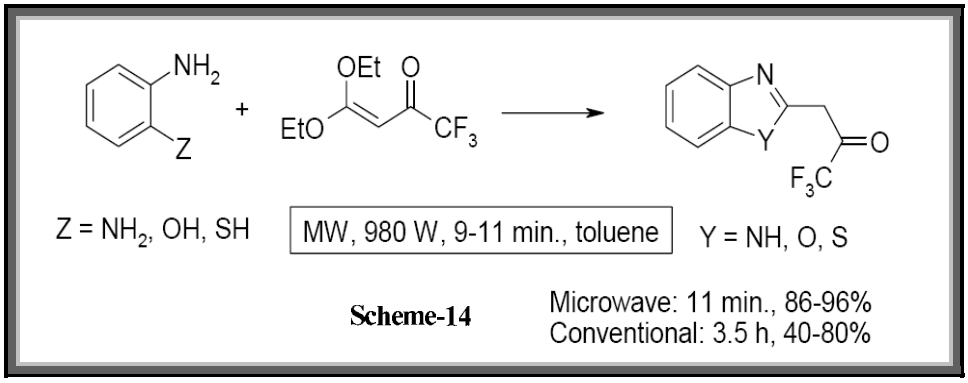


Scheme-13

1.4.2.2 Benzo-derivatives of five-membered rings

A. Benz-imidazoles, -oxazoles, and –thiazoles

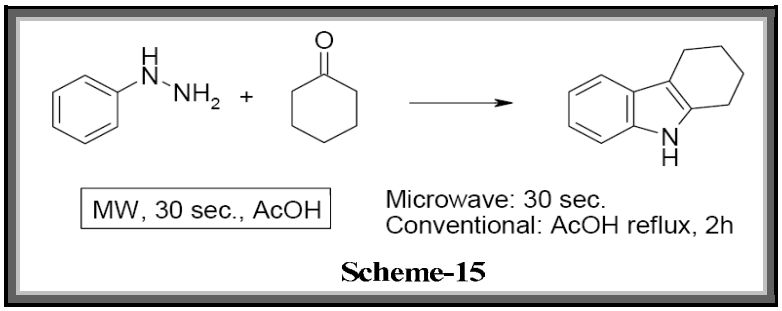
Ring closure reactions of appropriate *o*-substituted anilines to give benzimidazoles, benzoxazoles, and benzthiazoles takes place much faster and in significantly high yield under microwave conditions than conventionally [131] as shown in Scheme 14.



Scheme-14

B. Indoles

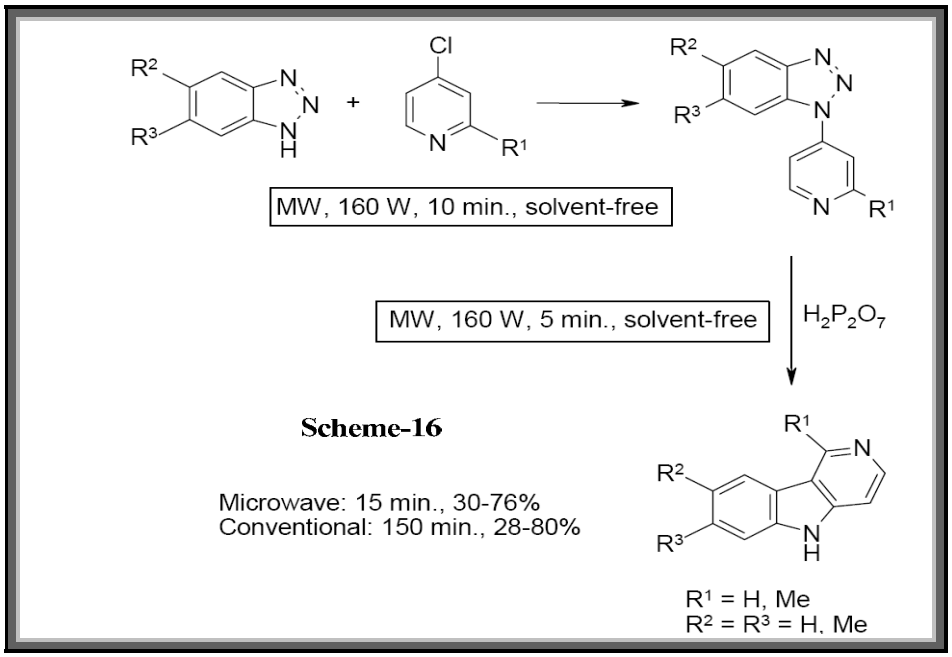
The classical Fischer-indole synthesis from an aryl hydrazine and a ketone is speeded-up by several 100-fold as documented in Scheme 15 [132].



Scheme-15

C. 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 16 where the 1-(4-pyridyl)benzotriazole is converted into a γ-carboline [133].



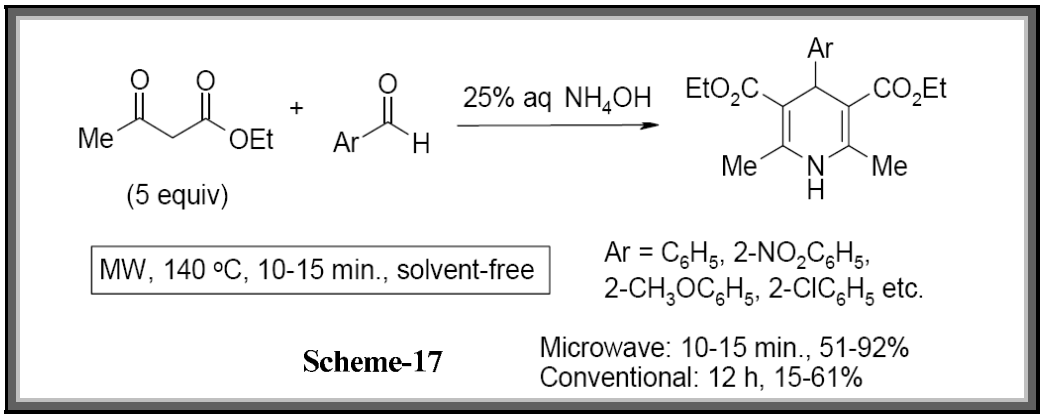
Scheme-16

1.4.2.3 Six-membered rings

A. 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

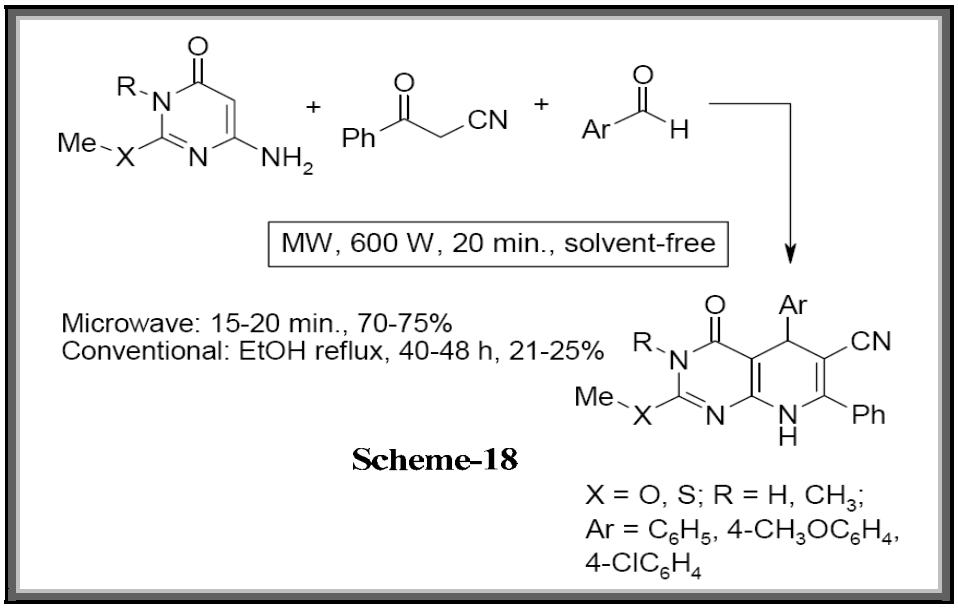
the heating times and also significantly increase the yields as shown in Scheme 17 [134].



Scheme-17

B. Dihydropyridopyrimidinones

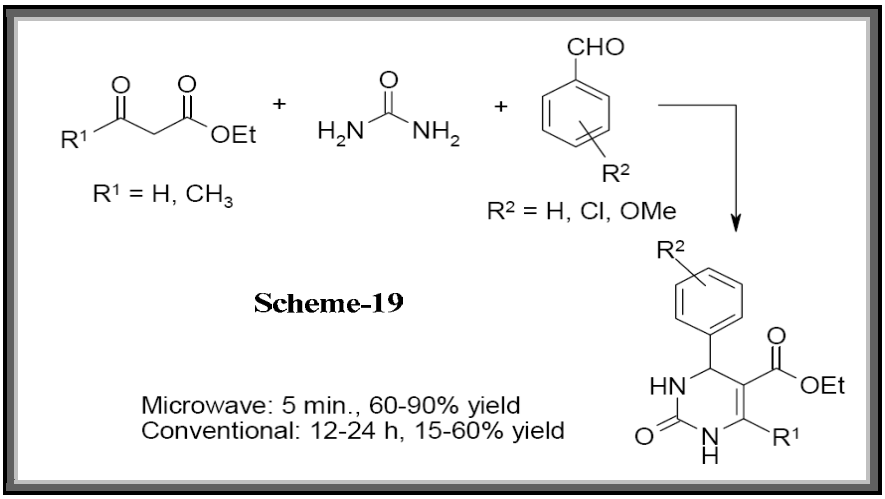
Dihydropyridopyrimidinones have been produced by ring annulations of aminopyrimidinones. Once again the reaction time is dramatically reduced and yields are much better with the solvent-free microwave conditions. (Scheme-18) [135].



Scheme-18

C. Dihydropyrimidines

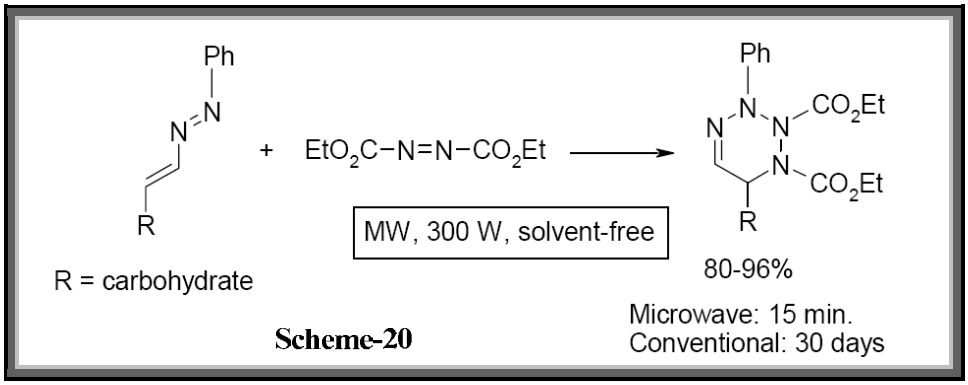
The Biginelli reaction is important for the preparation of dihydropyrimidine derivatives and excellent results are found for reactions carried out with microwave enhancement (Scheme 19) [136].



Scheme-19

D. 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 20 [137].

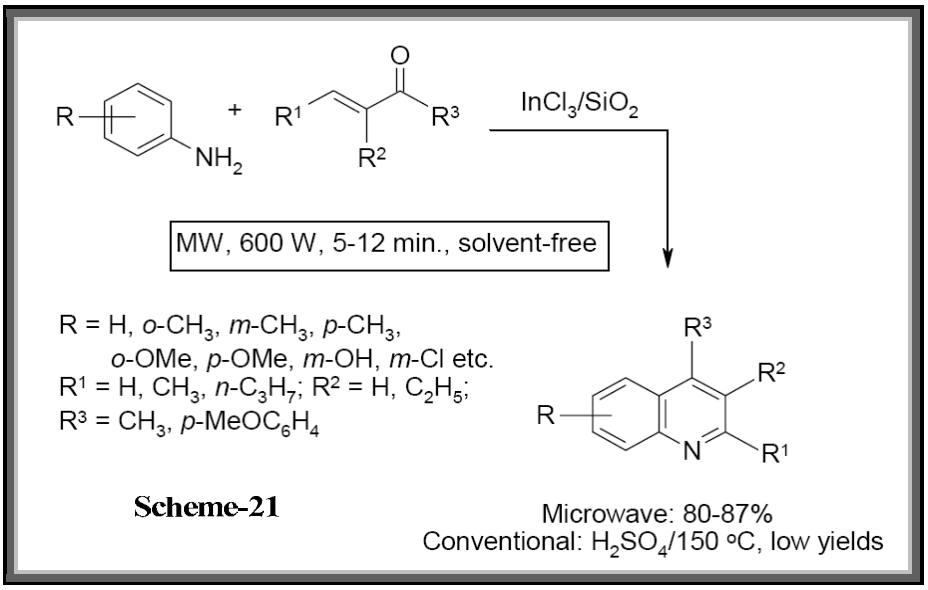


Scheme-20

1.4.2.4 Polycyclic six-membered rings

A. Quinolines

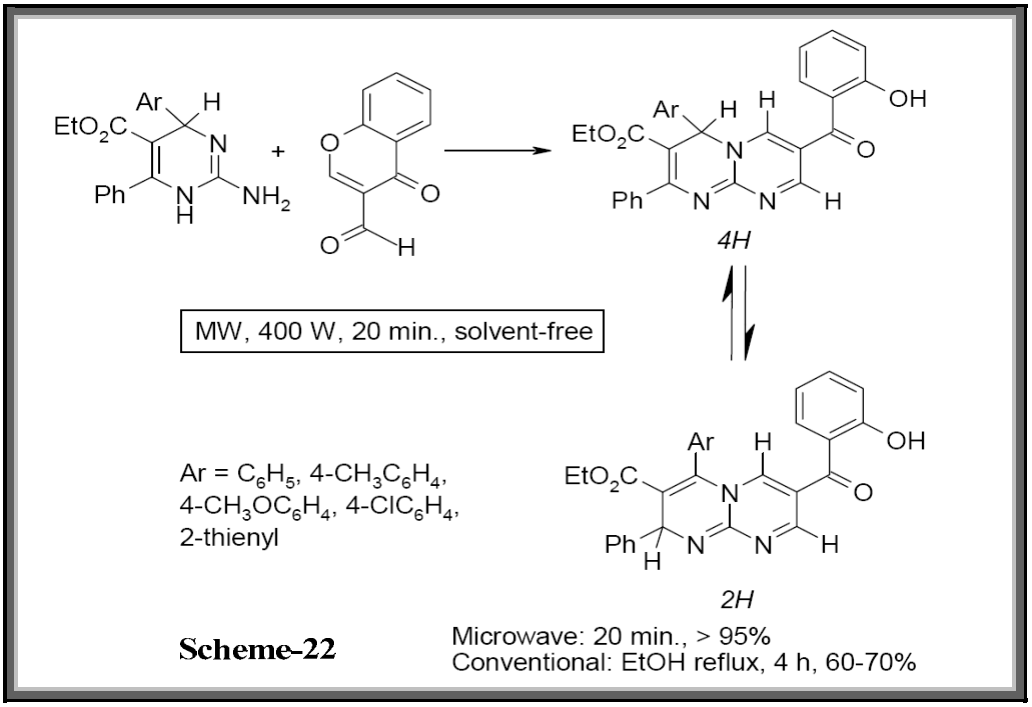
The Skraup synthesis has a bad reputation as it involves very messy conditions and gives only low yields of quinolines when carried out conventionally. Recently, it has been reported that microwave enhancement reduces the reaction time to a few minutes and allows high yields to be isolated (Scheme 21) [138].



Scheme-21

B. Pyrimido [1,2-a]pyrimidines

Pyrimido [1,2-*a*]pyrimidines are prepared from dihydroaminopyrimidines and chromone-3- aldehydes as is shown in Scheme 22 [139]. Although the conventional reaction must proceed in refluxing ethanol, reactions are much faster and better yields have been obtained with microwaves.

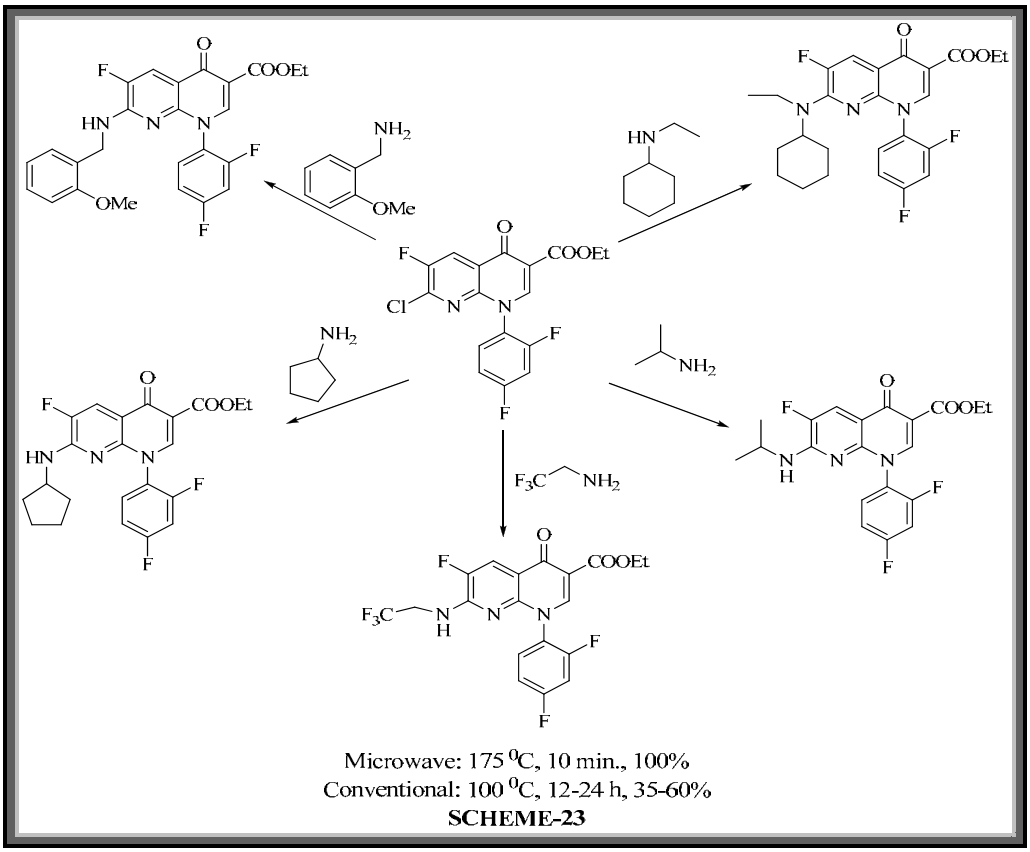


Scheme-22

1.4.2.5. Nucleophilic Substitution Reactions

A. Heterocyclic C-alkylations

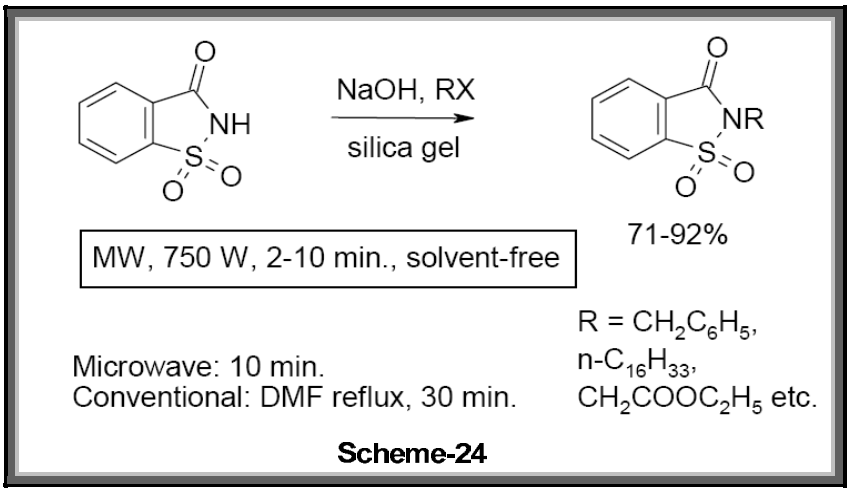
Nucleophilic substitution reactions can be speeded-up very considerably as is illustrated in Scheme 23 for a chloro-naphthyridine derivative [140].



SCHEME-23

B. Heterocyclic N-alkylations

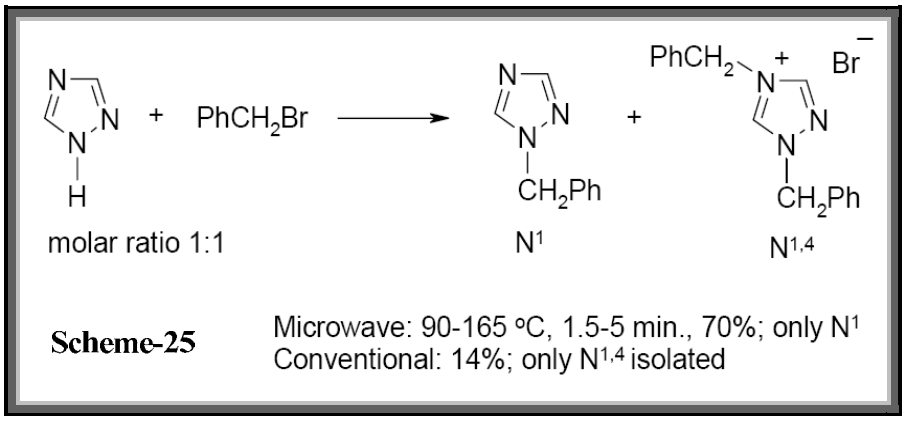
Another class of nucleophilic substitution is involved in heterocyclic *N*-alkylation which we have illustrated in Scheme 24. This shows that nucleophilic substitution on the nitrogen atom of saccharin is significantly speeded-up by microwave irradiation.



Scheme-24

C. Selective-alkylation

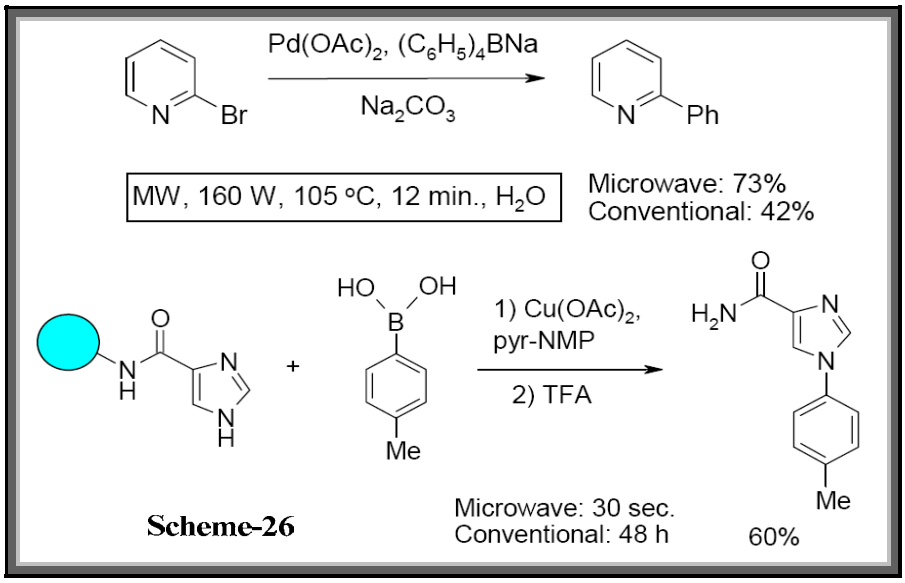
In Scheme 25, the results presented indicate that selectivity is achieved in the *N* alkylation of 1,2,4-triazole under microwave conditions where only the N^1-alkyl derivative was formed in contradistinction to the conventional conditions which give a considerable amount of the di-1,4-substituted compound [141].



Scheme-25

D. 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 26 to give significantly better yield in the presence of microwave irradiation. At the bottom of Scheme 26 another Suzuki coupling is speeded-up by a factor of 100 [142].

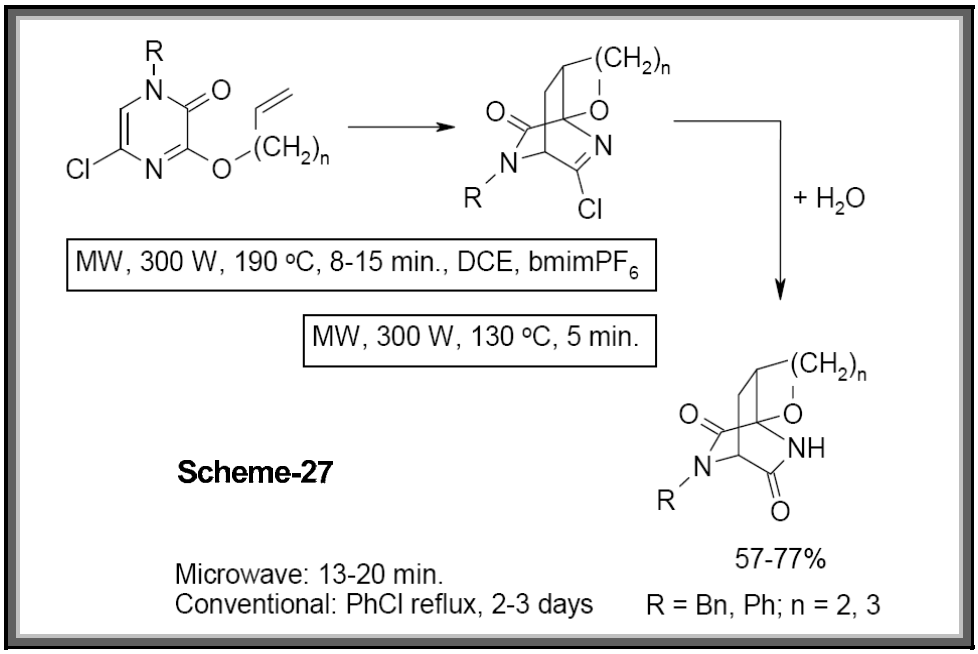


Scheme-26

1.4.2.6 Hetero-Diels–Alder reactions

A. Intramolecular reactions

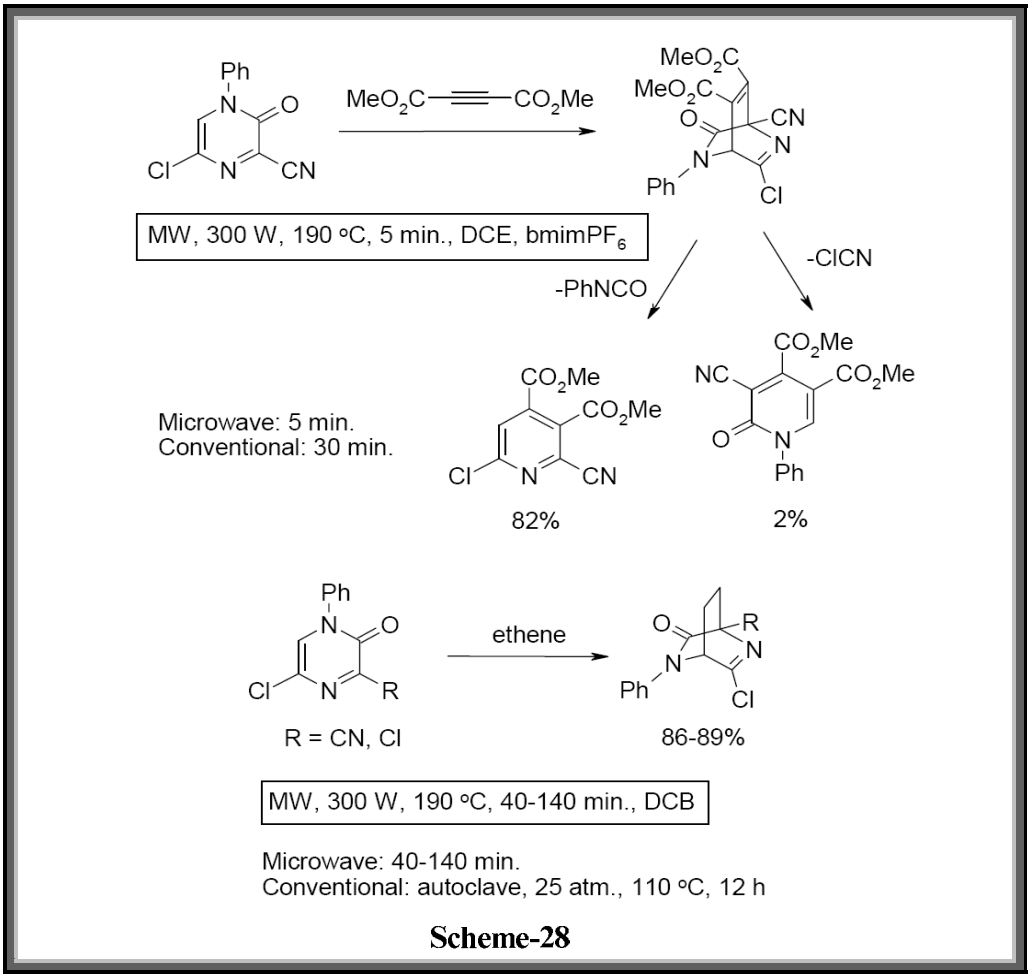
Hetero-Diels-Alder reactions involving cyclic components which lead to polycyclic ring systems are of great importance. An intramolecular example shown in Scheme 27 indicates that the reaction was accelerated by a factor of around 1000 by microwave irradiation [143].



Scheme-27

B. Intermolecular reactions

Scheme 28 shows two impressive examples of rate enhancement for intermolecular hetero-Diels−Alder reactions [144]. In the first example on the top of Scheme 28 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 28 where an autoclave is required under conventional conditions but which can be dispensed with when microwave acceleration is utilized.

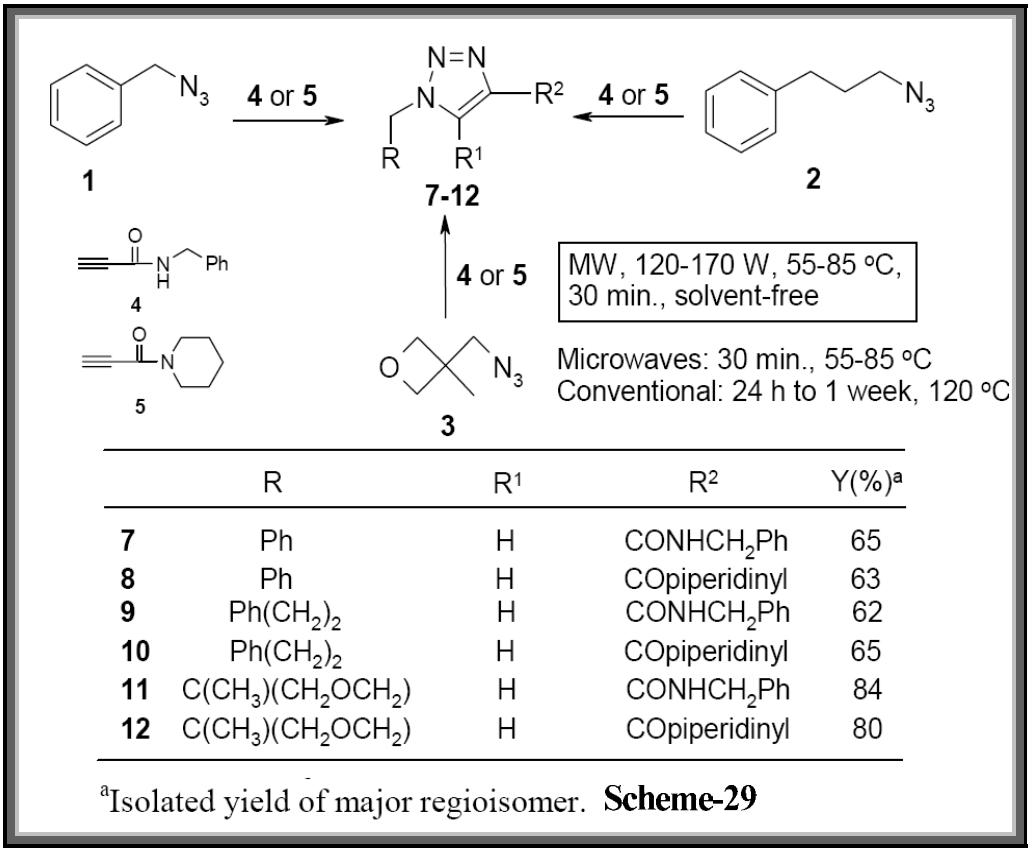


Scheme-28

1.4.2.7. 1, 3-Dipolar cycloaddition reactions

A. Synthesis of C-carbamoyl-1, 2, 3-triazoles

Recently, this laboratory group has been involved in microwave induced 1,3-dipolar cycloaddition of organic azides to acetylenic amides. As shown in Scheme 29 we were able to achieve these reactions under microwave conditions in a reasonable time at temperatures of around 70±15 ºC. Under conventional conditions the times were roughly 100 times as long and the temperature had to be taken up to 120 ºC [145].

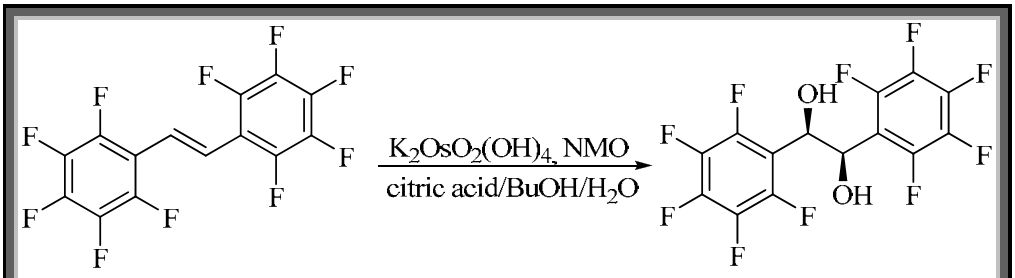


	R	R^1	R^2	Y(%)[a]
7	Ph	H	$CONHCH_2Ph$	65
8	Ph	H	COpiperidinyl	63
9	$Ph(CH_2)_2$	H	$CONHCH_2Ph$	62
10	$Ph(CH_2)_2$	H	COpiperidinyl	65
11	$C(CH_3)(CH_2OCH_2)$	H	$CONHCH_2Ph$	84
12	$C(CH_3)(CH_2OCH_2)$	H	COpiperidinyl	80

[a]Isolated yield of major regioisomer. **Scheme-29**

1.4.2.8 Oxidation

The osmium-catalyzed dihydroxylation reaction, the addition of osmium tetroxide to olefins to produce a vicinal diol, is one of the most selective and reliable organic transformations. Recent work by Sharpless, Fokin, and coworkers [146] has uncovered that electron-deficient olefins can be converted into the corresponding diols much more efficiently when the reaction medium is kept acidic (Scheme 30).



Scheme 30

1.5 AQUA MEDIATED ORGANIC SYNTHESIS (AMOS): A BRIEF REVIEW

The medicinal chemistry community has been under increased pressure to produce, in an environmentally benign fashion, the myriad of drugs required by society in short periods of time. Because of high molecular complexity in drug discovery processes accompanied by time constraints, the primary driver of pharmaceutical green chemistry has become the development of efficient and environmentally benign synthetic protocols. This can be achieved through the proper choice of starting materials, atom economic methodologies with a minimum number of chemical steps, the appropriate use of greener solvents and reagents, and efficient strategies for product isolation and purification. Thus, green chemistry has emerged as a discipline that permeates all aspects of synthetic chemistry. A major goal of this endeavor must then be to simultaneously maximize the efficient use of safer raw materials and to reduce the waste produced in the process [147].

There is a variety of approaches for the development of sustainable methods, which reflects the enormity and complexity of this field. Alternative reaction media is one of the ways to make a protocol greener. However, solvent replacement in itself may not be enough. The whole process must be well thought-out, and the solvent is only one part of this puzzle. The atom efficiency, energy uses and deployment of renewable resources must also be taken into account. Solvents define a major part of the environmental performance of processes in chemical industry and also impact cost, safety, and health issues. Being volatile and highly inflammable, they are the main root of environmental pollution and are high on the green-chemistry agenda

[148]. Use of solvents in reactions cannot be avoided as they are necessary for various processes like the mixing of reactants, constant and uniform supply of energy by transfer of heat, and in some cases, control of the regio- and chemo-selectivity of reactions. However, the use of organic solvents for isolation and purification of products (which involves the use of large amounts per mass of final products) can be prevented or minimized by developing atom-economic synthetic methods, which selectively generate the desired product without producing any by-products.

Environmental improvements, in terms of solvents, can be achieved by implementing several alternative methodologies as described below:

(1) Replacement of hazardous solvents with those that show superior ecological, health, and safety properties.

(2) Bio-solvents: solvents produced with renewable resources such as ethanol produced by fermentation of sugar containing feeds and starchy feed materials.

(3) Substitution of organic solvents with supercritical fluids such as CO_2 that are environmentally benign, and with benign ionic liquids that have low vapor pressure, and thus, curb release into the environment.

(4) Biphasic technologies: using fluorous and regenerable ionic liquids along with aqueous systems and supercritical carbon dioxide.

1.5.1 Is Water the Green Solvent?

The idea of ''green'' solvents expresses the goal to minimize the environmental impact resulting from the use of solvents in chemical production, thus identifying green solvents is a top priority for the organic chemist. Use of no-solvent, i.e. solvent free reactions is another solution, however, this may work for only a few reactions; a lack of reaction medium may lead to overheating of the reaction mixture, in view of the poorly Understood heat- and mass-transfer issues [149]. Biphasic technologies, using fluorous and ionic liquids [150] along with aqueous systems [151] and supercritical carbon dioxide, have formed the main thrust of this movement. However, the cost and toxicity of ionic liquids are big concerns in using them as a solvent [152]. Thus, naturally abundant water appears to be a better option because of its non-toxic, non-corrosive and non-flammable nature. Also, water can be contained

because of its relatively high vapor pressure as compared to organic solvents, which are favorable traits to render water as a sustainable alternative.

1.5.2 What are the limitations of water as a solvent?

The main difficulty with water as a solvent is that most organic substrates are insoluble in it, which makes the reaction mixture heterogeneous. This can be overcome by using phase transfer catalysts, but this will cause the process to be more expensive. Also, the isolation of products from aqueous medium is another concern. For this, evaporation of water from the reaction mixture may be an option, but this is not an energy-efficient process. However, some of these issues can be overcome by using microwave (MW) heating for reactions in aqueous medium.

1.5.3 How do microwaves promote the reaction in aqueous medium?

Water is rapidly heated to high temperatures under microwave irradiation, enabling it to act like a pseudo-organic solvent. Also, precise control of the reaction temperature can be achieved efficiently because of the very high heat capacity of water. MW-enhanced chemistry is based on the efficiency of the interaction of molecules in a reaction mixture (substrates, catalyst and solvents) with electromagnetic waves generated by a ‘‘microwave dielectric effect’’. This process mainly depends on the specific polarity of molecules. Since water is polar in nature, it has good potential to absorb microwaves and convert them to heat energy, thus accelerating the reactions in an aqueous medium as compared to results obtained using conventional heating [153].

This can be explained by two key mechanisms: dipolar polarization and ionic conduction of water molecules (Fig. 2). Irradiation of a reaction mixture in an aqueous medium by MW results in the dipole orientation of water molecules and reactants in the electric field.

This causes two distinguishing effects:

(i) Specific microwave effect: The electrostatic polar effects which produce the dipole–dipole type interaction of the dipolar water molecules and reactants with the electric field component of MW, resulting in energy stabilizations of an electrostatic nature (Fig. 2b). This concept of a specific MW (non-thermal) effect is controversial

and the subject of debate among various chemists. Recent studies by Kappe et al. have shown that this effect is essentially due to thermal phenomena and is thus, not non-thermal;[154] however, more in-depth study is required to obtain a definite answer.

(ii) Thermal effect: the dielectric heating that ensues from the tendency of dipoles (mostly water molecules in addition to reactants) to follow the inversion of alternating electric fields and induce energy dissipation in the form of heat through molecular friction and dielectric loss, which allows more regular repartition in reaction temperatures compared to conventional heating (Fig. 2c).

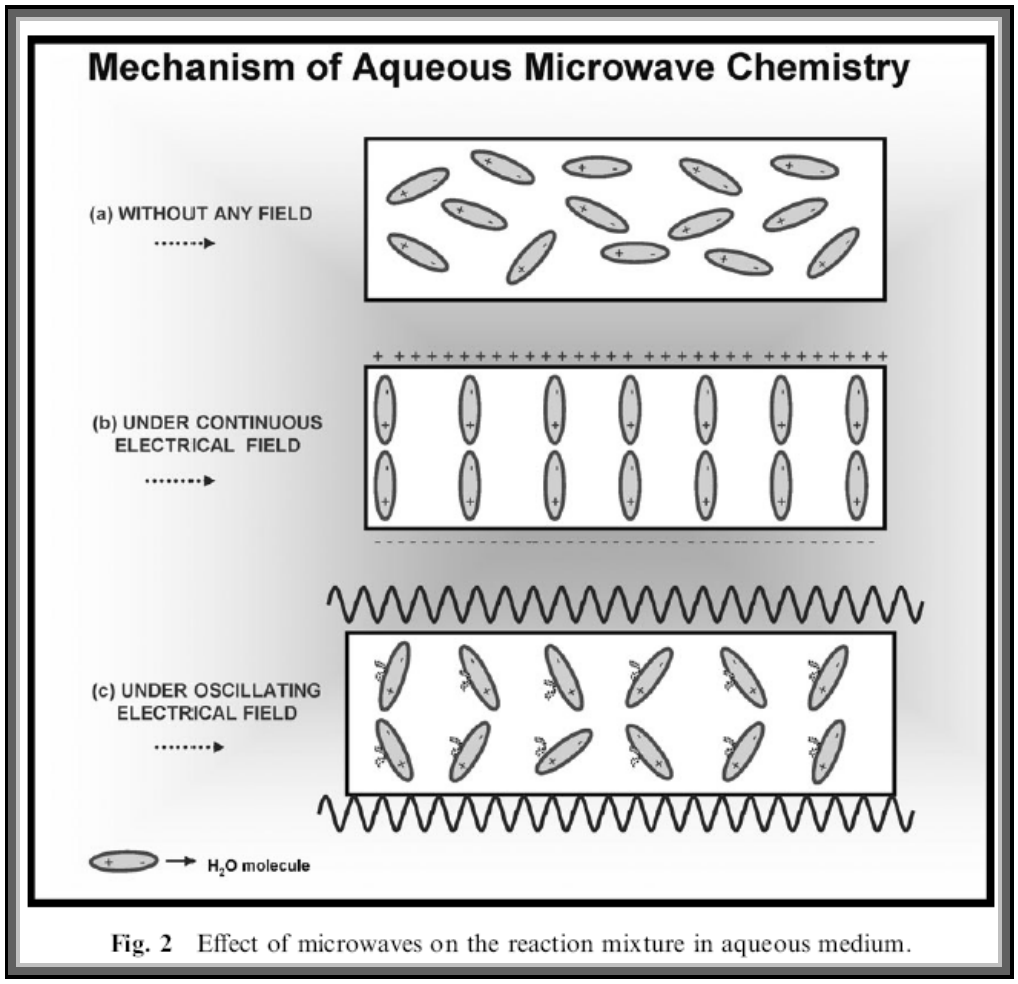


Fig. 2 Effect of microwaves on the reaction mixture in aqueous medium.

1.5.4 How does aqueous chemistry expedite Organic synthesis?

MW-assisted chemistry has blossomed into a useful technique for a variety of applications in drug discovery [155] and organic synthesis [156]. Although MW-assisted reactions in organic solvents have developed rapidly, the focus has now shifted to the

more environmentally benign methods, which use greener solvents and supported renewable catalysts. There are many examples of the successful application of MW-assisted chemistry to organic synthesis; these include the use of benign reaction media, solvent-free conditions, and the use of solid supported and reusable catalysts. As with most organic solvents, the loss tangent (tan *ä*) for water is strongly influenced by temperature. Since the dielectric constant _¢ for water drastically decreases with temperature (Table 2), the dielectric loss _¢¢ and therefore the loss tangent are also reduced.35 For that reason it is not a trivial affair to heat pure water to high temperatures under microwave conditions. While water can be heated rather effectively from room temperature to 100 °C, it is more difficult to superheat water in sealed vessels from 100 to 200 °C and very difficult to reach 300 °C by microwave dielectricheating.38 In fact, SCW is transparent to microwave radiation. Thus in this manner the organic reaction gets accelerated.

Table 2: Properties of Water Under Different Conditions

fluid	ordinary water (T < 150 °C, p < 4 bar)	near-critical water (NCW) (T = 150−350 °C, p = 4−200 bar)	supercritical water (SCW) (T > 374 °C, p > 221 bar)
temp (°C)	25	250	400
pressure (bar)	1	50	250
density (g cm^{-3})	1	0.8	0.17
dielectric constant, ϵ'	78.5	27.1	5.9
pK_W	14	11.2	19.4

[a] Data from ref 26.

The loss tangent of a solvent such as waters in other words the ability of the medium to convert electromagnetic energy into heat scan be significantly increased, for example, by addition of small amounts of inorganic salts. The electric component of an electromagnetic field causes heating by two main mechanisms: dipolar polarization and ionic conduction. When irradiated at microwave frequencies, the dipoles or ions of the sample align in the applied electric field. As the applied field oscillates, the dipole or ion field attempts to realign itself with the alternating electric field, and in the process, energy is lost in the form of heat through molecular friction

and dielectric loss. The amount of heat generated by this process is directly related to the ability of the matrix to align itself with the frequency of the applied field. From a chemical point of view, introduction of ions into a solution leads to a marked increase in dielectric heating rates due to the ionic conduction mechanism. Thus the organic reactions are accelerated.

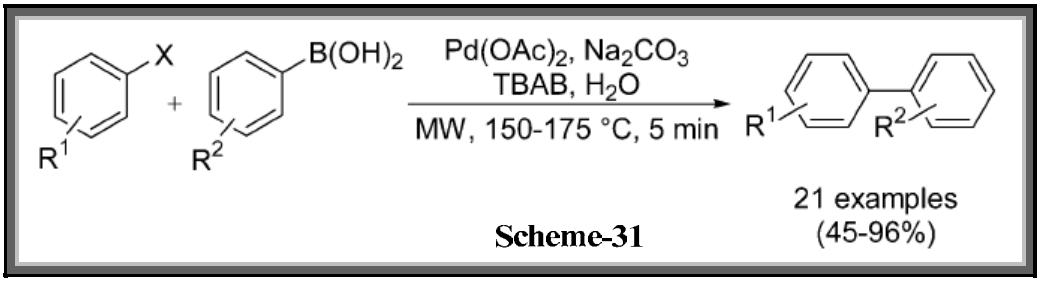
1.5.5 Some Examples of Microwave Assisted Organic Synthesis Using Water as Solvent

1.5.5.1 Transition Metal Catalyzed Reactions

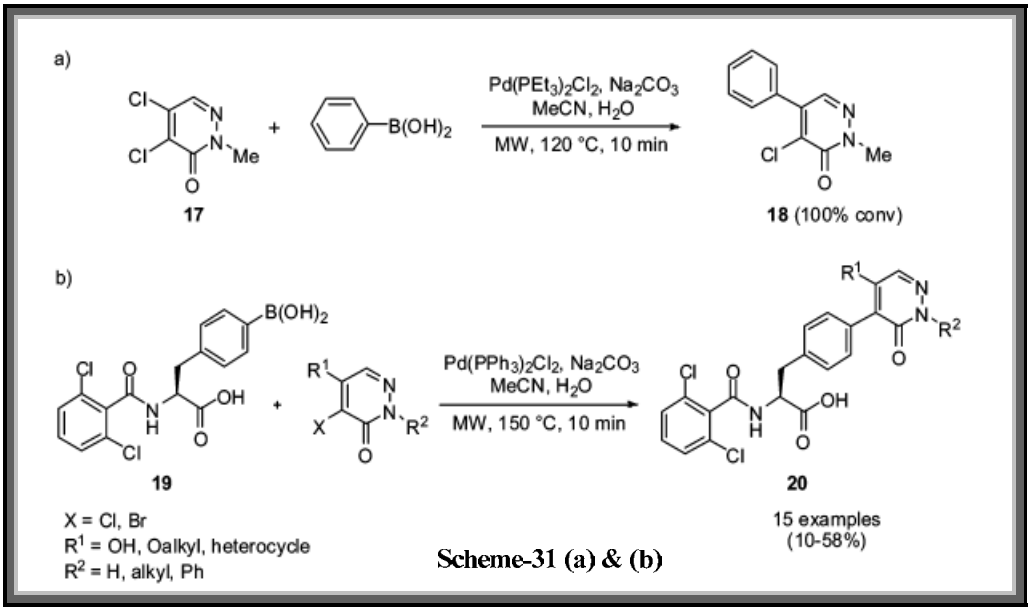
Homogeneous and heterogeneous transition-metal-catalyzed carbon-carbon and carbon-heteroatom bond-forming reactions represent one of the most important reaction types performed in MAOS. These reactions, which are known to need hours or days for completion, often in an inert atmosphere, can be conducted very efficiently in a rapid manner under microwave heating.

A. Suzuki Reaction

The Suzuki reaction (palladium-catalyzed cross-coupling of aryl halides with boronic acids) is one of the most often used C-C cross-coupling reactions and displays a convenient method for the synthesis of biaryls. Leadbeater and Marko reported in 2002 on the ligand free palladium-catalyzed Suzuki couplings of aryl halides with boronic acids using water as solvent [157]. Palladium acetate loadings as low as 0.4 mol % proved to be sufficient, and with addition of 1 equiv of the phase-transfer catalyst tetrabutylammonium bromide (TBAB), aryl bromides and iodides could be coupled successfully in high yields and short reaction times (Scheme-31).



Scheme-31

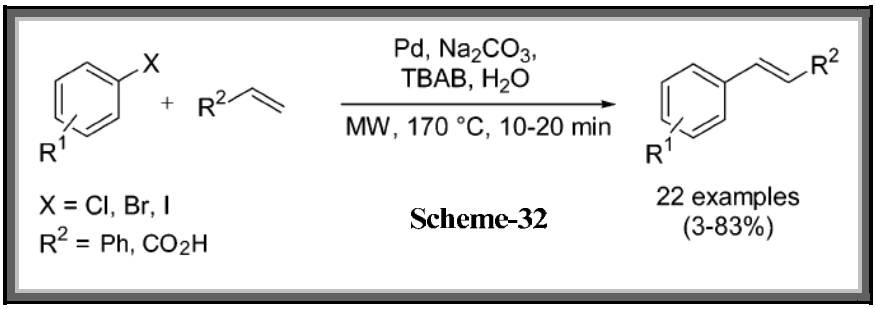


Scheme-31 (a) & (b)

Scheme 31 (a) & (b) are other 2 examples of Suzuki coupling wherein the scientists have synthesized the Pyridazinones successfully which by themselves proved to be a potent α_4-integrin receptor antagonists.

B. Heck Reaction

Palladium-catalyzed vinylic substitution, also known as the Heck reaction, is generally performed with aryl halides and alkenes. In recent years, development of "ligand-free" palladium-catalyzed protocols has gained much interest. Arvela and Leadbeater reported on Heck couplings of aryl halides with styrene and acrylic acid, respectively, applying their aqueous ultra low palladium protocol developed for Suzuki couplings (Scheme 14) [158]. Palladium concentrations down to 0.5-1 ppm are sufficient for the coupling at 170 °C for 10-20 min, although a limited substrate scope was observed. Interestingly better yields were obtained by stirring of the reaction since it is believed that the reaction takes place at the aqueous/organic interface, and with stirring the aryl halide would be exposed to the basic aqueous medium, resulting in faster decomposition [160]. A 10-fold scale up performing the reaction in a stop-flow microwave approach was possible with only a slight change in time (20 vs 10 min) and solvent. Here, a mixture of water/DMF 7:1 proved to be better with respect to pumping the reaction mixture through the lines [159]

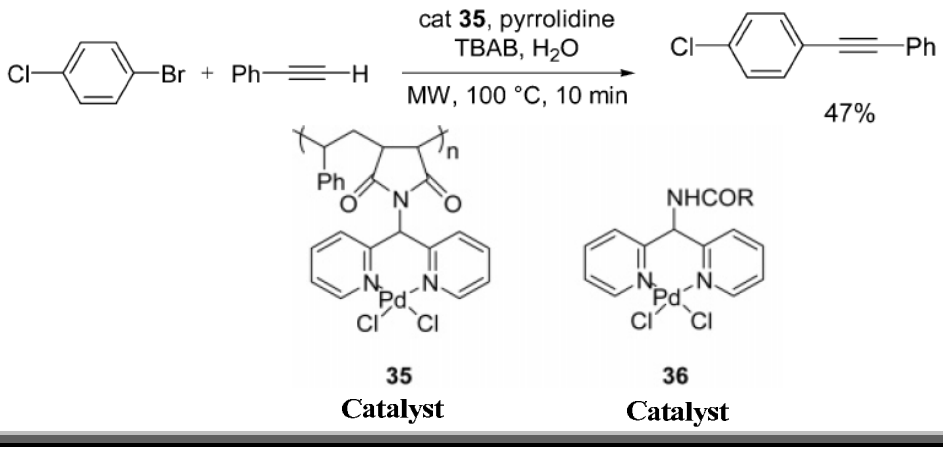


Scheme-32

C. Sonogashira Reaction

The Sonogashira reaction (palladium and copper co-catalyzed coupling of terminal alkynes with aryl and vinyl halides) is a general method for the preparation of unsymmetrical alkynes. In recent years, investigations toward the design of new catalyst systems or protocols for copper-free reactions have been made. The group of Na´jera has performed aqueous copper-free Sonogashira couplings of aryl bromides and iodides with phenylacetylene, applying both polymeric complex 35 and the monomeric 36 as catalyst (see Chart 2), pyrrolidine as base, and TBAB as additive [161]. Under conventional heating better yields in shorter reaction times could be achieved for the polymeric complex; additionally dimerization of the alkyne was decreased. Applying microwave heating for the reaction of 4-chloro-bromobenzene with phenylacetylene, lower yields were obtained for 0.1 mol % of catalyst **35** than for the monomeric **36** (47 vs 66%) (Scheme 33).

Scheme-33

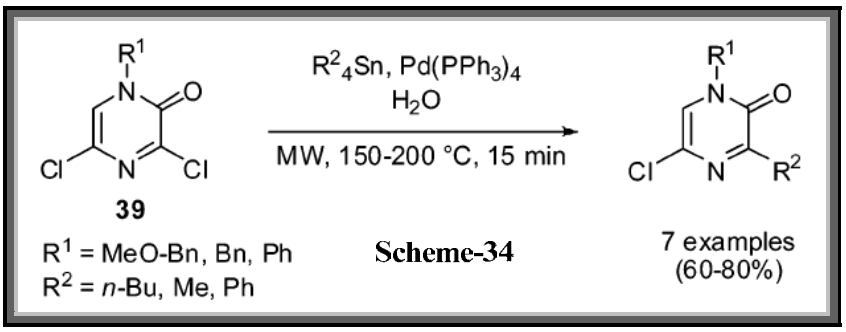


35
Catalyst

36
Catalyst

D. Stille Reactions

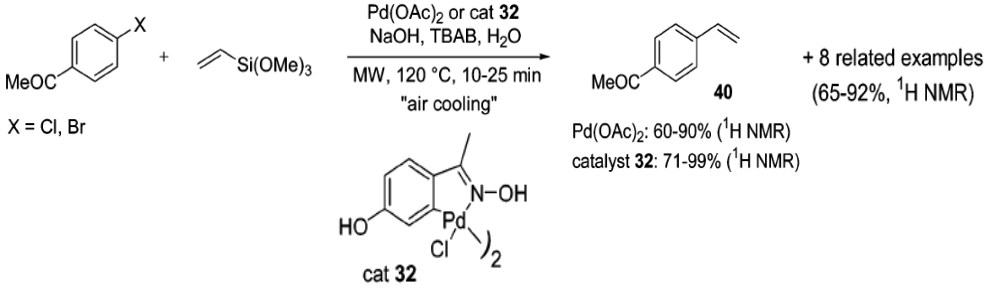
Very few examples are known of microwave-assisted Stille reactions involving organo-tin reagents as coupling partners.12-14 In the course of scaffold decorations of the 2(1*H*)-pyrazinone core **39**, the Stille reaction at the C-3 position was performed by Van der Eycken and co-workers (Scheme 34) [162] For tetraphenyl-tin a higher temperature (200 °C) had to be applied in order to achieve full conversions. A great acceleration compared to conventional heating in refluxing toluene could be reached (3 days vs. 15 min), albeit the yields being somewhat lower for the aqueous microwave synthesis.



E. Hiyama Reaction

The Hiyama reaction (palladium-catalyzed cross-coupling of organo-silicon compounds with organic halides) has become a good alternative to other coupling reactions which employ different organo-metallic reagents from an environmental point of view since the organo-silicon compounds are attractive because of their stability, ease of handling, and/or low toxicity [163]. Several types of organo-silicon reagents have been applied for this carbon-carbon bond-forming reaction such as alkyl-, fluoro-, chloro-, hydroxy-, and alkoxysilanes. In general, Hiyama couplings are promoted by the fluoride anion, usually obtained from tetrabutylammonium fluoride (TBAF), but recently it was found that inorganic bases like KOH, NaOH, and K_2CO_3 are also able to promote the reaction in water as solvent under fluoride-free conditions [164].

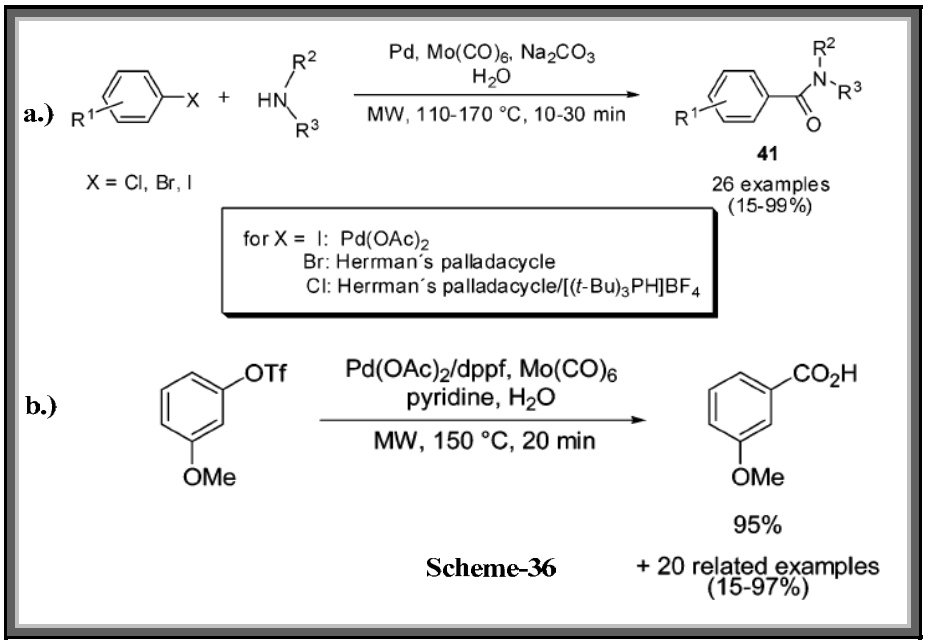
Scheme-35



F. Carbonylation Reaction

For the palladium (0)-catalyzed carbonylations of aryl halides to give aromatic acid derivatives (e.g., acids, amides, esters) the group of Larhed developed a rapid microwave assisted procedure where solid $Mo(CO)_6$ is used as carbon monoxide source [165]. Very recently the authors additionally showed that amino carbonylations can also be conducted in water as solvent, the amine being a better nucleophile than water [166]. Aryl iodides, bromides, and even the otherwise unreactive chlorides could be reacted with diverse primary and secondary amines to the aryl amide products in moderate to excellent yields (Scheme 36 a). The competing hydroxy carbonylation could be inhibited by fine tuning of the reaction parameters; in particular, the stoichiometry of aryl halide to amine was crucial for the successful reaction as well as the proper catalyst. With this general protocol, aryl iodides could be reacted at 110 °C whereas the bromides and chlorides needed the higher temperature of 170 °C and sometimes longer reaction times.

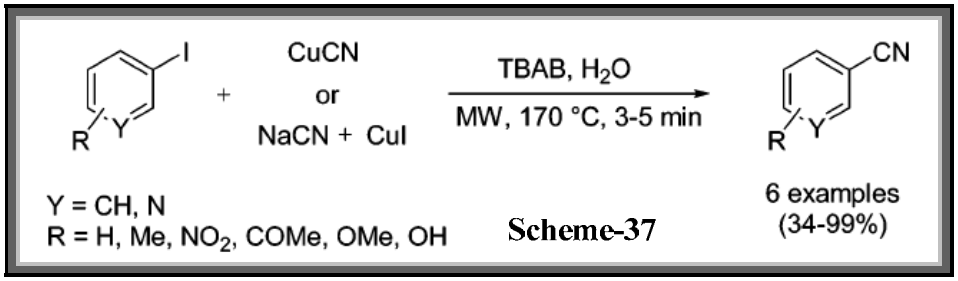
Aqueous hydroxycarbonylations of aryl and vinyl triflates were reported by Silvani and co-workers [167]. A concentration of 0.1 equiv of the catalyst/ligand system $Pd(OAc)_2$/dppf (1,1'-bis(diphenylphosphino)ferrocene), pyridine as base, and $Mo(CO)_6$ as CO source proved to be the best conditions (Scheme-36 b). By heating to 150 °C for 20 min, moderate to excellent yields for aryl carboxylic acids were achieved. Complete chemoselectivity was obtained for halogenated aryl triflates, affording only the halogenated aryl carboxylic acids.



Scheme-36

G. Cyanation Reaction

Preparation of aryl nitriles from aryl iodides using CuCN was disclosed by Leadbeater and co-workers (Scheme 37) [168]. Key to the success of this reaction is the addition of TBAB as phase-transfer agent and a high concentration of cyanide, resulting from a 1:2 ratio of aryl halide/CuCN. Conventional heating under identical conditions resulted in no product; also, activated aryl bromides did not show any conversion. The reaction can also be performed when less expensive NaCN in combination with CuI is employed, forming CuCN in situ.

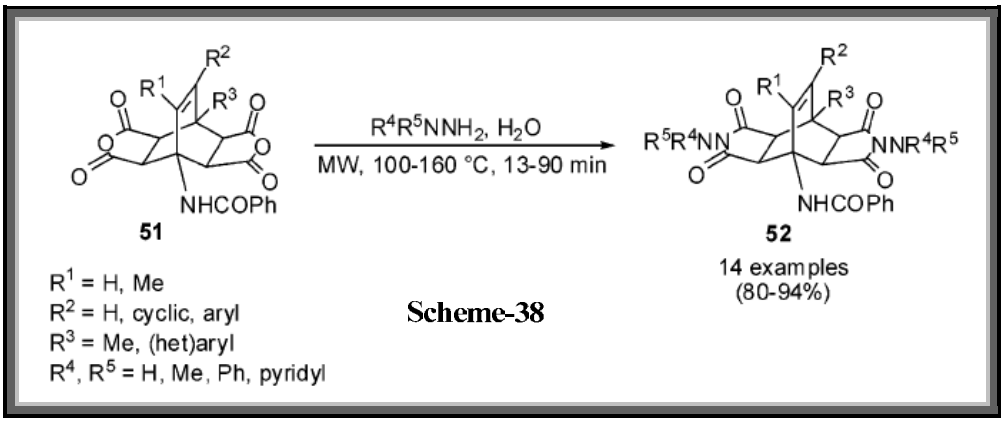


Scheme-37

1.5.5.2 N-, O-, S- Functionalization Reactions

A. N-Acylations

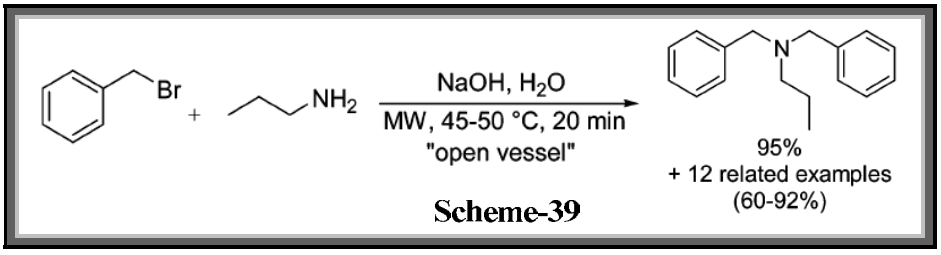
In the transformation of fused succinic anhydrides 51 with hydrazines to the fused *N*-aminosuccinimide derivatives of bicyclo[2.2.2]oct-7-enes 52, microwave heating in aqueous media proved to be very efficient (Scheme 38) [169]. Compared to conventional heating the reaction times could be reduced from several hours to 13-90 min, less hydrazine was necessary (2.2-2.4 vs 10 equiv), and most importantly, cleaner conversions were achieved, giving the products in high yields (80-94%).



Scheme-38

B. N-Alkylations

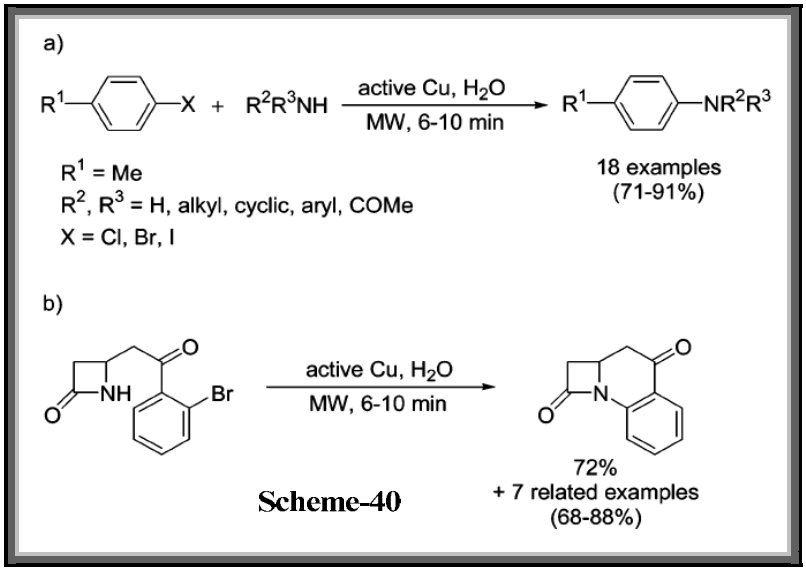
Varma and Ju reported on the synthesis of tertiary amines via *N*-alkylation of primary and secondary amines (aromatic, cyclic, and noncyclic) with alkyl halides (Scheme 39) [170]. By applying microwave heating (open vessel, 45-100 °C), not only could the reaction time be reduced from 12 h to 20-30 min but also formation of side products, mainly secondary amines, could be suppressed. Water as solvent, compared to solventless conditions, MeCN and PEG300, proved to be the best choice in regard to product yield and environmental friendliness.



Scheme-39

C. N-Arylation

Yadav and co-workers disclosed base-free inter- and intramolecular *N*-arylations promoted by active copper [171]. Reactions of aryl halides with amines, amides, imides (Scheme 40 a), and *â*-lactams (Scheme 40 b) proceeded under very mild conditions. Key to the successful intramolecular *N*-arylations of *â*-lactams is the absence of a base since decomposition of the starting material is otherwise observed. Interestingly, a protocol with irradiation for 2 min at 85-90 °C and subsequent mixing for 2 min outside the microwave instrument was applied (this irradiation-mixing cycle was repeated until completion of the reaction was detected; times given in Scheme 40 correspond to total irradiation times). Similar or lower yields, especially in the case of *â*-lactam derivatives, were achieved by performing the reaction under solvent-free microwave conditions.



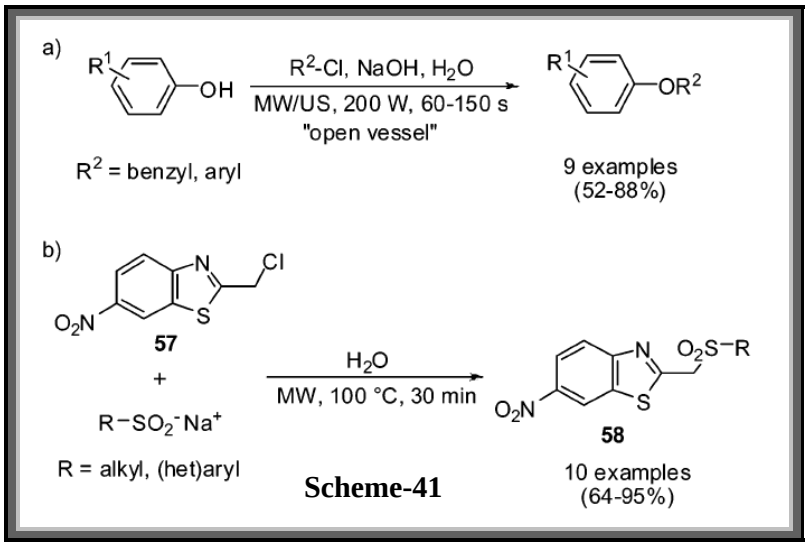
Scheme-40

D. O- & S- Functionalization

A combined microwave and ultrasound (US) protocol for the Williamson ether synthesis from phenols and aryl or alkyl chlorides, respectively, was disclosed by Song and Peng (Scheme 41 a) [172]. This rather uncommon combination, which is performed in a custom-built instrument, proved to give higher yields in much shorter reaction times compared to only microwave heating or sonication [173]. With an ultrasound power of 50 W and a microwave power of 200 W, diphenyl and benzyl phenylethers were obtained in moderate to good yields very rapidly in 60-150 s. A

second "green" aspect in this heterogeneous synthesis is the absence of an otherwise required phase-transfer catalyst.

The group of Vanelle reported formation of new sulfonylmethylbenzothiazole derivatives which show significant cytotoxic activity [174]. The synthesis proceeds via aqueous *S*-alkylation of sodium salts of diverse substituted sulfinic acids with 2-chloromethyl-6-nitrobenzothiazole (Scheme 41b). All experiments were also conducted under conventional heating at the same temperature (100 °C), affording products in similar or lower yields in 24 h. Hence, the authors assumed that specific microwave effects due to a more polar transition state are responsible for the higher yields achieved by microwave heating.



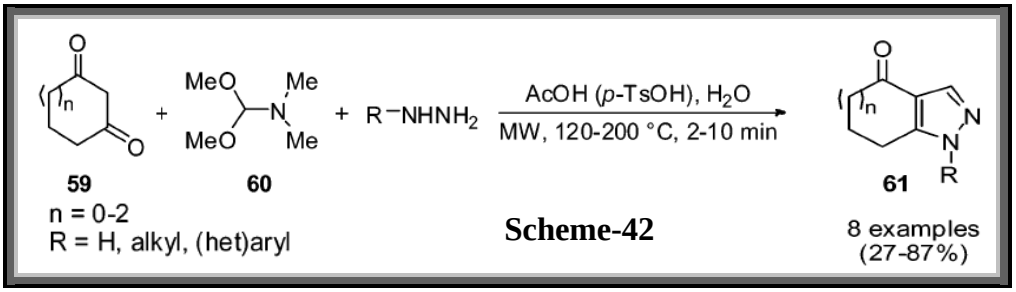
Scheme-41

1.5.5.3 Heterocyclic Synthesis

A. Five membered N-Heterocycles

Molteni and co-workers described the three-component, aqueous one-pot synthesis of fused pyrazoles by reacting cyclic 1,3-diketones with *N*,*N*-dimethylformamide dimethyl acetal (DMFDMA) and a suitable bidentate nucleophile like a hydrazine derivative (Scheme 42) [175] The reaction proceeds via initial formation of an enamino ketone in situ followed by a tandem addition-elimination/cyclodehydration step. An amount of 2.6 equiv of acetic acid is necessary to ensure a clean conversion at 200 °C within 2 min. For 1,3- cyclopentanedione (*n*) 0), *p*-toluenesulfonic acid has to be used instead of AcOH at lower temperatures but

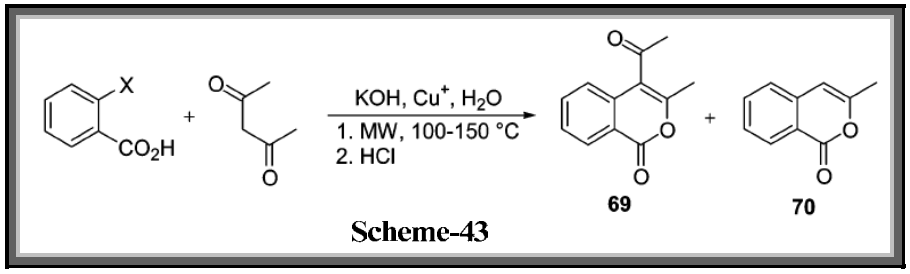
with longer irradiation time (120 °C, 10 min) to afford the corresponding pyrazole in 27% yield. Pyrimidines and isoxazoles could be synthesized as well applying the same protocol employing amidines and hydroxylamine as nucleophiles.



Scheme-42

B. Six Membered O-Heterocycles

Aromatic substitution by activated methylene compounds (1,3-diketones) with base and stoichiometric amounts of a copper(I) catalyst leads to different isochromenone derivates depending on the temperature, pressure, and nature of the activating methylene groups which was shown by the Bryson group (Scheme 43). Under standard reflux conditions (NaH, Cu+) in THF, isochromene is obtained as the main product after acidification, whereas under microwave irradiation (KOH, Cu+) in water at 100-150 °C (3-14 bar) it is the minor product and deacylated isochromene the main product (55-70%) due to cleavage of the acyl group in the high-temperature water media.

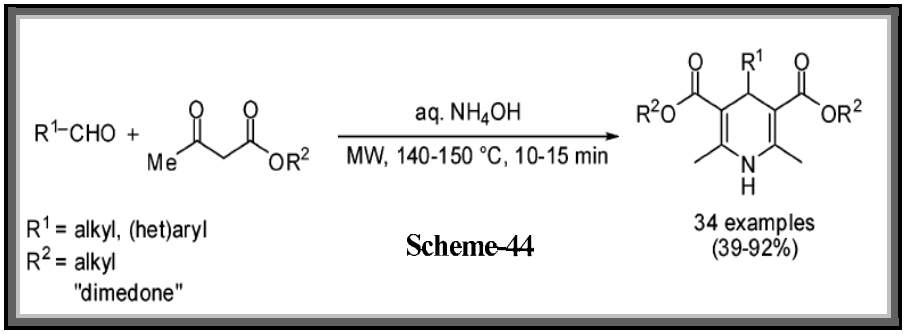


Scheme-43

C. Six Membered N-Heterocycles

A well-known method for the preparation of Heterocycles is the Hantzsch dihydropyridine (DHP) synthesis. Ohberg and Westman presented a fast procedure for this multicomponent, one-pot condensation of an aldehyde, *â*-ketoester, and aqueous ammonium hydroxide, which was used as both reagent and solvent (Scheme

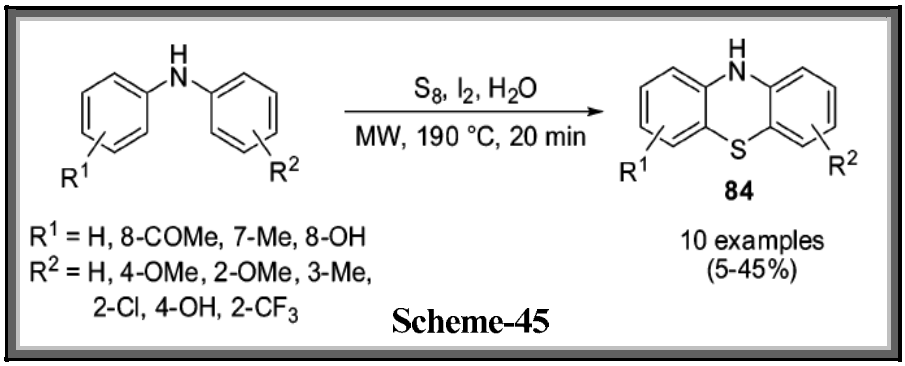
44) [176]. Best yields were obtained by exposing the reaction mixture to microwave heating at 140-150 °C for 10-15 min. Additionally, a small library of 24 compounds as prepared by applying a fully automated microwave instrument within hours.



Scheme-44

D. Six membered N, S- Heterocycles

In order to evaluate the structure-activity relationship for the binding of phenothiazine derivatives to HIV-1 TAR RNA the group of James synthesized a small focused library of 10*H*-phenothiazines with novel substitution patterns around the ring system (Scheme 48) [177]. The synthesis proceeded by an iodine-catalyzed reaction of diarylamines with sulfur in doubly distilled water at 190 °C within 20 min in acceptable to moderate yields. Due to the hydrophobicity of the 10*H*-phenothiazine products, they directly precipitated upon cooling and could be isolated by filtration.

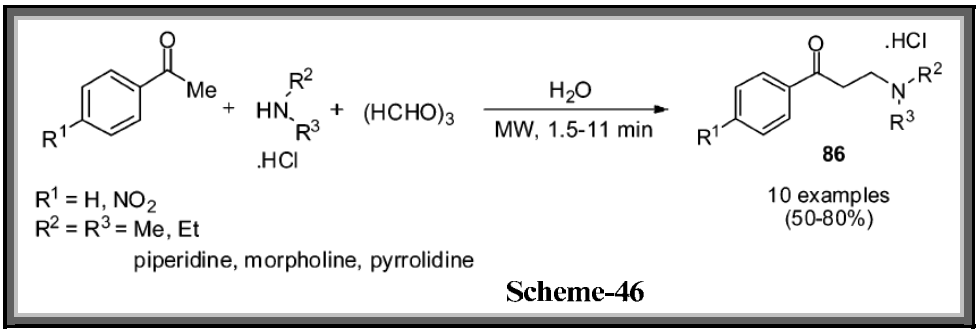


Scheme-45

1.5.5.4 Mannich Type Multi component Reaction

The Mannich reaction is one of the most important transformations leading to *â*-aminoketones. Although the reaction is powerful, it suffers from some disadvantages, such as the need for drastic conditions, long reaction times, and sometimes low yields of products. The group of Song reported on the Mannich

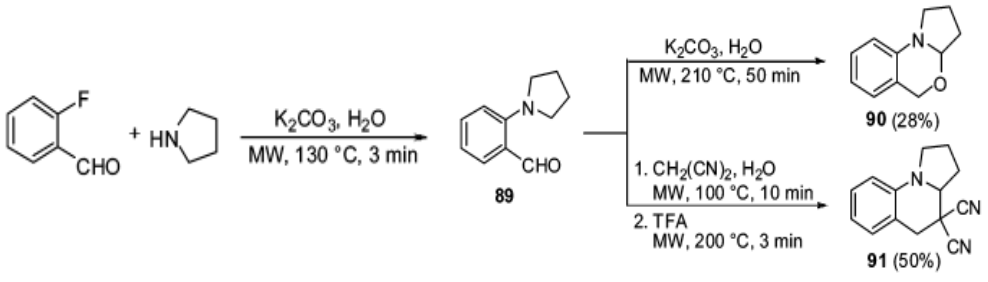
reaction of acetophenones, secondary amines in the form of their hydrochloride salt, and trioxymethylene as formaldehyde source (Scheme 46) [178].



Scheme-46

1.5.5.5 Nucleophilic Aromatic Substitution

The group of Van der Eycken explored the synthesis of pyrido-fused heterocycles which was performed in *n*-BuOH as solvent under microwave irradiation and usually consists of three steps: nucleophilic substitution, Knoevenagel condensation, and ring closure applying the *tert*-amino effect [179]. In a case study, the authors were successful in performing all three reactions in water as solvent. Nucleophilic substitution of *o*-fluoro-benzaldehyde with pyrrolidine at 130 °C for 3 min gave intermediate, which was found to be converted to the pyrrolobenzoxazine by further heating at 210 °C for 50 min in 28% yield (Scheme 47).

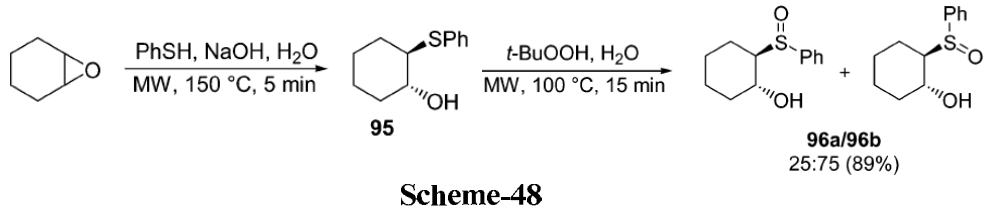


Scheme-47

1.5.5.6 Epoxide ring opening reaction

Recently, Pironti and Colonna described the synthesis of *â*-hydroxy sulfides via the aqueous thiolysis of epoxides with thiophenol in the presence of a catalytic amount of NaOH (Scheme 48) [180] The ring opening proved to be completely anti stereoselective, and the trans products were obtained in excellent yields (six

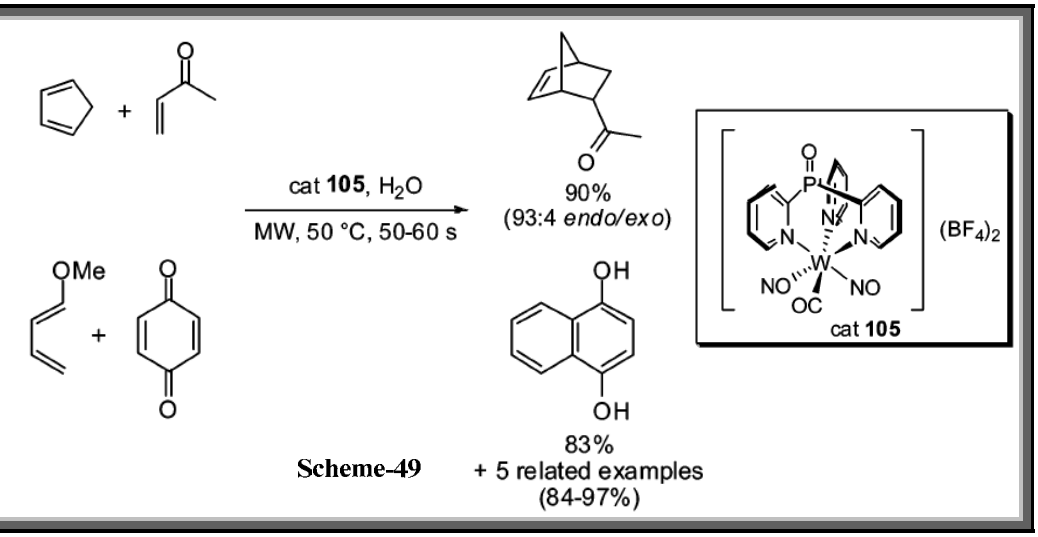
examples, 85-98%). Additionally, a one-pot procedure was developed for the synthesis of *â*-hydroxy sulfoxide.



Scheme-48

1.5.5.7 Diels Alder Cycloaddition Reaction

Enhanced rate accelerations in Diels-Alder cycloadditions due to the combined effects of a water-soluble organotungsten Lewis acid catalyst (105), water as solvent, and microwave heating were observed by Yu and co-workers [181]. All reactions were completed in less than 1 min at 50 °C employing 3 mol % of the Lewis acid catalyst 105 (Scheme 49). When the ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate (bmimPF6) is employed as solvent, even higher accelerations were possible, the reactions being completed within 30 s. Another advantage of the ionic liquid medium is the higher degree of catalyst recovery. Lewis acid 105 can be reused up to 10 times without significant activity loss, whereas a 20% decrease in conversion after the sixth cycle was obtained for the water recovery.



Scheme-49

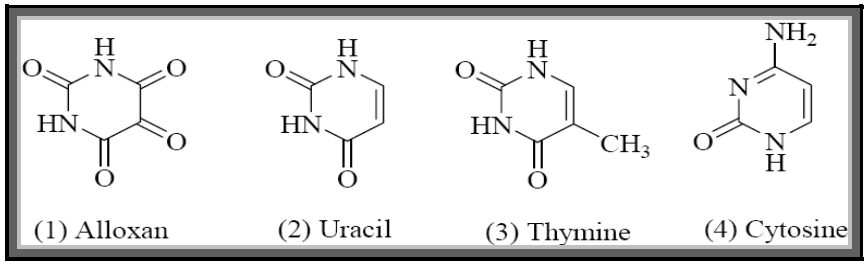
1.6 Biological and Medicinal significance of Pyrimidines and other related heterocyclic scaffolds

Many heterocyclic structures have been identified in various ways and they have shown potent biological activity starting from classical vitamins to modern drugs / receptor based drug molecules. A fair review of these heterocyclic scaffolds is mentioned here in a concise manner especially related to the subsequent chapters on synthetic aspects.

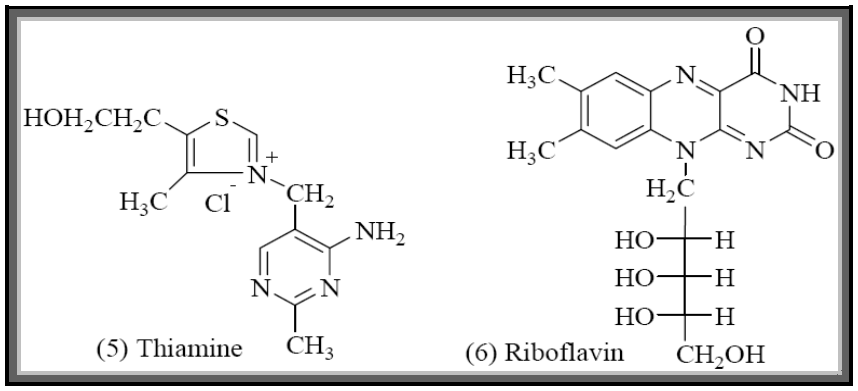
1.6.1 Biological significance

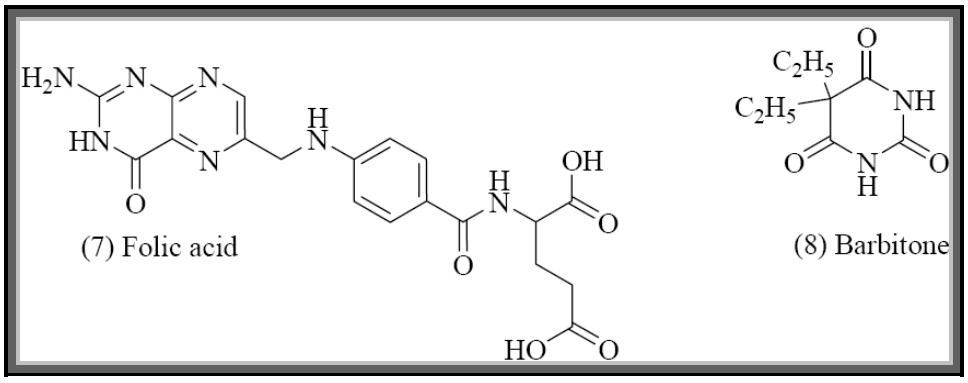
Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of AIDS.

Alloxan (1) is known for its diabetogenic action in a number of animals [182]. Uracil (2), thymine (3) and cytosine (4) are the three important constituents of nucleic acids.



The pyrimidine ring is found in vitamins like thiamine (5), riboflavin (6) and folic acid (7) [183]. Barbitone (8), the first barbiturate hypnotic, sedative and anticonvulsant are pyrimidine derivatives.



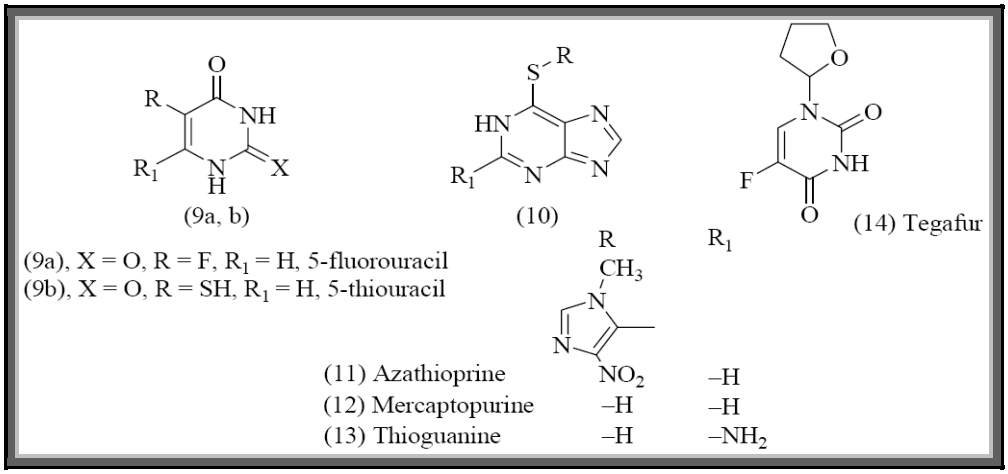


1.6.2 Medicinal Significance.

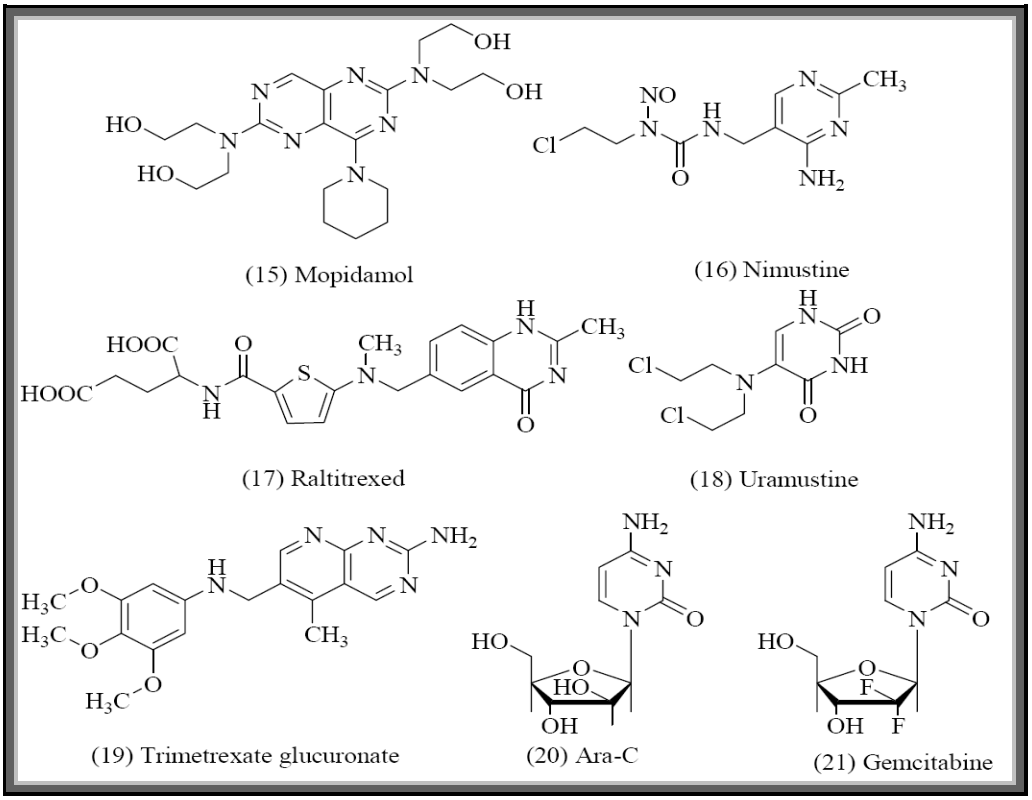
During the last two decades, several pyrimidines derivatives have been developed as chemotherapeutic agents and have found wide clinical applications.

1.6.2.1 Antineoplastic / Anticancer agents

There are a large number of pyrimidine-based anti metabolites. Usually, they are structurally related to the endogenous substrates that they antagonize. The structural modification may be on the pyrimidine ring or on the pendant sugar groups. One of the early metabolites prepared was 5-fluorouracil (5-FU, 9a) [184, 185], a pyrimidines derivative. 5-Thiouracil (9b) also exhibits some useful antineoplastic activities [186]. The antineoplastic compounds [187] possessing the guanine nucleus (10) like azathioprine (11) [188], mercaptopurine (12) [189], thioguanine (13) [190], tegafur (14) [191], etc. were discovered after formulation of the antimetabolite theory by Woods and Fildes in 1940. These drugs prevent the utilization of normal cellular metabolites.

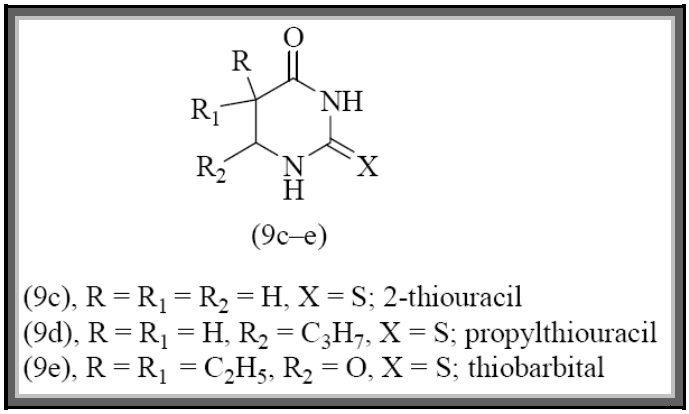


There are many more in recent times, like mopidamol (15) [192], nimustine (16) [193], raltitrexed (17) [194], uramustine (18) [195] and trimetrixate (19) [196]. 1-*β*-DArabinosylcytosine (Ara-C, 20) [197] is also an example of a pyrimidine antimetabolite in which the sugar is arabinose having a beta configuration. It is mainly used as an anticancer agent and also exhibits significant therapeutic effects in patients with herpes virus infections and herpes encephalitis. Gemcitabine (21), a pyrimidine antimetabolite, shows excellent antitumour activity against murine solid tumours [198].



1.6.2.2 Drugs for Hyperthyroidism

2-Thiouracil (9c) and its alkyl analogue, thiobarbital (9e) are effective drugs against hyperthyroidism. Propylthiouracil (9d) is used as a drug for hyperthyroidism with minimum side effects [199].

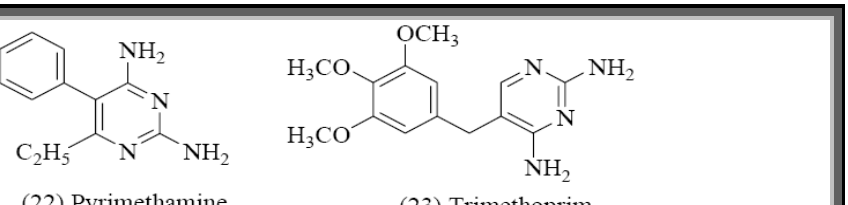


(9c–e)

(9c), R = R_1 = R_2 = H, X = S; 2-thiouracil
(9d), R = R_1 = H, R_2 = C_3H_7, X = S; propylthiouracil
(9e), R = R_1 = C_2H_5, R_2 = O, X = S; thiobarbital

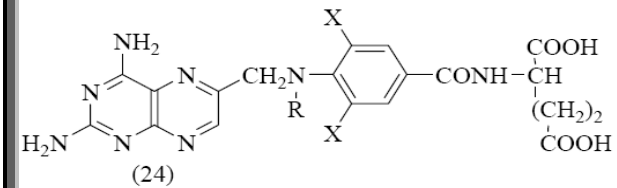
1.6.2.3 Antifolates, Antibacterial, & Antiprotozoals

In 1948, Hitchings made an important observation that a large number of 2,4-diaminopyrimidines and some 2-amino-4-hydroxypyrimidines are antagonists of folic acid [200]. Since then, a large number of 2,4-diaminopyrimidines have been synthesized as antifolates. It was eventually proved that these pyrimidines are inhibitors of the enzyme dihydrofolate reductase (DHFR) [201, 202]. Notable amongst the 2,4-diaminopyrimidine drugs are pyrimethamine (22), a selective inhibitor of the DHFR of malarial plasmodia; trimethoprim (23), an antibacterial drug which selectively inhibits bacterial DHFR and most importantly, the very potent but non selective DHFR inhibitors, methotrexate (24a) and aminopterin (24b), both used in cancer chemotherapy [203]. 3',5'-dichloromethotrexate (24c), which is less toxic and more readily metabolized than methotrexate, has recently been introduced for anticancer therapy [204]. Brodimoprim (25) is also found to be an effective antibacterial compound [205].

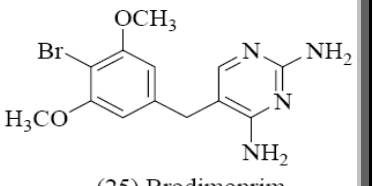


(22) Pyrimethamine

(23) Trimethoprim



(24)

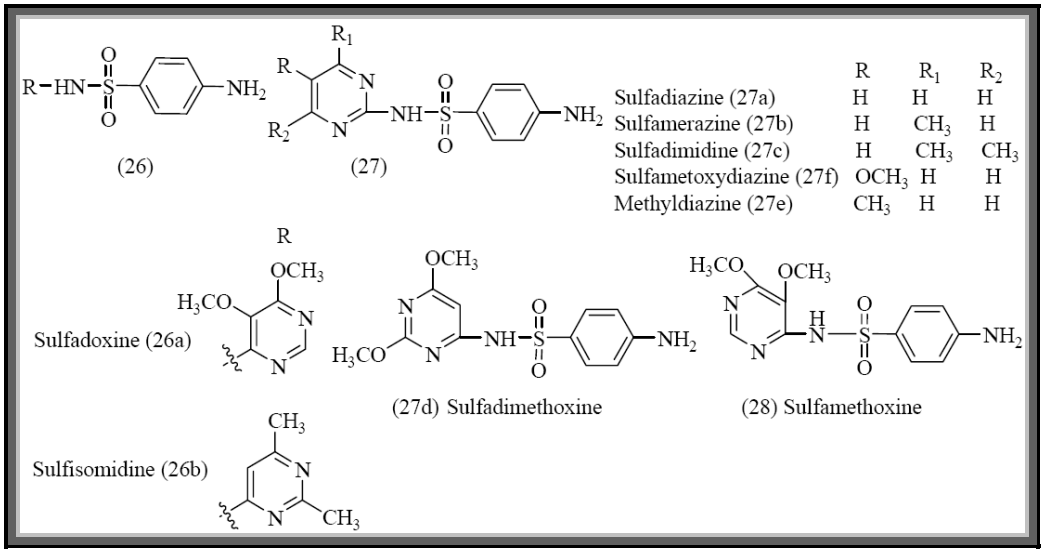


(25) Brodimoprim

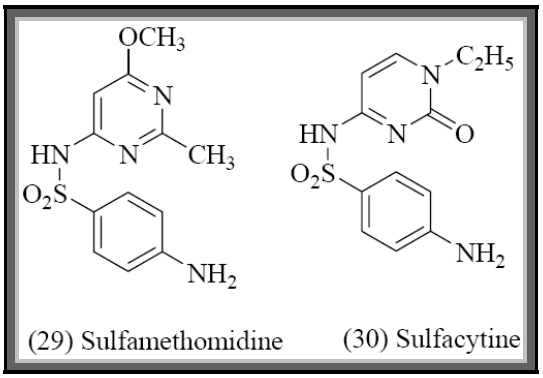
(24a) R = CH_3, X = H; Methotrexate
(24b) R = X = H; Aminopterin
(24c) R = CH_3, X = Cl; 3',5'-dichloromethotrexate

1.6.2.4 Sulfa Drugs

Pyrimidine derivatives of sulfa drugs, namely sulfadiazine, sulfamerazine and sulfadimidine are superior to many other sulfonamides and are used in some acute UTIs, cerebrospinal meningitis and for patients allergic to penicillins [206]. Sulfonamide–trimethoprim combinations are used extensively for opportunistic infections in patients with AIDS [207]. Sulfadoxine (26a) [208], a short and intermediate acting sulfonamide with a half-life of 7–9 days is used for malarial prophylaxis. Sulfisomidine (26b) with a half-life of 7 h is used as a combination sulfa therapy in veterinary medicine [209]. Sulfadiazine (27a), sulfamerzine (27b) and sulfadimidine (27c) possess good water solubility and therefore carry minimum risk of kidney damage, which makes them safe even for patients with impaired renal functions.



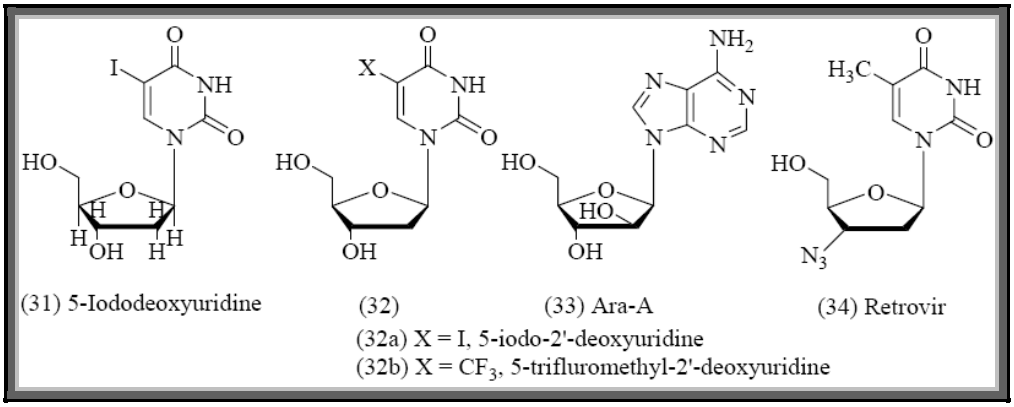
In 1959, sulfadimethoxine (27d) [210] was introduced with a half-life of approximately 40 h. The related 4-sulfonamidopyrimidine, sulfamethoxine (28) [210] having two methoxy groups at 5 and 6 positions, has by far the longest half-life of about 150 h. Methyldiazine (27e) [210] has a half-life of 65 h. Also, sulfamethoxy diazine (27f) [210] possesses good half-life. A new broad-spectrum sulfonamide, sulfamethomidine (29) [210] is relatively nontoxic and patients do not need extra fluid intake or alkalization. Sulfacytine (30) has been reported to be 3–10 times more potent than sulfaisoxazole and sulfisodimidine [210].



(29) Sulfamethomidine (30) Sulfacytine

1.6.2.5 Antivirals & Anti-AIDS

Recently, pyrimidine derivatives have generated widespread interest due to their antiviral properties. 5-Iododeoxyuridine (31) [211] is an antiviral agent of high selectivity.



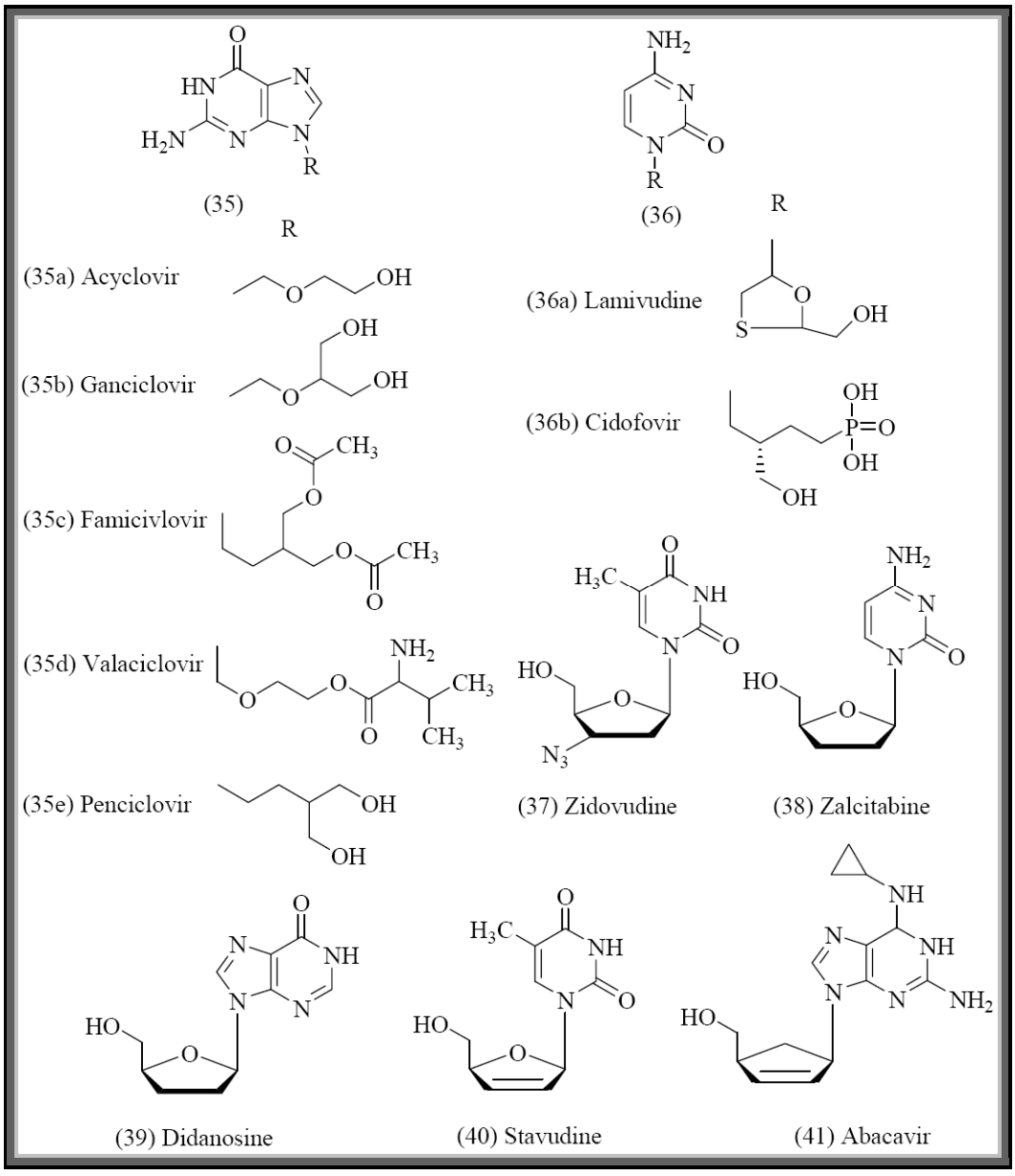
(31) 5-Iododeoxyuridine (32) (33) Ara-A (34) Retrovir

(32a) X = I, 5-iodo-2'-deoxyuridine
(32b) X = CF_3, 5-trifluromethyl-2'-deoxyuridine

IDU (5-iodo-2'-deoxyuridine) (32a) has been extensively utilized for viral infections. 5-Trifluromethyl-2'-deoxyuridine (F3 TDR, 32b) has been found useful against infections resistant to IDU therapy [211]. Ara-A, 9-*β*-D-arabinofuranosyl adenine (33), a relatively new antiviral drug, is effective against herpes infections of eye, brain and skin. It is especially effective against IDU-resistant herpes virus [211].

Some purine nucleosides are equally noteworthy. Retrovir (AZT-16, 34) is a potent inhibitor of the *in vivo* replication and cytopathic effects of HIV and has been recently approved for use against AIDS and severe ARC [212]. At present, Acyclovir (35a) is the only remedy for genital herpes. The oral formulation of Acyclovir is effective against both first and second degree recurrence genital herpes with minimal

side effects [213]. Ganciclovir (35b) [214] has shown good *in vivo* activity against HCV1&2.

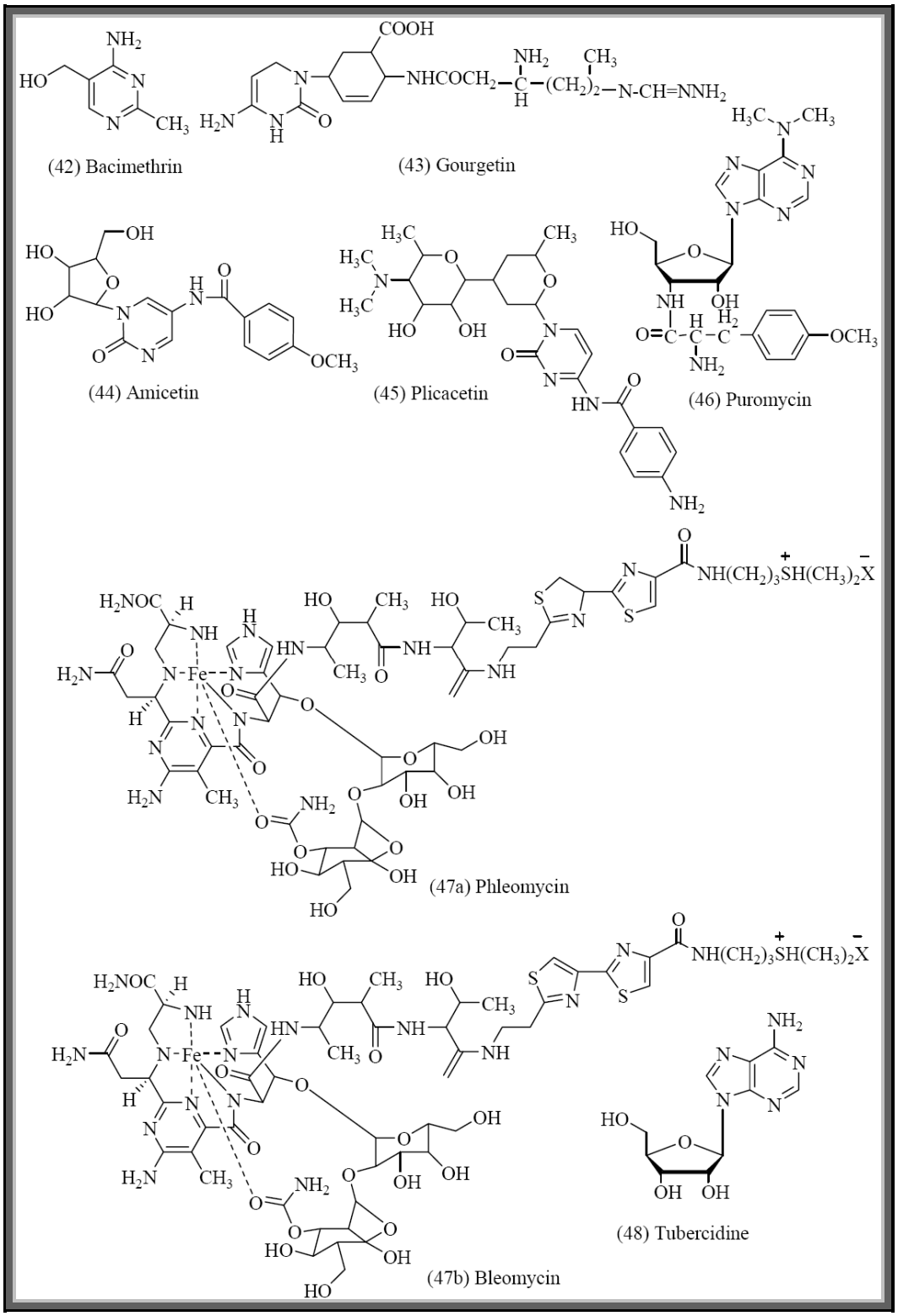


Several members of a series of acyclic nucleosides, which contain a fused pyrimidine ring (mainly purine), are found to be effective antivirals. Famiciclovir (35c) and valaciclovir (35d) are drugs used for several DNA viruses, including HSV types 1 and 2, Varicella-zoster virus and Epstein-Barr virus [215]. Penciclovir (35e) [216] is useful for topical treatment of recurrent herpes, *Libialis*. Cidofovir (36b) [216], an antimetabolite for deoxycytosine triphosphate is used for the treatment of cytomegalovirus (CMV) in AIDS patients. Lamivudine (36a) [216] is an effective anti-

AIDS drug when used in combination with zidovudine (37) [216]. Zidovudine [217] is an analogue of thymidine in which the azido group is substituted at the 3-position of the dideoxyribose moiety. It is active against RNA tumour viruses (retroviruses) that are the causative agents of AIDS and T-cell leukaemia. It is used in AIDS and AIDS related complex (ARC) to control opportunistic infections by raising absolute CD4+ lymphocyte counts. Also, zalcitabine (38) [217] is another useful alternative drug to zidovudine. It is given in combination with zidovudine, when CD4+ cell count falls below 300 cells/mm3. Didanosine (39) [218] is a purine dideoxynucleoside, which is an analogue of inosine. Didanosine inhibits HIV RT and exerts a virustatic effect on the retroviruses. Combined with zidovudine, antiretroviral activity of didanosine is increased. Stavudine (40) [218] is a pyrimidine nucleoside analogue that has significant activity against HIV-1 after intracellular conversion of the drug to a D4T-triphosphate. It is more effective than zidovudine or didenosine for treatment in patients for delaying the progression of HIV infection. It is recommended for patients with advanced HIV infection. Abacavir sulfate (41) [218] was approved in 1998 as a NRTI (Nucleoside Reverse Transcriptase Inhibitor) to be used in combination with other drugs for the treatment of HIV and AIDS. The major use of abacavir appears to be in combination with other NRTIs.

1.6.2.6 Antibiotics

There are few examples of pyrimidine antibiotics. The simplest of all is bacimethrin (5-hydroxymethyl-2-methoxypyrimidin-4-amine) (42), which is active against several staphylococcal infections [219]. Gourgetin (43), a cytosine derivative is active against mycobacteria as well as several Gram-positive and Gram-negative bacteria [220]. There are more derivatives of cytosine, namely amicetin (44) and plicacetin (45), which exhibit activity against acid fast and Gram-positive bacteria as well as some other organisms [219]. Puromycin (46) has a wide spectrum of antitrypanosomal activity. Aminoglycoside antibiotics phleomycin (47a), bleomycin (47b) and related families are wide-spectrum antibiotics containing the pyrimidine ring. Another antibiotic tubercidine (48) is reported to exhibit antitumour properties [220]. In addition, they have antineoplastic activity. Bleomycin is already in clinical use against certain tumours like Hodgkin's lymphoma and disseminated testicular cancer [221].



(42) Bacimethrin

(43) Gourgetin

(44) Amicetin

(45) Plicacetin

(46) Puromycin

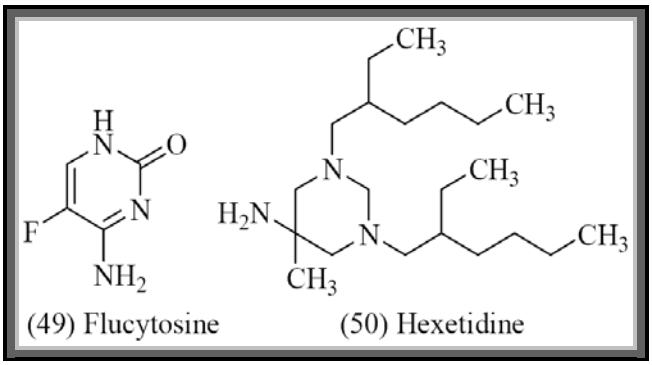
(47a) Phleomycin

(47b) Bleomycin

(48) Tubercidine

1.6.2.7 Antifungals

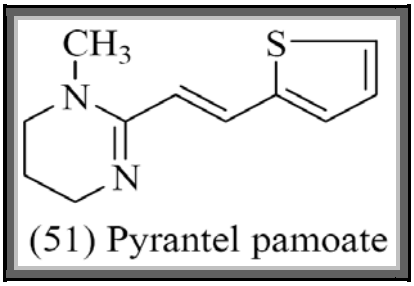
Pyrimidines also exhibit antifungal properties. Flucytosine (49) [222] is a fluorinated pyrimidine used as nucleosidal antifungal agent for the treatment of serious systemic infections caused by susceptible strains of candida and Cryptococcus [223]. Hexetidine (50) [224] is mainly used for the treatment of aphthous ulceration.



(49) Flucytosine (50) Hexetidine

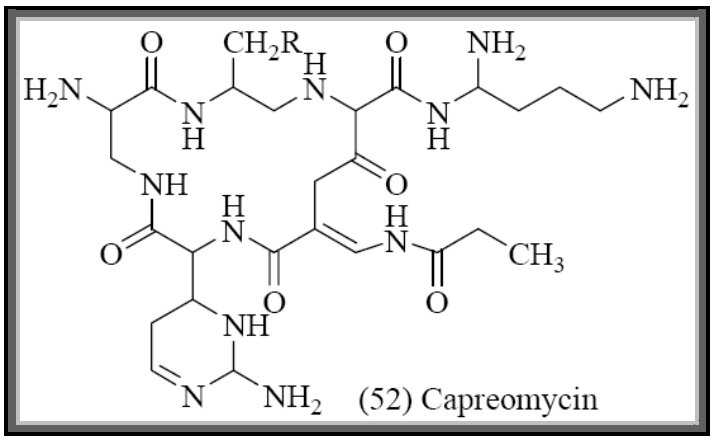
1.6.2.8 Anthelmentics

These drugs have the ability of ridding the body of parasitic worms. Pyrantel pamoate (51) is a depolarizing neuromuscular blocking agent that causes spastic paralysis in helminthes and is employed in the treatment of infestations caused by pinworms and roundworms [225].



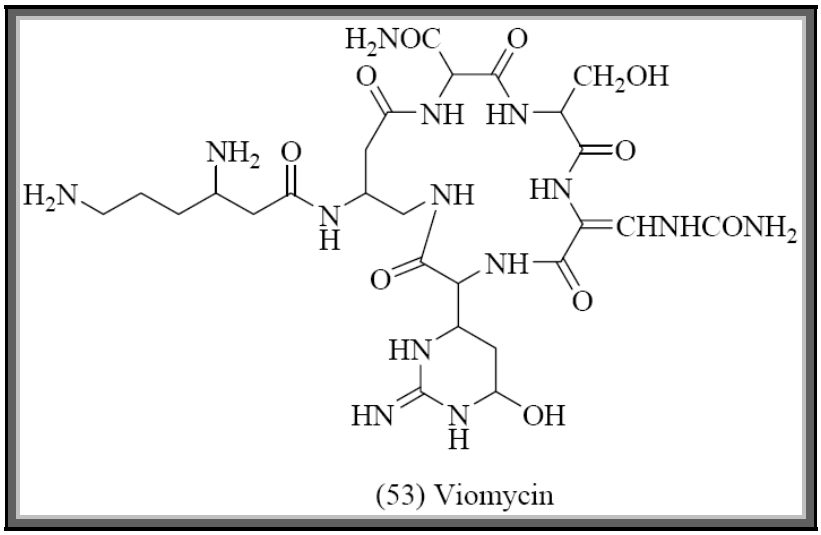
(51) Pyrantel pamoate

1.6.2.9 Antitubercular Drug



(52) Capreomycin

Capreomycin (52) produced by *Streptomyces capreolus* is a second-line bacteriostatic antituberculin drug containing pyrimidine [226, 227].

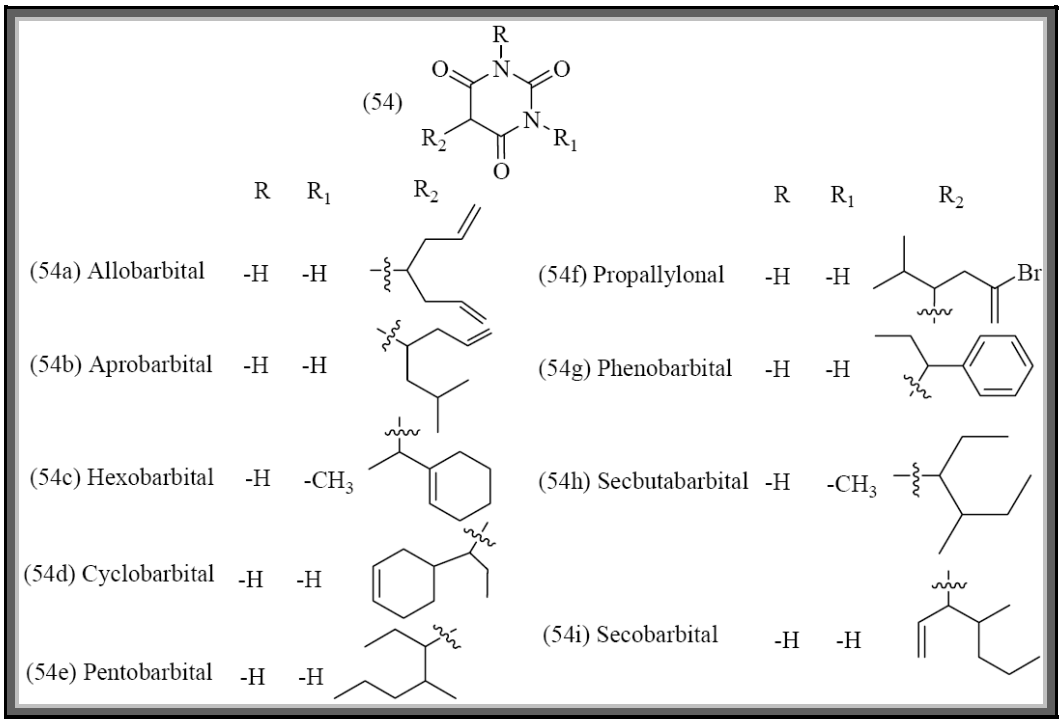


(53) Viomycin

Viomycin (53) is more tuberculostatic than *p*-aminosalicyclic acid. It is effective in the treatment of experimental tuberculosis.

1.6.2.10 CNS active agents

A. Sedative / Hypnotic / Antiepileptic agents

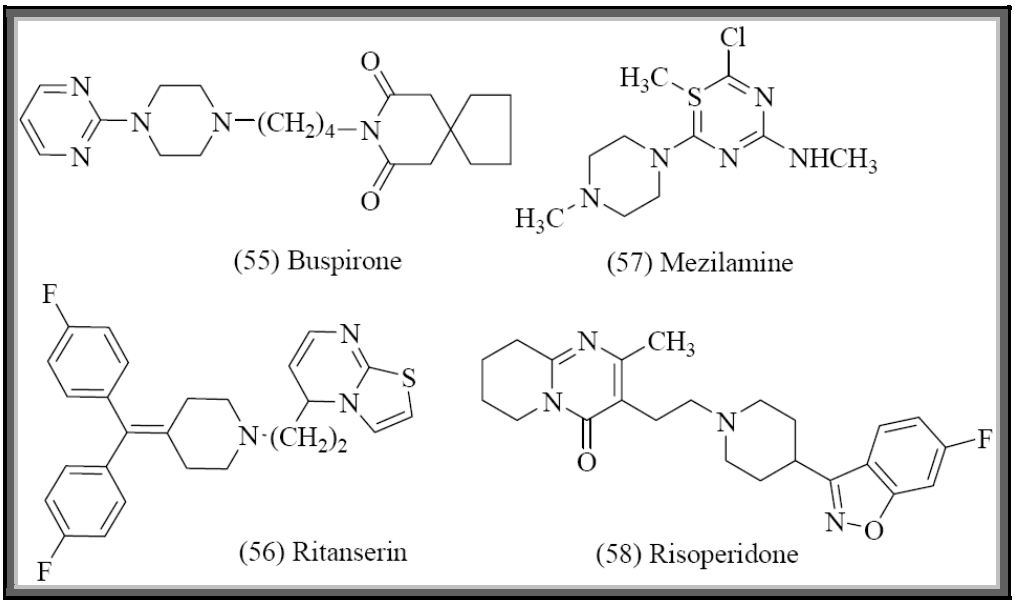


Agents of the anxiolytic, sedative and hypnotic group include a wide variety of barbiturates (54a–i) used as sedative and hypnotics and are classified as drugs having short, intermediate and long duration of action [228, 229]. Allobarbital (54a), aprobarbital (54b), pentobarbital (54e), phenobarbital (54g) and secobarbital (54i) are frequently used clinically as hypnotic barbiturates [230]. Hexobarbital (54c), cyclobarbital (54d) and propallylonal (54f) are some of the current drugs in the market used as sedative hypnotics [231]. Barbiturates as sedative hypnotics have a long and fascinating history. In fact Eli Lilly [232] patented secbutabarbital (54h) in 1932, while barbitone (8), the first of the barbiturates was introduced in 1903.

B. Anxiolytic Agents

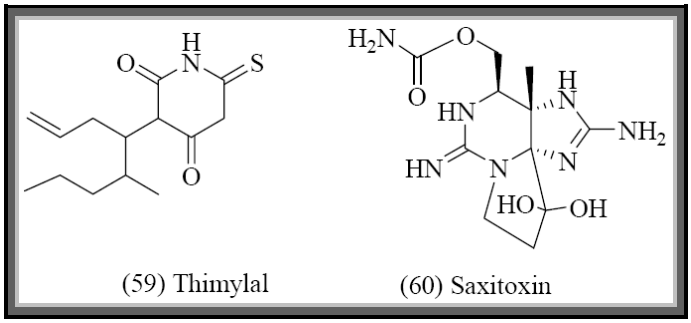
Few of the pyrimidine derivatives are also used as anxiolytics. Most important of these is buspirone (55), indicated in the management of anxiety disorders accompanied with or without depression. It lacks sedative, anticonvulsant and muscle relaxant effects and most importantly abuse potential [233]. Buspirone lacks affinity

to benzodiazepine receptors, but binds avidly to one subclass of serotonin receptors, the 5-HT1A subtype [234, 235]. Ritanserin (56), a 5HT2 antagonist with anxiolytic activity is a pyrimidine derivative [236]. A simple pyrimidine derivative, mezilamine (57) is classified as an antipsychotic agent [237]. Risoperidone (58) is an antipsychotic drug, which is a structural hybrid of butyrophenone and can be used as anxiolytic, antidepressant and antiparkinsonian drug [238].



C. Pyrimidine Anaesthetics

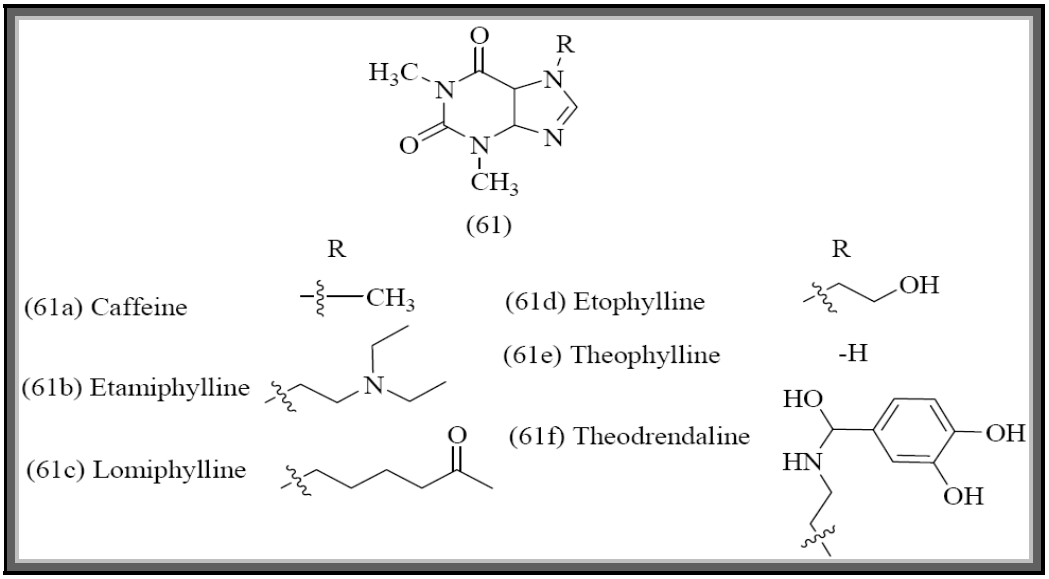
Thimylal (59) is a short acting general anaesthetic drug, which is also a pyrimidines analogue [239. 240].



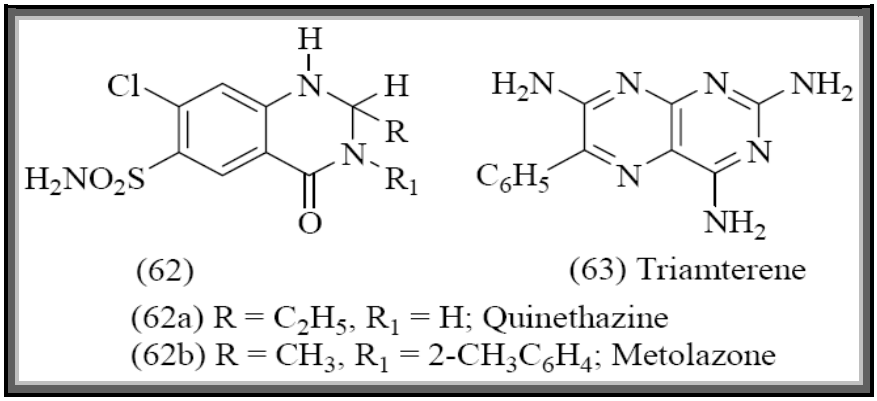
Saxitoxin (60) is a naturally occurring pyrimidine containing anaesthetic agent, but is too toxic to be of clinical use. Saxitoxin is isolated from some marine dinoflagellates.

D. Diuretics & Uricosurics

Several xanthine derivatives (61) containing fused pyrimidine ring systems like caffeine (61a) [241], etamiphylline (61b) [242], lomiphylline (61c) [243], etophylline (61d) [244], theophylline (61e) and theodrendaline (61f) [245] are known to promote a weak diuresis by stimulation of cardiac function and by a direct action on the nephron, acting as adenosine receptor antagonists.



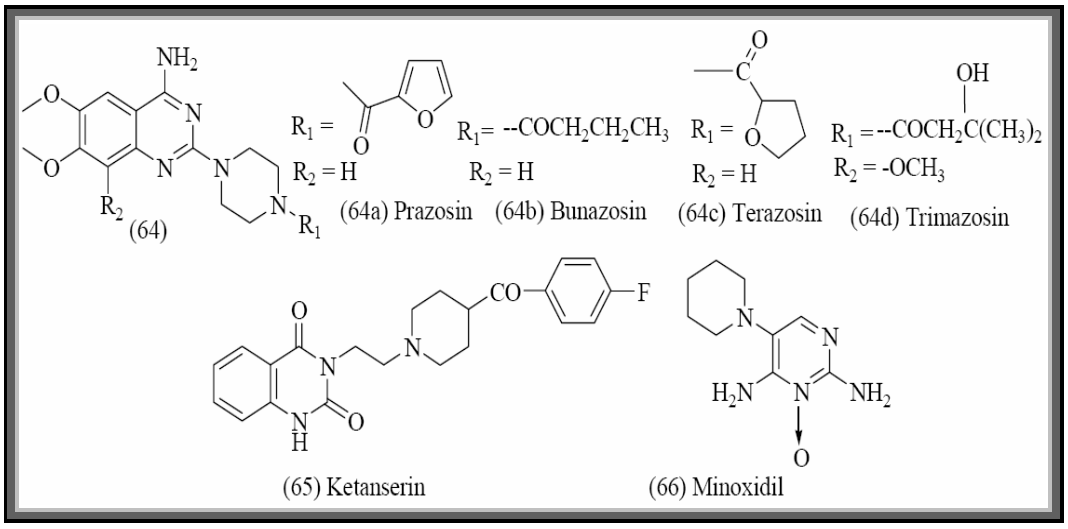
There are a few examples of diuretics which contain a pyrimidine ring. Noteworthy are quinethazine (62a), metolazone (62b) [246] and triamterene (63) [247].

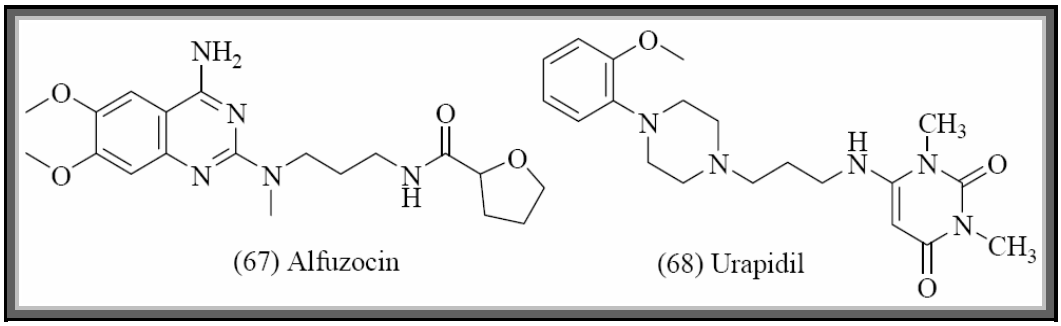


1.6.2.11 Cardiac Agents

A. Antihypertensive agents

Several pyrimidine ring-containing drugs have exhibited antihypertensive activity. Prazosin (64a), a quinozoline derivative, is a selective α1-adrenergic antagonist [[248, 249]. Its related analogues bunazosin (64b) [250], terazosin (64c) [251] and trimazosin (64d) [252] are potent antihypertensive agents. Another quinazoline derivative, ketanserin (65) [253] having a similar effect is an antagonist of both *a*1-adrenergic and serotonin-S2 receptors. Its mechanism of action however is still controversial. A triaminopyrimidine derivative, minoxidil (66), whose mechanism of action and therapeutic action are similar to Prazosin, has been introduced in therapy for its side effects, in the treatment of alopecia, male baldness [254]. Besides these, some more pyrimidine derivatives given below were found to be antihypertensives [255, 256].

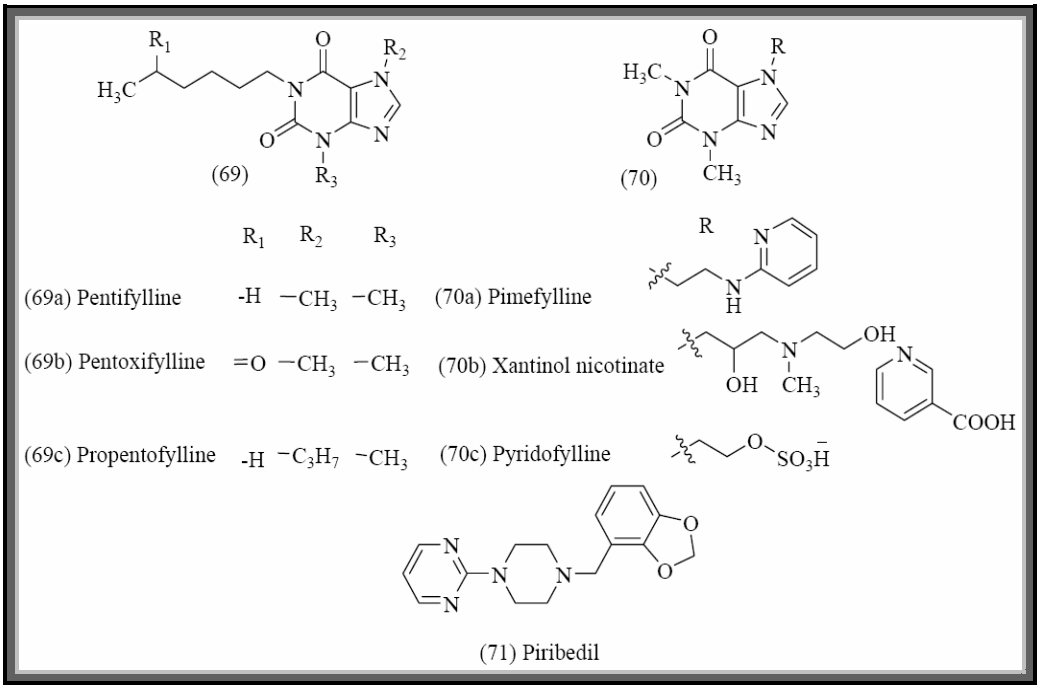




Alfuzocin (67), a prazosin analogue and an α1-adrenoceptor antagonist as well as urapidil (68) are used especially in urinary obstruction caused by benign prostate hyperplasia.

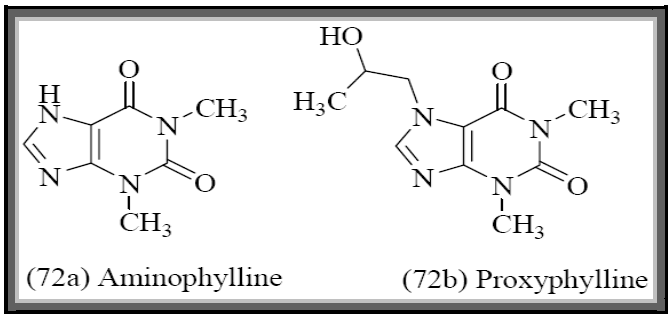
B. Vasodialators

A series of xanthine derivatives are used as peripheral and cerebral vasodilators. Especially, pentifylline (69a) and pentoxifylline (69b) are used in cardiovascular disorders [257]. Other derivatives like xantinol nicotinate (70b) [258], a vasodilator with general properties like nicotinic acid used in cerebral and peripheral vascular disorders and pimefylline (70a) and pyridofylline (70c) [259] are noteworthy. A new dopamine receptor stimulant, pirebidil (71) [260] is reported to have produced significant improvement in ADL (Activity of Daily Living) in patients suffering from Parkinson's syndrome.



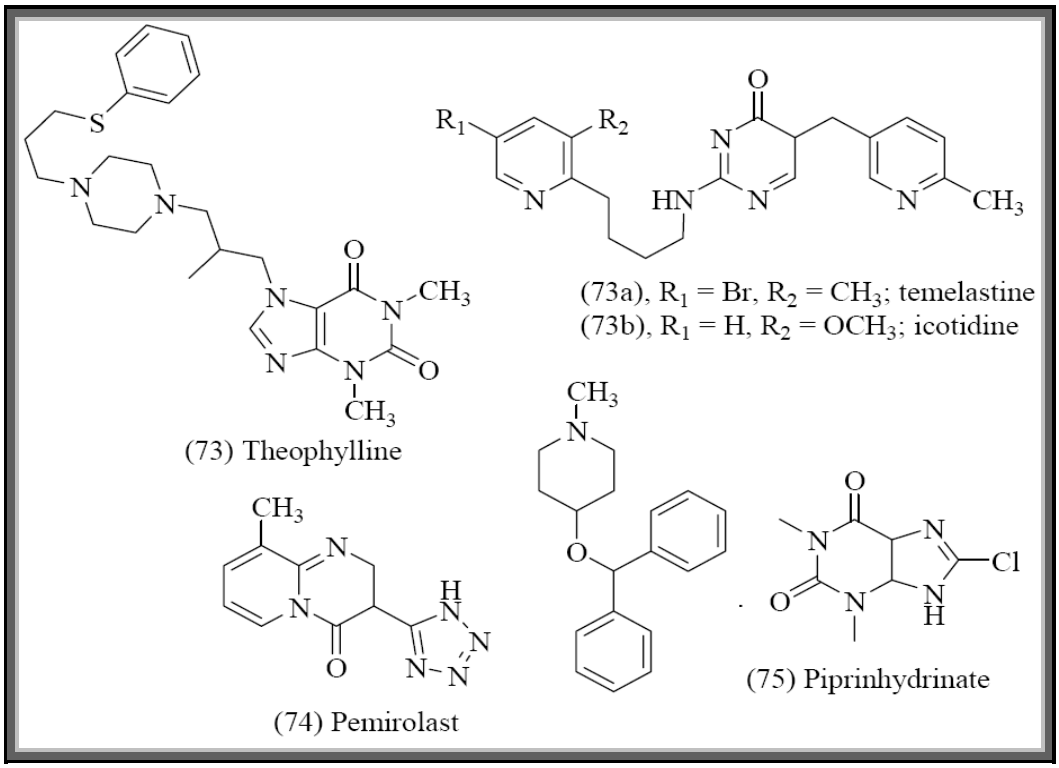
C. Cardiotonics / Bronchodialators

Several xanthine derivatives *viz.*, theophylline (61e), aminophylline (72a) [261] and proxyphylline (72b) [261] exhibit good bronchodilator activity.



1.6.2.12 Antihistaminic Pyrimidines

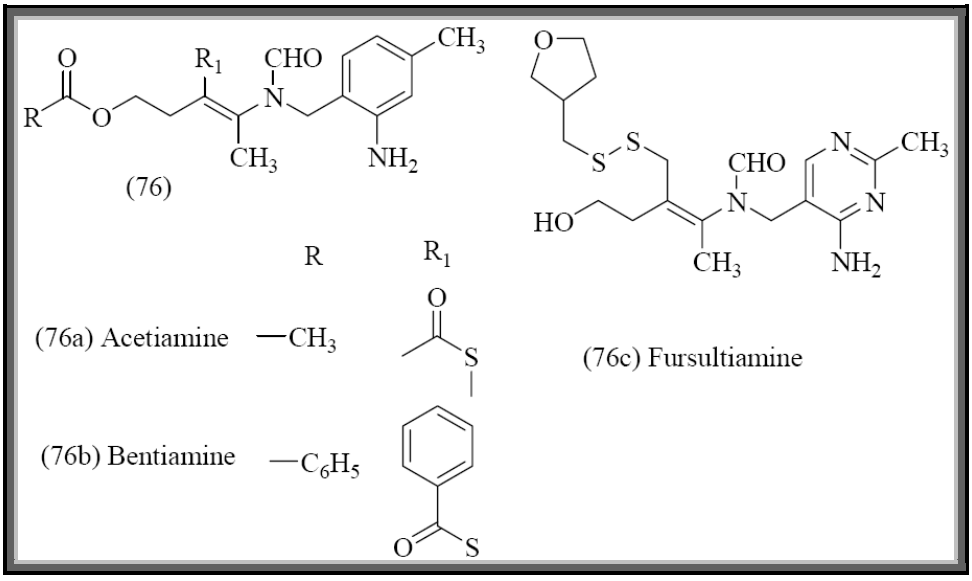
Theophylline (73) is ten times more potent than either astemizole or terfenadine in its affinity for H1-histamine binding site and appears to be devoid of CNS activity [262]. Another pyrimidine containing antihistaminic drug, temelastine (73a) is comparable to mepyramine [263]. Radiolabelled studies have indicated that it does not penetrate the CNS appreciably. Icotidine (73b), a structural analogue of temelastine lacks CNS activity and is a dual antagonist of both H1 and H2 receptors [264].



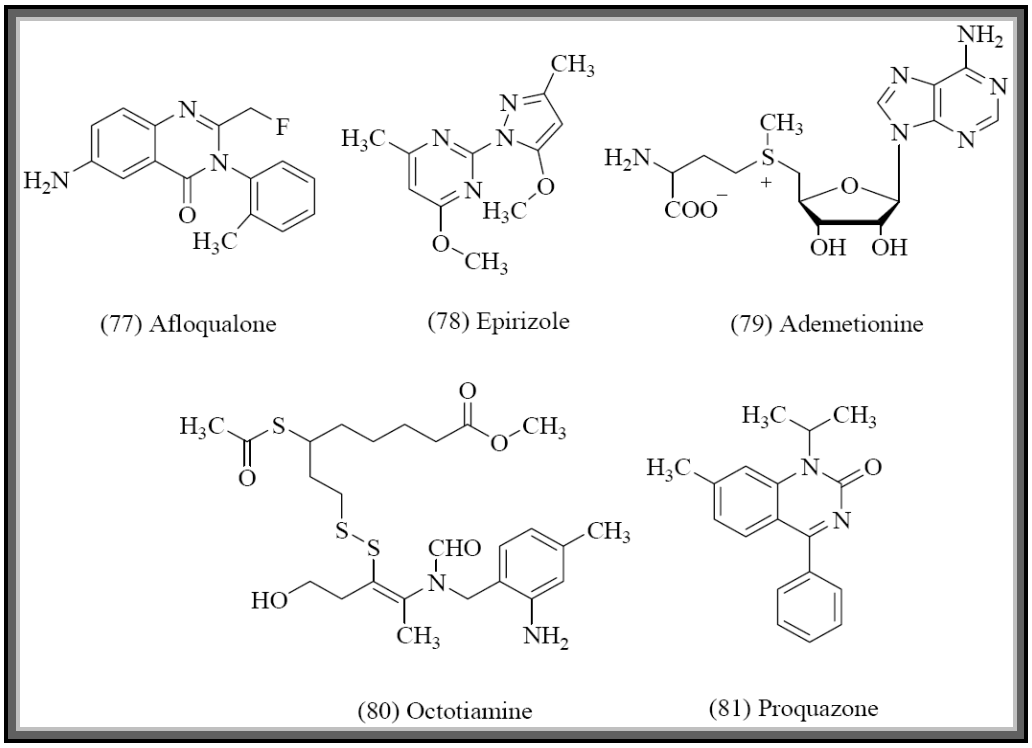
Pemirolast (74) [265], a new oral nonbronchodilator antihistaminic agent is also a pyrimidine derivative. It has demonstrated sufficient antihistaminic activity to warrant its use in severe asthma. Another compound, piprinhydrinate (75) [266] is also a pyrimidine derivative.

1.6.2.13 Analgesics / NSAID drugs

Acetiamine (76a) [267], bentiamine (76b) and fursultiamine (76c) [268] are new lipid-soluble forms of thiamine (vitamin B1) having therapeutic use in beriberi, polyneuritis, encephalopathy, pain, malnutrition and alcoholism and especially in the treatment of long-standing insulin-dependent diabetes mellitus. Fursultamine has been reported to inhibit the arachadonic acid cascade-line activation and reverse the increase in CBF (Coronary Blood Flow).



Afloqualone (77) [269] has been evaluated as a successful anti-inflammatory agent with lower back pain patients. Epirizole (78) [270], another NSAID, is suggested to be a COX-2 inhibitor. Ademetionine (79) [271] is primarily used in conjunction to glucosamine and chondroitin therapy. Octotiamine (80) [272], a vitamin B1 derivative also exhibits anti-inflammatory activity. Proquazone (81) [273], a condensed pyrimidin-2-one derivative has been reported to exhibit good NSAID potential.



1.6.2.14 Metabolic Elelctrolytes

Orotic acid (82) [274], a simple pyrimidine derivative and its mineral forms are used in metabolic therapy, especially for cardiovascular patients to prevent heart failure in cardiomyopathy. Oroate is needed as a key intermediate in biosynthesis of pyrimidines nucleotides, [275] which are building blocks for DNA and RNA required for the final protein synthesis.

1.6.3 Conclusion.

Pyrimidines occupy a distinct and unique place in our life. This heterocyclic moiety has great biological and medicinal significance. A large array of pyrimidine drugs possesses a variety of medicinal properties. These properties include anticancer, antibacterial, antiprotozoal, antimicrobial, antiviral, antihypertensive, antihistaminic, anti-inflammatory, analgesic, CNS-active to metabolic adjuvant.

Keeping in mind various biomedical applications and with a view to further assess the pharmacological profile of bi/tricyclic aromatic heterocycles related to pyrimidines, a new class of Chromeno Pyrimidinones have been synthesized in the frame work of this doctoral thesis.

1.7 THIAZOLIDINONE: A MAGIC MOIETY

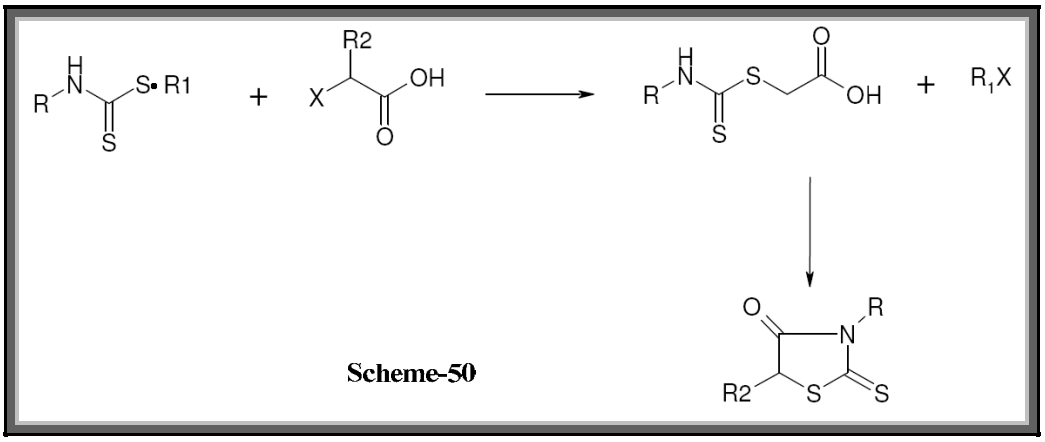
Thiazolidinone, a saturated form of thiazoles with carbonyl group on fourth carbon, has been considered as a magic moiety (wonder nucleus) which posses almost all types of biological activities. This diversity in the biological response profile has attracted the attention of many researchers to explore this skeleton to its multiple potential against several activities. Thiazolidinones are the derivatives of thiazolidine which belong to an important group of heterocyclic compounds containing sulfur and nitrogen in a five member ring. A lot of research work on thiazolidinones has been done in the past. The nucleus is also known as wonder nucleus because it gives out different derivatives with all different types of biological activities. Numbers of methods for synthesis by using various agents are available in the references.

1.7.1 Chemistry of Thiazolidinone

Considerable confusion concerning the structure of 4-thiazolidinones exists in the early literature and noncyclic formulas were at first proposed for pseudothiohydantoin and for rhodanine [276]. 4-thiazolidinones are derivatives of thiazolidine with a carbonyl group at the 4 position [277]. Substitution is possible at 2, 3 and 5 position. Various optical and geometrical isomers are reported in the references [278]. A series of regioselective isomers has been reported in some works [279, 280]. The carbonyl group of 4- thiazolidinone is highly unreactive. But in few cases 4-thiazolidinone on reaction with Lawesson's reagent gives corresponding 4-thione derivatives [281]. A detail study of tautomerism in 2- imnothiazolidine-4-one has been done by *Akerblom E.* [282].

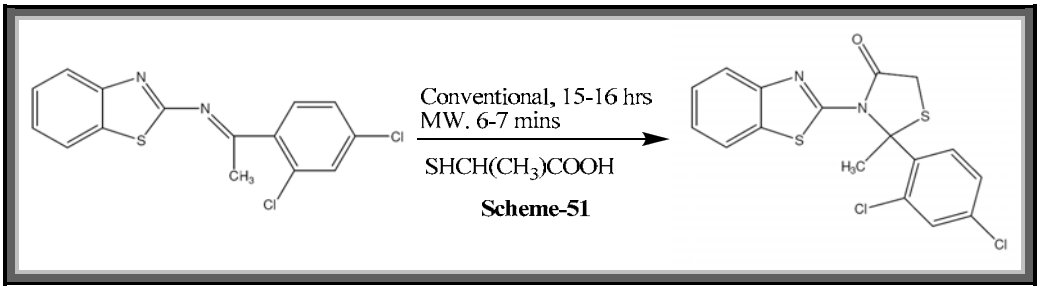
1.7.2 Synthesis of 4-Thiazolidinone

Several methods for syntheses are available in literature which involves conventional one pot, two pot synthesis [283] and microwave as well as combinatorial syntheses methods. The dithiocarbamates formed by the reaction of primary amine with carbon disulfide in the presence of base react with haloalkanoic acid in the presence of $NaHCO_3$ to give substituted 2-thiono-4- thiazolidinones as presented in the scheme 50.



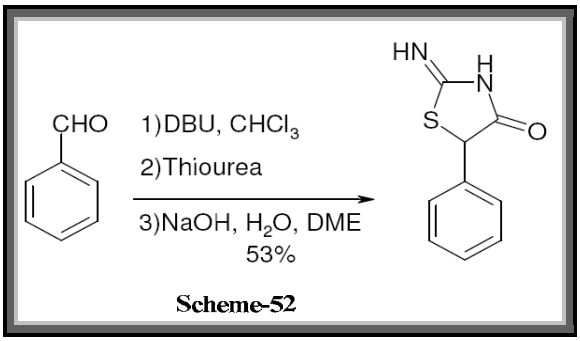
Scheme-50

The synthesis of 2-imino-4- thiazolidinones-4-14C has been reported by using thiourea and sodium salt of labeled monochloroacetic acid [284]. Another method of synthesis of 4- thiazolidinones is by use of thiocyanate, alkyl isothiocyanate with hydrazide/ acetamide followed by the treatment with ethyl bromoacetate and sodium acetate [285]. Schiff's bases obtained by the condensation of ketones and amines also react with mercaptoacetic acid to give 2,2-disubstituted-4-thiazolidinones [286]. Desai KR *et al* [287] has carried out the microwave assisted synthesis of thiazolidinone from the Schiff's bases (scheme 51) by using thiolactic acid. The products were synthesized by conventional and microwave synthesis and the yield were compared with each other. They concluded that the percent yield with the microwave irradiated synthesis was better than the conventional.



Scheme-51

One pot three component synthesis containing aldehyde, thiourea and chloroform (scheme 52) to give 2-amino- 4-thiazolidinone derivatives was also reported [288]. Various imino thiazolidinones were developed by using different reagents with different reaction conditions.



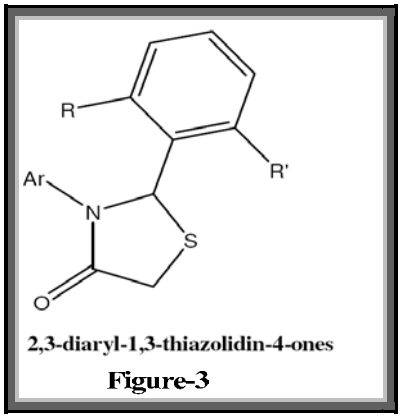
Scheme-52

Use of task specific ionic liquid as synthetic equivalent of ionic liquid phase matrices for the synthesis of small library of 4-thiazolidinone is also possible. Ethylene glycol is functionalized in good yields with 4- (formylphenoxy) butyric acid by using DCC/ DMAP catalyst. The synthesis was performed by one pot three component condensations under microwave dielectric heating [289, 290]. Lot of work has been done on the microwave dielectric heating based techniques either one step three components or two step processes [291-295]. Microwave method is easiest and rapid method of synthesis. The yield of product obtained is better than the conventional technique. Generally environmentally benign catalysts are used for the synthesis which helps in the less pollution and lower wastage of the reagents.

1.7.3 Pharmacological importance of 4-Thiazolidinone

A. Anti-HIV activity

The anti-HIV activity of several series of 2,3-diaryl-1,3-thiazolidin-4-ones (Fig.3) has been studied. Which are reported as a new family of antiviral agents acting as NNRTI's with minimal cytotoxicity [296-298].



2,3-diaryl-1,3-thiazolidin-4-ones

Figure-3

2-adamantyl-substituted thiazolidin-4- ones (Fig. 4) were synthesized and evaluated for activity against HIV-1 (IIIB) and HIV-2(ROD) in CEM cell cultures, by taking Nevirapine as reference compounds [299].

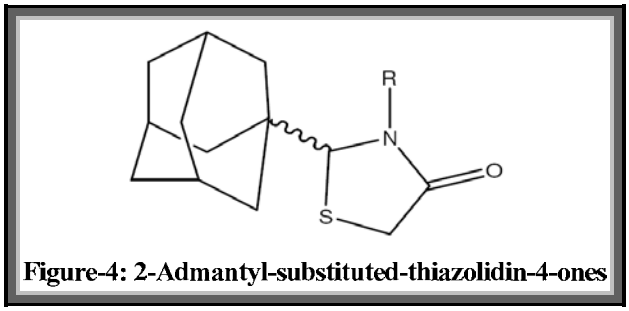


Figure-4: 2-Admantyl-substituted-thiazolidin-4-ones

Some researchers reported 2-(2,6- dibromophenyl)-3-heteroaryl-1,3-thiazolidin- 4-one derivatives as shown in the Fig 5. A positive correlation between size of the halogen substituent and HIV-RT inhibitory activity was taken as logic for the synthesis [300].

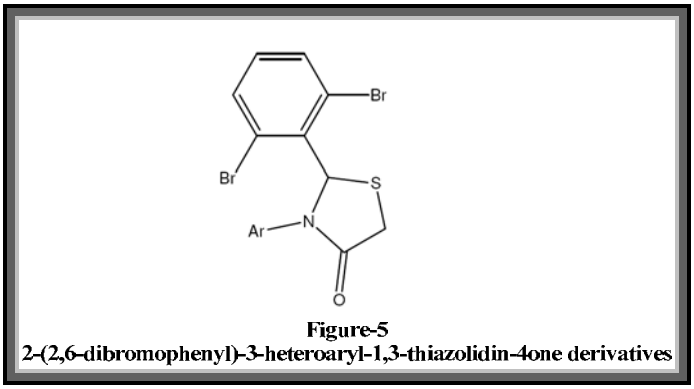


Figure-5
2-(2,6-dibromophenyl)-3-heteroaryl-1,3-thiazolidin-4one derivatives

Microwave-assisted synthesis of 2,3- diaryl-1,3-thiazolidin-4-ones (Fig.6) was performed in order to achieve striking reductions in reaction times, better yields, cleaner reactions [301].

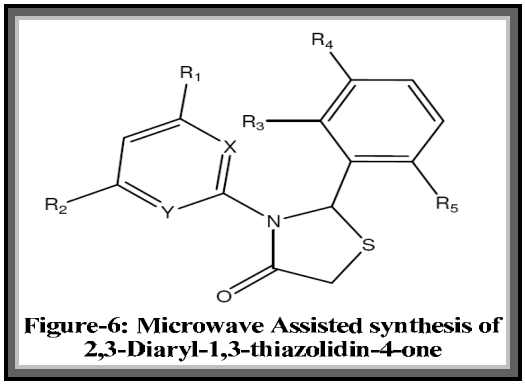


Figure-6: Microwave Assisted synthesis of 2,3-Diaryl-1,3-thiazolidin-4-one

Recently prediction of Anti-HIV activity of 1,3,4-thiazolidinone dervatives were made on the basis of QSAR. CoMFA and CoMSIA were the two models used for the analysis. Based on the structures and biodata of previous thiazolidinone analogs, 3D-QSAR studies have been performed with a training set consisting of 96 molecules [302].

B. Anticonvulsant activity

Number of articles were found for the anticonvulsant potential of 4-thiazolidinones where substitution on 2,3 and 5 positions were done. Most of the compounds were found to exhibit protection against pentylenetetrazole induced seizures [303-308]. Researchers reported the synthesis, characterization, and anticonvulsant evaluation of new N,N'-bis(arylidene)dihydrazide (Fig. 7) and bis*(4*-thiazolidinone) (Fig. 8) derivatives. Upto 90% protection was observed in the pentylenetetrazole seizure [309].

Synthesis of newer thiadiazolyl and thiazolidinonyl quinazolin-4(3H)-ones was done in 2002 by *kumar, A.* [310]. The compounds were screened for their anticonvulsant activity and were compared with the standard drugs, phenytoin sodium, lamotrigine and sodium valproate. Out of the 30 compounds the most active compound was 3-({4-[2-(m-methoxy-hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-1,3,4-thiadiazol-2-yl}methylamino)-2-methyl-6-bromoquinazolin- 4(3H)-one.

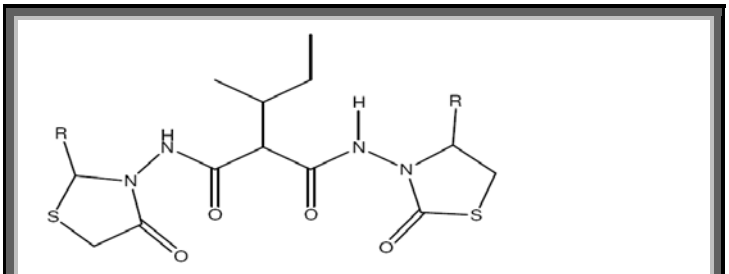


Fig. 7: N,N'-bis(arylidene)dihydrazide

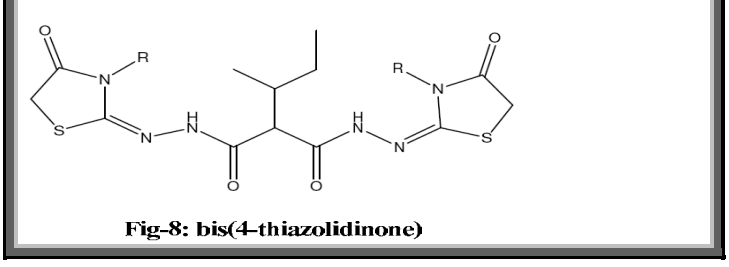


Fig-8: bis(4-thiazolidinone)

Recently anticonvulsant activity of clubbed Thiazolidinone-barbituric acid and Thiazolidinone-triazole derivatives have been reported [311]. The compound in (Fig 9), substituted with different phenylthiazolidinonyl amino moieties at the 5 position of barbituric acid, has shown varying degrees of anticonvulsant activity. While 3-(2-chloroacetyl)-2- arylimino-5-[(*Z*)-arylmethylidene]-1,3-thiazolan-4-ones on treatment with 5-(1-phenoxyethyl)-4H-1,2,4-triazole-3-thiol in identical conditions provided a set of bulkier derivatives which have also shown the anticonvulsant potential (Fig 10).

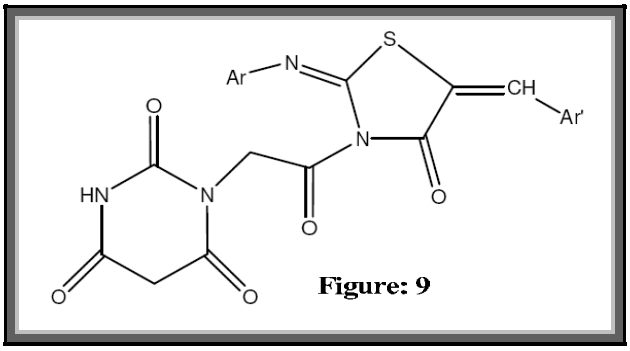
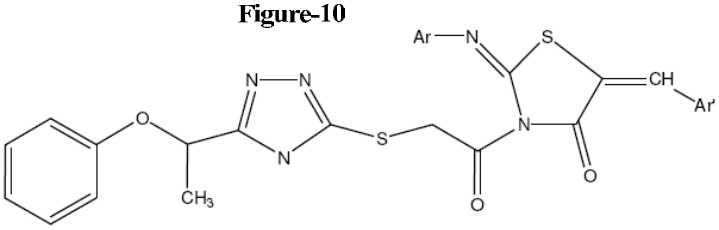


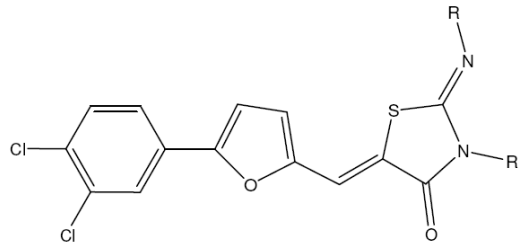
Figure: 9

Figure-10



C. Antimicrobial activity

Bhoot et al have synthesized 2-(ptolylimino)-3-(4-tolyl)-5-[5_-(3,4-dichlorophenyl)-2_-furylidene]-4-thiazolidinone (Fig. 11) and derivatives as an antimicrobial agents. Compounds were screened *in vitro* for their antimicrobial activity towards variety of bacterial strains such as *B. mega, S. aureus, E. coli, P. vulgaris* and fungi such as *Aspergillus niger* at a concentration of 40 µg. In conclusion remarkable inhibition was observed in compounds bearing R=phenyl, 2-methoxyphenyl, 2- methylphenyl, 3-methylphenyl 4-nitrophenyl substituents [312].

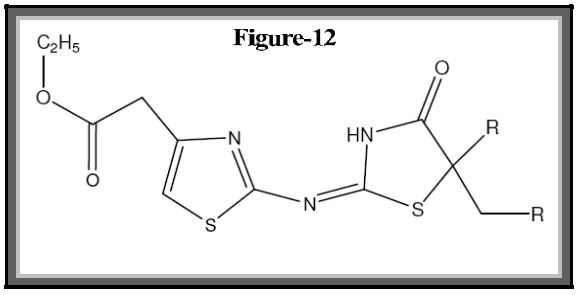


2-(p-tolylimino)-3-(4-tolyl)-5-[5-(3,4-dichlorophenyl)-2 -furylidene]-4-thiazolidinone

Figure-11

Various 5-substituted 5-(N,N-disubstituted aminomethyl)-2-[(4-carbethoxymethylthiazol-2-yl)imino]-4-thiazolidinones (Fig. 12) were synthesized by Altintas et al. Derivatives were screened for their in vitro antibacterial activity against Staphylococcus aureus ATCC 6538, Staphylococcus epidermidis ATCC 12228, Escherichia coli ATCC 8739, Klebsiella pneumoniae ATCC 4352, Pseudomonas aeruginosa ATCC 1539, Salmonella typhi, Shigella flexneri and Proteus mirabilis ATCC 14153 using disk diffusion [313].

Figure-12



Desai and Desai have synthesized five membered sulfur containing heterocyclic derivatives 2-(aryl)-3-[2-(benzothiazolylthio)-acetamidyl]-4-oxo-thiazolidines (Fig. 13). All the compounds have been screened for their antibacterial activity against *Escherchia coli* (Gram−ve), *Staphylococcus aureus* and *Bacillus substilis* (Gram +ve) [314].

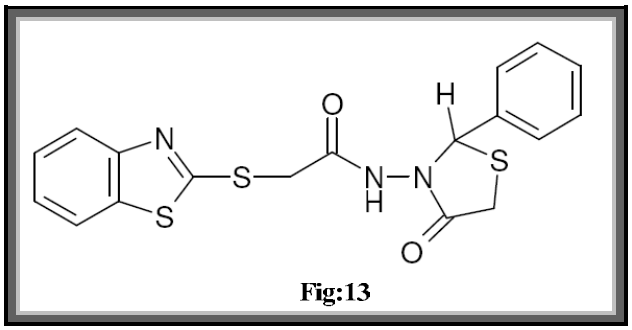


Fig:13

A series of 2-(substituted phenyl)-3-[4-(2,4-dichloro-5-fluorophenyl)-6-(2-thienyl)pyrimidine-2-yl-ureido]-5*H*/methyl/carboxymethyl-4-thiazolidinones (Fig. 14) were prepared. All the derivatives were screened for antibacterial activity [315]. Number of other researchers also synthesized and screened 4-thiazolidinone derivatives for antimicrobial potential [316-322].

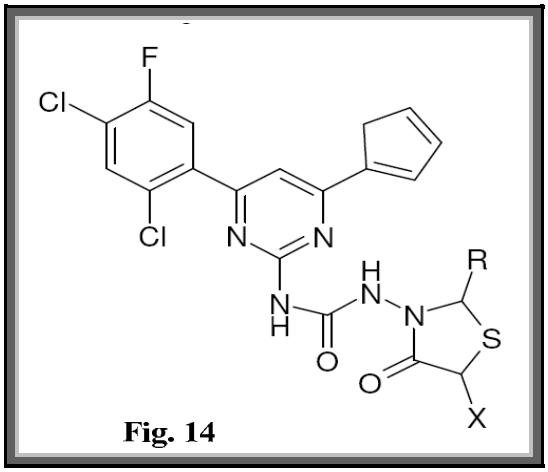


Fig. 14

***D*. Follicle stimulating hormone (FSH) receptor agonist activity.**

Follicle stimulating hormone (FSH) is a 38 kDa protein that triggers maturation of ovarian follicles in women and spermatogenesis in men. It is released from the anterior pituitary gland, following stimulation by gonadotropin releasing hormone (GnRH), and serves as the naturally occurring agonist of the FSH receptor.

Yanofsky SD *et al.* have shown the allosteric activation of FSH receptor, by screening unbiased combinatorial chemistry libraries of thiazolidinone derivatives (Fig. 14), using a cAMPresponsive luciferase reporter assay [323]. They also have shown that discrete modifications in the chemical structure of the thiazolidinone agonists produced compounds with different pharmacological properties [324]. This

was done by preparing substituted 5-alkyl [325], Gama lactam substituted [326] 4-thiazolidinone derivatives.

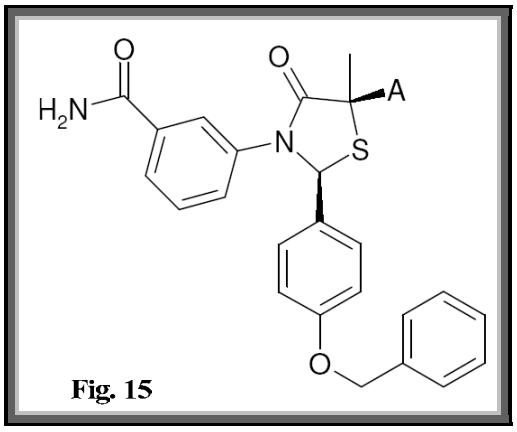


Fig. 15

E. Anti cancer activity & Anti proliferative activity

Ten cytoselective compounds have been identified from 372 thiazolidinone analogues (Fig. 16) by applying iterative library approaches. These compounds selectively killed both non-small cell lung cancer cell line H460 and its paclitaxel-resistant variant H460taxR at an IC50 between 0.21 and 2.93 _M while showing much less toxicity to normal human fibroblasts at concentrations up to 195 _M. A pharmacophore derived from active molecules suggested that two hydrogen bond acceptors and three hydrophobic regions were common features [327].

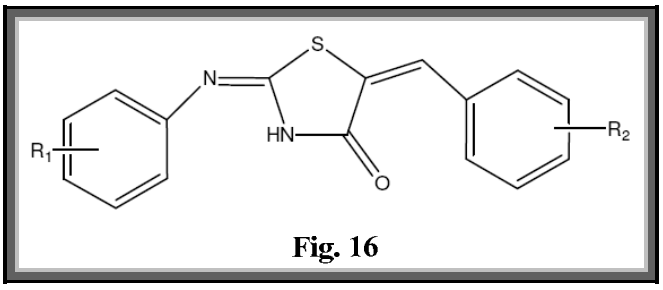


Fig. 16

Gududuru has synthesized a series of 2-aryl-4-oxothiazolidin-3-yl amides and were evaluated for ability to inhibit prostate cancer cells. Few potent compounds were detected, which were effective in killing prostate cancer cells with improved selectivity compared to serine amide phosphates [328]. Various 4-thiazolidinone derivatives were synthesized for in vitro antiproliferative activity on five cell lines of human colon cancers, obtained from the American type culture collection [329-333].

Thiazolidinone amides, carboxylic acids, serine amides were synthesized and tested for possible anticancer activity [334].

F. Anti-inflammatory activity

Sparatore has synthesized aromatic Schiff bases and 2,3-disubstituted-1,3-thiazolidin-4-one derivatives (Fig. 17) as anti-inflammatory agents. Both types of compounds displayed good level of activity against carrageenan induced edema in rat hind paw, while only moderate activity was observed in the writhing test in mice [335].

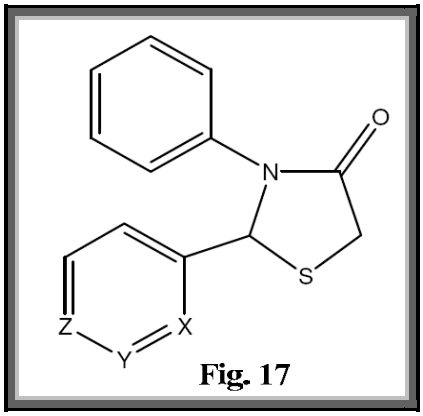


Fig. 17

Kumar A has synthesized 3-[4'-(pchlorophenyl)-thiazol-20-yl]-2-[(substitutedazetidinone/thiazolidinone)-aminomethyl] -6-bromoquinazolin-4-ones (Fig. 18). Some of the compounds have shown satisfactory anti-inflammatory activity [336].

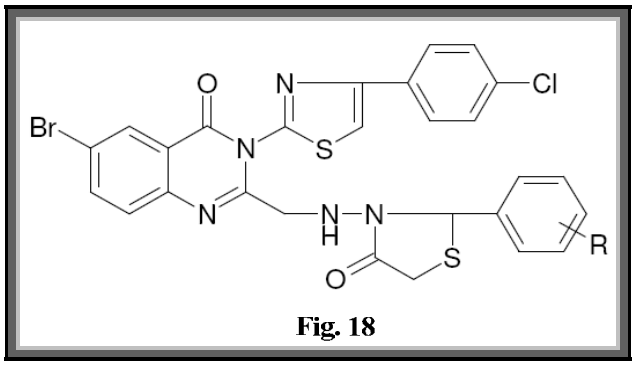


Fig. 18

A series of 4-thiazolidinone compounds, represented by LY178002 (5-[3,5-bis(1,1-dimethylethyl)- 4- hydroxyphenyl]methylene-4- thiazolidinone), have been described as potent inhibitors of cyclooxygenase and 5-1ipoxygenase, also an

inhibitor of phospholipase A 2 and cellular production of LTB4 by human polymorphonuclear leukocytes (PMNL). The results indicate that LY178002 is more effective in suppressing bone damage than the edema [337].

Ottana *et al* investigated 3,3’-(1,2-ethanediyl)-bis[2-aryl-4-thiazolidinone] derivatives, which showed interesting stereo selective anti-inflammatory/ analgesic activities, suggesting that they might preferentially interact with inducible COX-2 isoform [338]. Synthesized 2-imino-4-thiazolidinones and 5-arylidene- 2-imino-4-thiazolidinones were tested for in vivo anti-inflammatory activity in models of acute inflammation such as carrageenan-induced paw edema and pleurisy assay in rats [339, 340]. All derivatives exhibited significant activity levels. In addition, the ability of such a new class of anti-inflammatory agents to inhibit COX isoform was assessed in murine monocyte/macrophage J774 cell line assay.

Newbould studied the anti-inflammatory activity of 2-[(butoxycarbonyl) methylene]-4-thiazolidinone. The compound was found to be devoid of activity against most models of acute inflammation. However it partially inhibited Carageenan induced edema in the rat and prevented completely the development of secondary lesions in the rats injected with adjuvant in the footpad [341]. Geronikaki AA *et al* [342] has performed computer aided discovery of anti-inflammatory potential of 4-thiazolidinones by using PASS (Prediction of Activity Spectra for Substances), a tool for drug discovery.

G. CFTR inhibitor

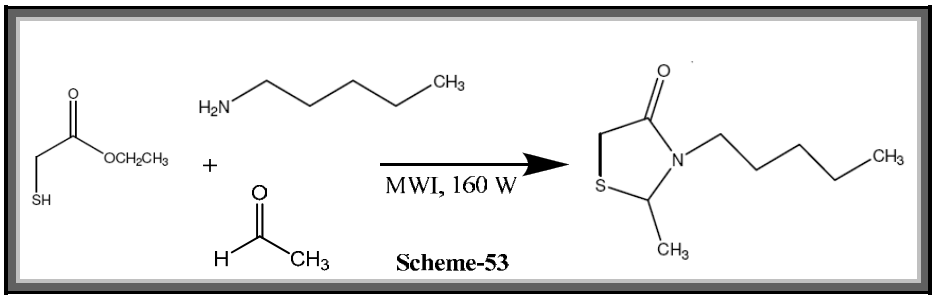
The cystic fibrosis trans membrane conductance regulator (CFTR) is a cAMP-regulated chloride channel, which when mutated can produce the hereditary disease cystic fibrosis. CFTR inhibition is a potential strategy for therapy of secretory diarrheas [343]. Tonghui Ma [344] have shown that the 4- thiazolidinones also have CFTR inhibitory potential. The purpose of the study was to identify high affinity CFTR inhibitors for application to studies of CF disease mechanisms and to the treatment of secretory diarrheas. The primary screening of 50,000 diverse compounds identified a small set of putative inhibitors of the 2-thioxo-4- thiazolidinone compound class. These compounds were unrelated structurally to known CFTR activators and to the CFTR inhibitors diphenylamine-2- carboxylate (DPC), 5-nitro-2(3-phenylpropyl-amino) benzoate (NPPB) and glibenclamide. The most potent CFTR inhibitor identified by screening of library of structural analogs had a K1 of about 300nM for inhibition of Cl-current in human airway cells. Inhibition was rapid, reversible and voltage dependant.

Sonawane ND88, has synthesized thiazolidinone 3-[(3-trifluoromethyl) phenyl]-5-[(4-carboxyphenyl) methylene]-2-thioxo-4-thiazolidinone (CFTRinh-172) which inhibits cystic fibrosis trans membrane conductance regulator (CFTR) chloride channel conductance with sub-micromolar affinity and blocks cholera toxin-induced intestinal fluid secretion. Greatest CFTR inhibition potency was found for 3-CF3 and polar group-substituted-phenyl rings, and a thiazolidinone core. Two compounds with CFTR inhibition potency and solubility >180 lM(>10-fold more than CFTRinh-172) were identified: Tetrazolo- 172, containing 4-tetrazolophenyl in place of 4-carboxyphenyl, and Oxo-172, containing thiazolidinedione in place of the thiazolidinone core. The same researchers and their co workers have shown the CFTR inhibitory activity of thiazolidinone derivatives using computational as well as conventional methods [345].

H. Miscellaneous applications

Apart from pharmacological applications the 4-thiazolidinones have also been used in synthesis. One of the older uses was in the synthesis of merocyanine dyes which extend the sensitivity of silver halide emulsions to wavelengths within the

visible region of the spectrum. Pawelczyk *et. al.* have synthesized the 4-thiazolidinone derivatives by microwave method as a new fragrant substances and unsaturated analogs of jasmines. The n-pentylamine was mixed with acetaldehyde. The mixture was stirred at room temperature under condenser. After 1 h ethyl thioglycolate (or thioglycolic acid) was added. Reagents were irradiated for 5 min with 160 W by microwaves in a flask with condenser and further treated with ethyl acetate.



Scheme-53

I. Conclusion

The literature reveals that 4-thiazolidinone has diverse biological potential, and the easy synthetic routes for synthesis have taken attention of the chemists, pharmacologists and researchers. The anticancer and anti HIV activities are the most encouraging activities for the pharmacists. Also the research in anticonvulsant, FSH agonistic and CFTR inhibitory activity has given positive results. By the present scenario it can be concluded that 4- thiazolidinones have a great potential which remain to be disclosed till date.

Thus, keeping in mind this potential some novel thiazolidin-4-ones containing the indoline nucleus have been synthesized and their biological activity has also been checked.

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CHAPTER – 2

Section-A

Aqua mediated and microwave assisted synthesis of 2-Amino-7-hydroxy-4-(substituted phenyl)-4*H*-chromene-3-carbonitriles

2.1 BENZOPYRAN- Nature's Preferred "Privileged" Structure

The term "privileged structure" was first introduced in 1988. A privileged structure was defined as "a single molecular framework able to provide ligands for diverse receptors." It was envisaged, that the privileged structures could be a valuable alternative in the search for new receptor ligands by suitably decorating these substructures. Since then, an increasing number of sub structural frameworks have been described as privileged structures, including indoles, aryl piperazines, spiro phenylpiperidines, biphenyls, benzopyranes and 1,4-Dihydropyridines. These privileged structures have since then, deliberate or not, been used extensively in medicinal chemistry programs to identify new ligands.

From the outset, it was clear that ultimate success of these efforts was contingent on the proper choice of a privileged natural product motif to be used as a starting point. In deliberating, a structure was required which was found as a subunit in a large number of natural products with diverse biological activities, and this template needed to accommodate the installation of a maximum degree of diversity. Furthermore, the scaffold should contain one or more rigid ring systems such that substituents would be presented to potential binding targets in a well-defined fashion.
A search of chemical abstracts revealed the 2,2-Dimethyl- 2*H*-benzopyran moiety to be present in more than 4000 compounds including natural products and designed structures. The search of chemical abstracts was performed on SciFinder.

The relatively high incidence of this benzopyran unit (and its derivatives, vide infra) in natural products is partially attributable to the numerous prenylation and cyclization reactions in many polyketide biosynthesis pathways. Examining the characteristics of many biologically active, natural products like benzopyran compounds, reveals their diverse structural properties, and more importantly, their wide ranging biological actions, suggesting that derivatives of the benzopyran motif may be capable of interacting with a variety of cellular targets. In addition, the fact that many of the structures are active in cell-based assays suggested that derivatives of the benzopyran unit remain sufficiently lipophilic to cross cell membranes, a key feature of any biologically relevant small molecule library [1].

Chromene derivatives are an important class of compounds, widely present in plants, including edible vegetables and fruits [2]. Numerous bioactive natural products have been identified, and the presence of the chromene-based structure has been associated with the capacity to prevent disease [3]. Synthetic analogues have been developed over the years, some of them displaying remarkable effects as pharmaceuticals [4], including antifungal [5], anti-microbial [6], molluscidial [7], anticoagulant, spasmolytic, diuretic, anticancer and antianaphylactic characteristics [8]. Moreover, nitrogen-containing heterocycles[9, 10] are also of broad pharmaceutical interest and significance, which justifies our continuing efforts in designing novel heterocyclic molecules of biological importance.

Also, the development of environmentally benign, efficient and economical methods for the synthesis of biologically interesting compounds remains a significant challenge in synthetic chemistry. The chemical industry is one of the major contributors to environment pollution, owing to the use of hazardous chemicals and in particular large amounts of flammable, volatile and often toxic organic solvents. Green chemistry emphasizes the need for environmentally clean synthesis, which involves improvement in selectivity, high atom efficiency, elimination of hazardous reagents, and easy separation with recovery and reuse of reagents. As a result, volatile organic solvents are being replaced by non-toxic, nonvolatile media such as ionic liquids, polyethylene glycol, and water. Alternatively, the reactions are carried out under solvent-free conditions. The phenomenal response, as evident from the growing number of publications, in order to achieve this goal is overwhelming. It is more advantageous to carry out reactions under solvent-free conditions [11]. Particularly, in recent years, reactions under solvent-free conditions have continuously attracted the attention of researchers both from academia and industry. This is due to the fact that without solvent, reactions usually need shorter reaction time, simpler reactors, and require simple and efficient workup procedures.

Multicomponent reactions, on the other hand, have become very popular in the discovery of biologically active novel compounds due to its simple experimentation, atom economy and high yields of the products [12].

2-Amino-4H-pyran derivatives represent an important class of compounds. They are often used in cosmetics and pigments, and utilized as potentially biodegradable agrochemicals [13, 14, 15]. Polyfunctionalized 4H-pyrans also constitute a structural unit of many natural products [16, 17] and biologically interesting compounds which possess various pharmacological activities [18], such as antiallergic [14], antitumor [19] antibacterial [20, 21, 22, 23, 24]. 4H-Pyran derivatives are also potential calcium channel antagonists [25] which are structurally similar to biologically active 1, 4-dihydropyridines.

2.1.1 Some previous synthetic attempts

Earlier 2-Amino-4H-pyrans were synthesized by the cyclization of arylidene malononitriles with b-dicarbonyl compounds in the presence of base such as piperidine [26], morpholine, pyridine [27], triethylamine [28, 29], sodium methoxide, or 1,1,3,3-tetramethylguanidine. Most of these methods also involve use of volatile solvents, require longer reaction time (approx. 12 h) and difficult to recover catalysts. Below given are a few synthetic route taken by scientists to prepare the 2-Amino chromene derivatives.

U. Sankappa Rai et al.[30] have reported a synthesis (Fig. 1) in ethanol using triethyl amine to first get 2-Imino-2H-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 hours.

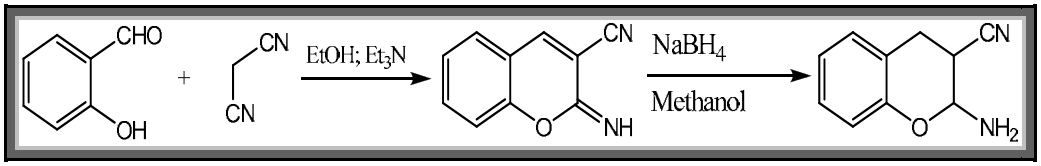


Fig. 1

D. Kumar et. al [31] have reported an environmentally benign synthetic process using Magnesium oxide as the catalyst and by process of grinding (Fig. 2). 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-cyano chromene 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.

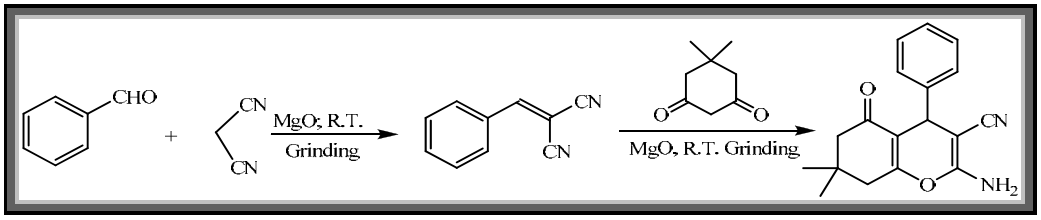


Fig. 2

Similarly, *Hamad. M. Al Matar et. al* [32] have synthesized many compounds of this class using chitosan as the catalyst (Fig.3).

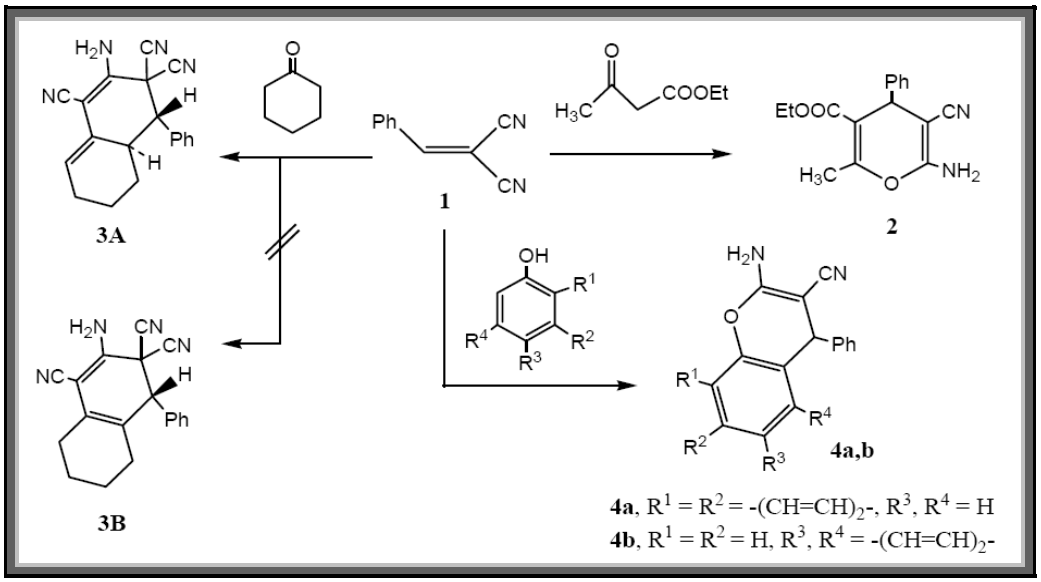


Fig. 3

More so ever they have also studied the formation of the exact product i.e. 2-Amion-3-cyano-7-hydroxy-4H-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 fig. 4.

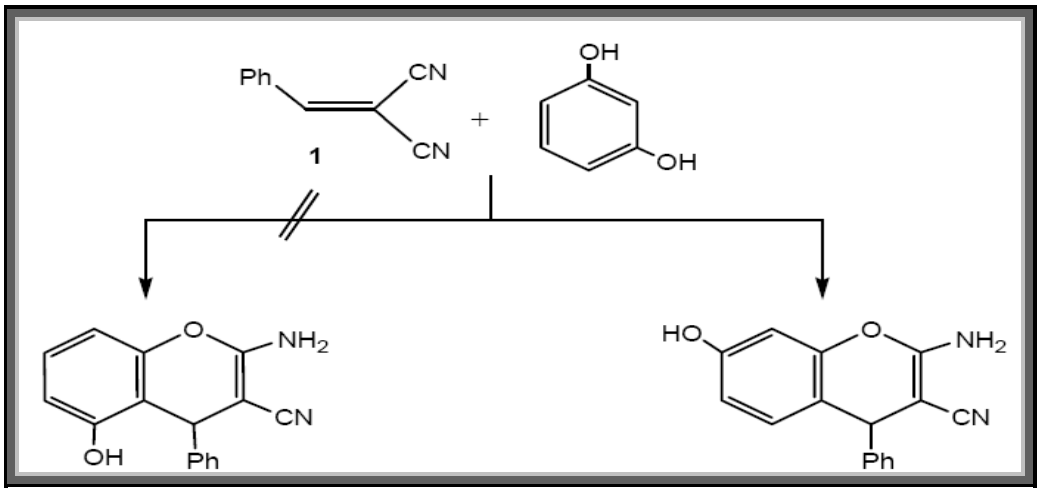


Fig. 4

They have also prepared many such compounds using the same methodology but different starting materials as shown in fig. 5, 6, and 7.

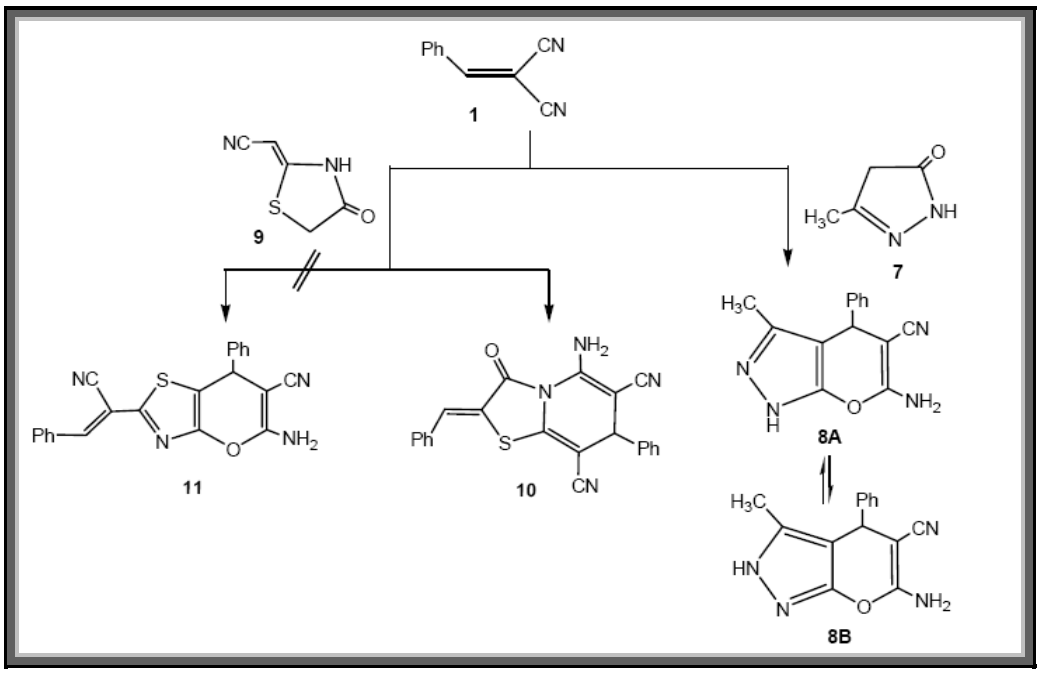


Fig. 5

The above results have been proved using the NOE, HMBC and HMQC calculations using the Proton NMR as well as C^{13} NMR spectrums. The other schemes that they have followed were as follows.

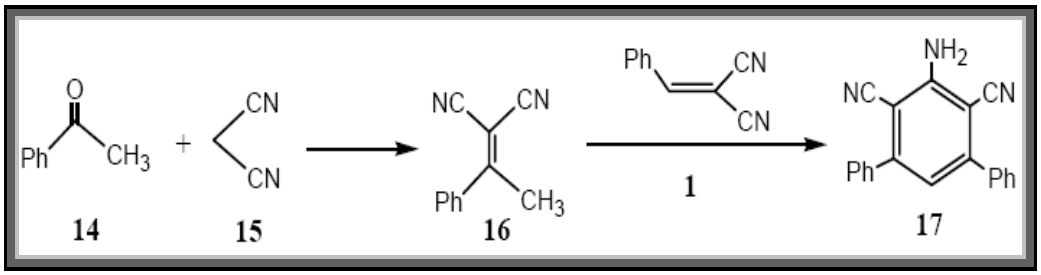


Fig. 6

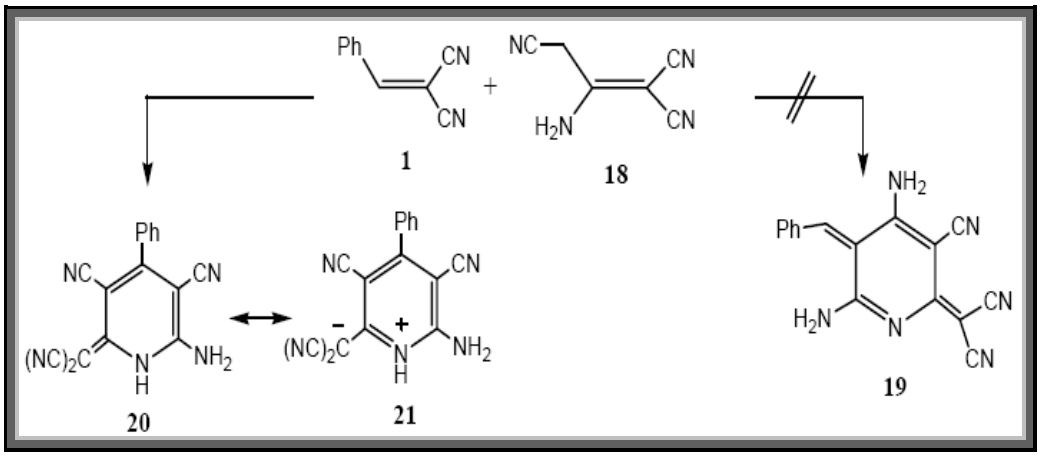


Fig. 7

Naliyapara et.al [33] have extended this work using 4-Hydroxy coumarin as a starting product (Fig.9)

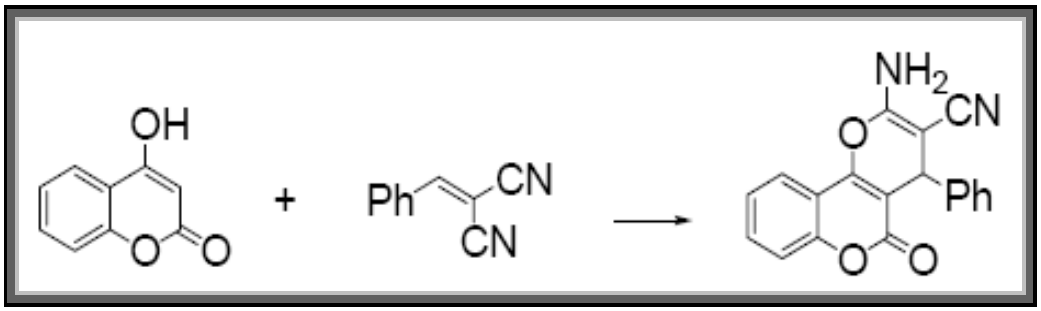


Fig. 8

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 fig. 9 and 10.

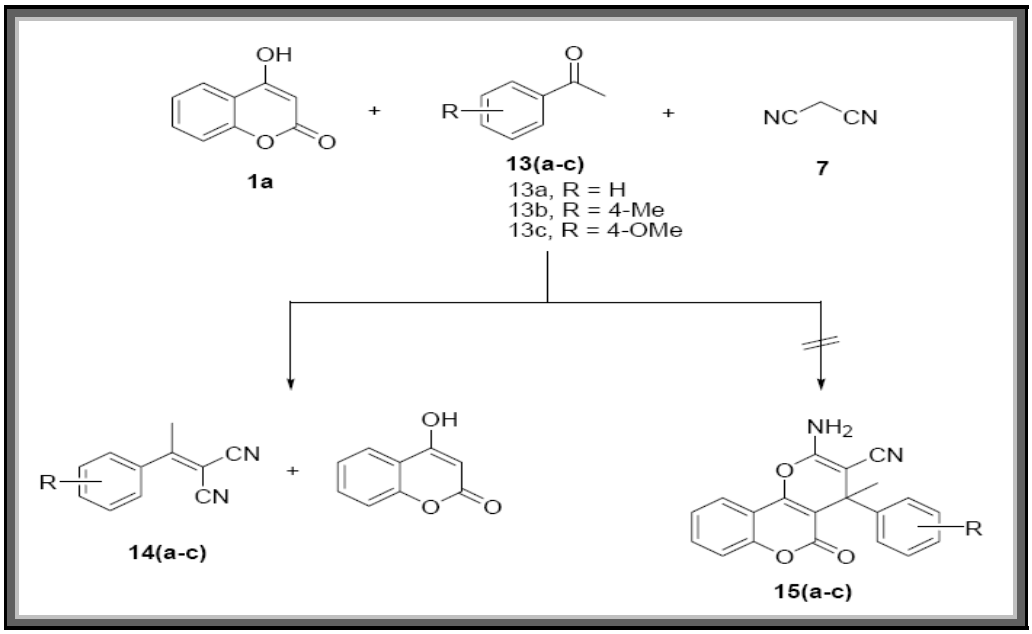

OH
O
O
1a
+
R
O
13(a-c)
13a, R = H
13b, R = 4-Me
13c, R = 4-OMe
+
NC
CN
7
R
CN
CN
14(a-c)
+
OH
O
O
NH2
CN
O
R
O
O
15(a-c)

Fig. 9

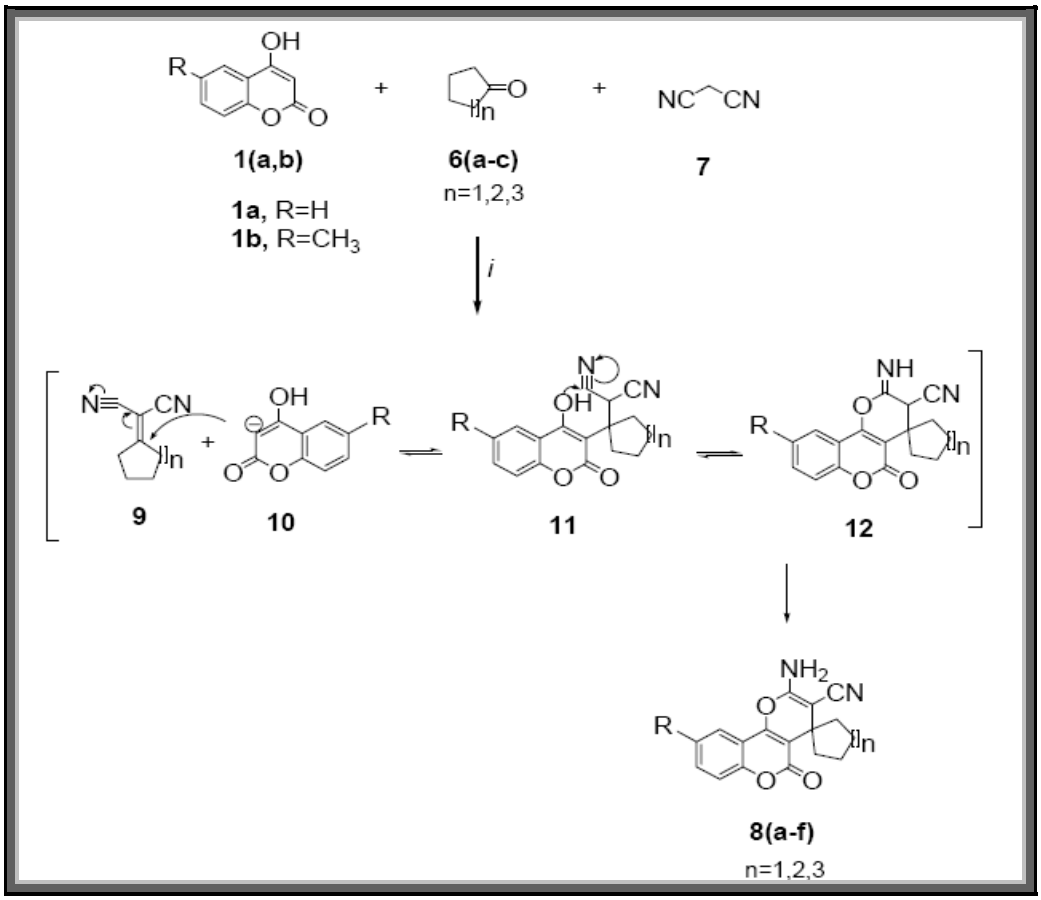

R
OH
O
O
1(a,b)
1a, R=H
1b, R=CH3
+
O
n
6(a-c)
n=1,2,3
+
NC
CN
7
i
N
CN
n
9
OH
R
O
O
10
N
OH
CN
R
n
O
O
11
NH
CN
O
R
n
O
O
12
NH2
CN
O
R
n
O
O
8(a-f)
n=1,2,3

Fig. 10

Scientists have also taken up Gewald reaction to prepare the similar class of compounds but instead of pyrans they have prepared the five membered heterocycles like thiol and furans [34, 35].

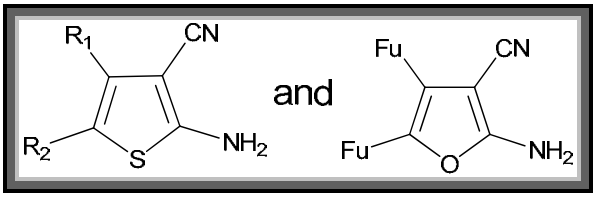


Fig. 11

Kidwai. M. et. al [36] has prepared the same class of the compounds using water as a solvent and potassium carbonate as the required base catalyst.

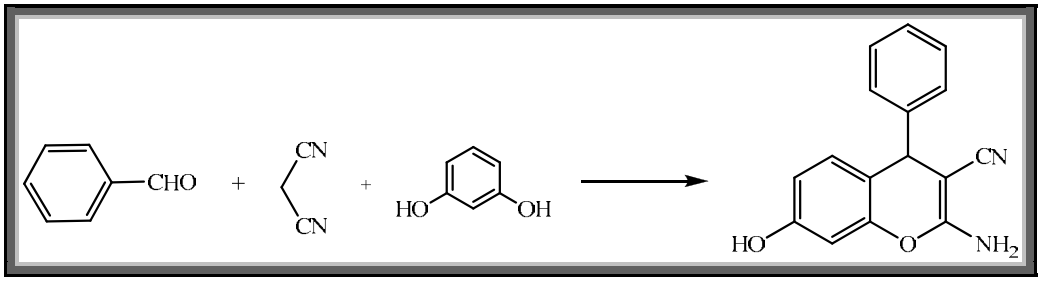


Fig. 12

A total of 8 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-4H-chromene dervatives.

2.2 AIM OF CURRENT WORK

Since many years this laboratory has been working on moieties like chromenes, coumarins, quinolines as well as dihydropyridines and dihydropyrimidines. Some significant results were obtained in Anti-TB[a], Anti Diabetes, Anti Neoplastic[b], Anti-HIV[c] and Multidrug Resistance Reversal[d] activities. Chromenes and their derivatives are well known naturally occurring oxygen-containing heterocyclic compounds which perform important biological functions in nature. It is known that certain natural and synthetic chromene derivatives possess important biological activities, such as antitumor, antihepatotoxic, antioxidant, anti-inflammatory, antispasmolytic, estrogenic and antibacterial activities. These applications have stimulated a continuous search for the synthesis of new compounds in this field and led already to the appearance of some drugs on the market.

Moreover, environment calls on the entire research edifice to define long-term strategic goals for clean chemistry and to reduce the amount of pollutants produced including organic solvents whose recovery is mandated by evermore strict laws. To reduce the dependence on ecologically unsafe chemicals, it is most advantageous to carry out reactions in aqueous media. Water is the cheapest abundantly available solvent. Indeed, water is recognized as an attractive medium for many organic reactions. Reactions in aqueous media are generally environmentally safe, devoid of

[a] Synthesis, in vitro anti-tubercular activity and 3D-QSAR study of 1,4-dihydropyridines. Atul T Manvar, M.Sc; Raghuvir R Pissurlenkar, M Pharm; Vijay R Virsodia, PhD; Kuldip D Upadhyay, PhD; Dinesh R Manvar; Arun K Mishra; Hrishikesh D Acharya; Alpesh R Parecha; Chintan D Dholakia; Anamik K Shah; Evans Clifton Coutinho, Ph.D..Molecular Diversity, **2009** [Epub ahead of print].

[b] Synthesis of 1-(2, 6-dichlorophenyl)-3-methylene-1,3-dihydro-indole-2-one derivatives and invitro anti cancer evaluation against SW620 colon cancer cell line. Vijay Virsodia, Atul Manvar, Kuldip Upadhyay, Rajesh Loriya, Denish Karia, Manu jaggi, Anu Singh, Rama Mukharjee, Mushtaque S. Shaikh, Evans C. Coutinho, Anamik Shah. European Journal of Medicinal Chemistry, 44(3), 1355-1362, **2009**

Synthesis and Biological Activity of Stable and Potent Antitumor Agents, Aniline Nitrogen Mustards Linked to 9-Anilinoacridines via a Urea Linkage. Naval Kapuriya, Kalpana Kapuriya, Xiuguo Zhang, Ting-Chao Chou, Rajesh Kakadiya, Yu-Tse Wu, Tung-Hu Tsai, Yu-Ting Chen, Te-Chang Lee, Anamik Shah, Yogesh Naliapara, Tsann-Long Su. Bioorganic & Medicinal Chemistry, 16, 5413-5423, **2008**.

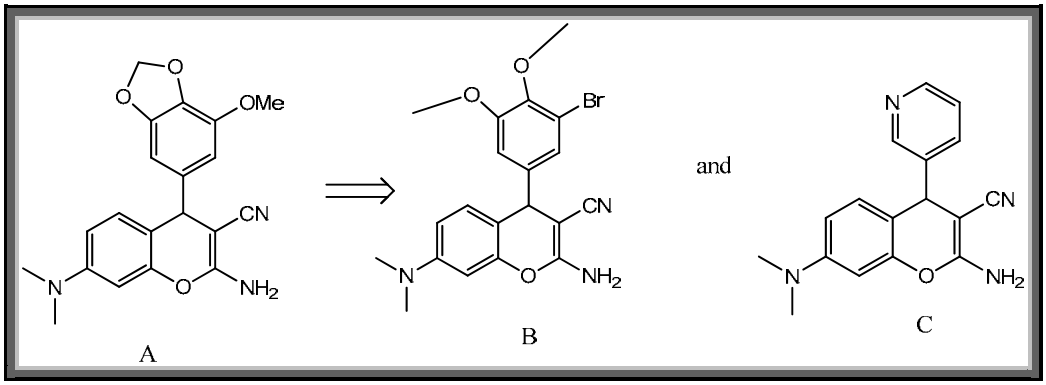
[c] Synthesis and anti-HIV activity of some 3-acetyl/acetoacetyl-4-hydroxy benzopyran-2-ones: An in vitro evaluation by Denish Karia, Atul Manvar, Vinay Trangadia, Anamik Shah. Organic chemistry an Indian Journal, 3(4), 170-175, **2007**.

[d] DP7, a novel Dihydropyridines MDR reverter, shows only weak inhibititory activity on human CYP 3A enzyme(s). Paola D'Elia, Francesco De Matteis, Stefania Dragoni, Anamik Shah, Giampietro Sgaragli, Massimo Valoti. European Journal of Pharmacology, 614(1-3), 7-13, **2009**.

any carcinogenic effects, simple to handle, comparatively cheaper to operate, and especially important in industry. Further, coupling of this solvent-free synthesis with microwave irradiation (MWI) has associated benefits of shorter reaction times, uniform heating, higher yields, enhanced selectivity, and associated ease of manipulation.

2-Amino-chromenes represent an important class of compounds being the main components of many natural occurring products and are widely employed as cosmetics, pigments, [37, 38] and potential biodegradable agrochemicals [39]. Fused chromenes are biologically active compounds with a wide spectrum of activities viz. antimicrobial, [40] antiviral [41, 42], mutagenicity,[43] antiproliferative, [44] sex pheromone,[45] antitumor,[46] and central nervous system activity [47].

Kemnitzer, W. & Cio Kai *et.al.* discovered a new series of 4-Aryl-4H-chromene as Apoptosis inducers using a cell and caspase based high throughput screening assay [48].

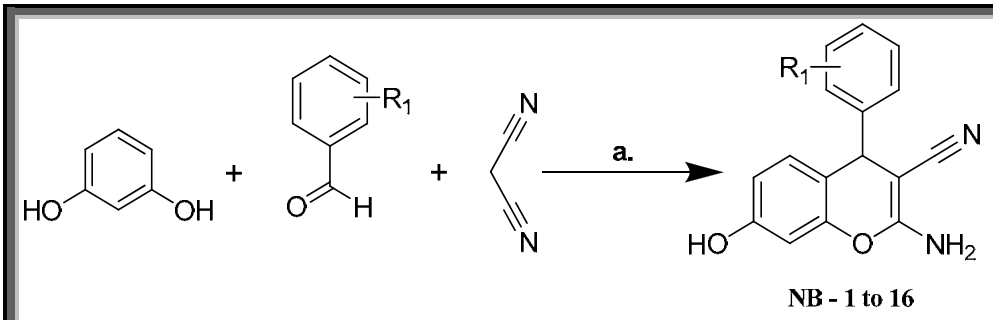


A novel cell- and caspase-based HTS assay,2-Amino-3-cyano-7-(dimethylamino)-4-(3-methoxy-4,5-methylenedioxyphenyl) -4*H*-chromene (A) has been identified as a potent apoptosis inducer. Compound A was found to induce nuclear fragmentation and PARP cleavage, as well as to arrest cells at the G_2/M stage and to induce apoptosis as determined by the flow cytometry analysis assay in multiple human cell lines (e.g. Jurkat, T47D). Through structure−activity relationship (SAR) studies of the 4-aryl group, a 4- and 7-fold increase in potency was obtained from the screening hit A to the lead compounds 2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-7-(dimethylamino)-4*H*-chromene (B) and 2-Amino-3-

cyano-7-(dimethylamino)-4-(5-methyl-3-pyridyl)-4*H*-chromene (C), with an EC_{50} of 19 and 11 nM in the caspase activation assay in T47D breast cancer cells, respectively. The 2-Amino-4-aryl-3-cyano-7-(dimethylamino)-4*H*-chromenes also were found to be highly active in the growth inhibition MTT assay, with GI_{50} values in the low nanomolar range for compound B. Significantly, compound B was found to have a GI_{50} value of 2 nM in the paclitaxel resistant, *p*-glycoprotein over expressed, MES-SA/DX5 tumor cells. Functionally, compound B was found to be a potent inhibitor of tubulin polymerization and to effectively inhibit the binding of colchicine to tubulin.

Thus, in view of the diverse therapeutic activity of chromenes and in continuation to our ongoing endeavor aimed at developing new selective and environmentally benign methodologies using MWI, we report herein the synthesis of substituted 2-Amino-4H-chromene, a scaffold from which a diverse range of other biologically important New Chemical Entities (NCE's) could be generated.

2.3 REACTION SCHEME



NB - 1 to 16

Reagents & Conditions: a.) K_2CO_3 , H_2O , MWI-320 watts, Open Vessel, 2 to 4 mins.,

Aqua Mediated Synthesis of Substituted 2-Amino-3-cyano-4H-chromene derivatives.

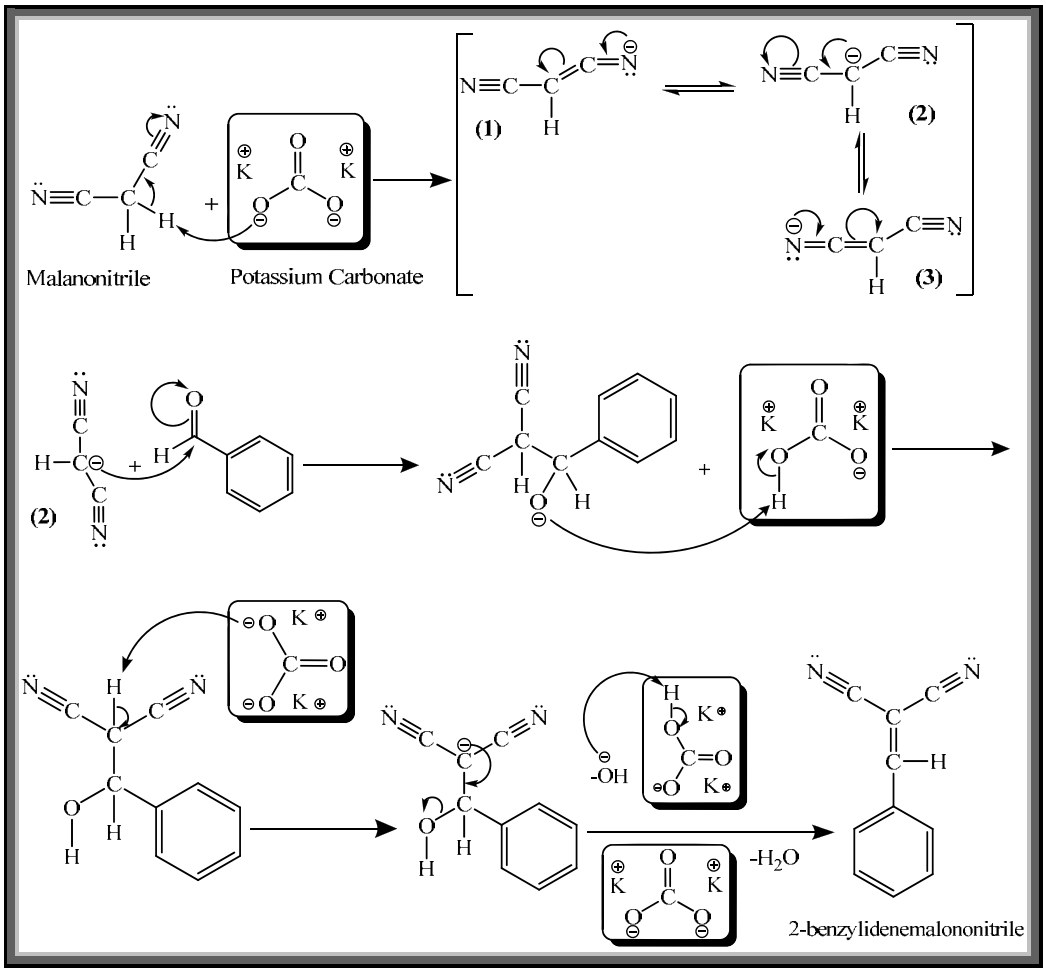
2.3.1 *Physical Data Table*

Code	R_1	M. F.	M. W.	M. P. ^0C	Time (min)	Yield %	R_f
NB-1	4-OCH_3	$C_{17}H_{14}N_2O_3$	294.30	210-212	2:10	92	0.46
NB-2	H	$C_{16}H_{12}N_2O_2$	264.27	142-144	2:30	84	0.48
NB-3	3-Br	$C_{16}H_{11}BrN_2O_2$	343.17	152-154	3:30	86	0.52
NB-4	3-Cl	$C_{16}H_{11}ClN_2O_2$	298.72	178-180	3:40	87	0.54
NB-5	3-NO_2	$C_{16}H_{11}N_3O_4$	309.27	158-160	3:30	94	0.46
NB-6	3-OCH_3, 4-OH	$C_{17}H_{14}N_2O_4$	310.30	162-164	3:50	85	0.51
NB-7	3-OC_2H_5, 4-OH	$C_{18}H_{16}N_2O_4$	324.33	176-178	4:00	84	0.56
NB-8	4-$N(CH_3)_2$	$C_{18}H_{17}N_3O_2$	307.34	156-158	4:10	88	0.50
NB-9	4-NO_2	$C_{16}H_{11}N_3O_4$	309.27	172-174	3:40	92	0.58
NB-10	3,4-OCH_3	$C_{18}H_{16}N_2O_4$	324.33	204-206	2:40	95	0.48
NB-11	4-CH_3	$C_{17}H_{14}N_2O_2$	278.30	172-174	2:50	86	0.53
NB-12	2-OH	$C_{16}H_{12}N_2O_3$	280.27	166-168	3:30	83	0.58
NB-13	Furyl	$C_{14}H_{10}N_2O_3$	254.24	154-156	4:00	80	0.49
NB-14	2-Cl	$C_{16}H_{11}ClN_2O_2$	298.72	182-184	4:00	92	0.53
NB-15	4-Cl	$C_{16}H_{11}ClN_2O_2$	298.72	194-196	4:00	91	0.59
NB-16	4-F	$C_{16}H_{11}FN_2O_2$	282.26	148-150	3:30	84	0.51

TLC solvent system for R_f = Toluene:Ethyl acetate - 7:3. Microwave Irradiation: 320 Watts

2.4 PLAUSIBLE REACTION MECHANISM

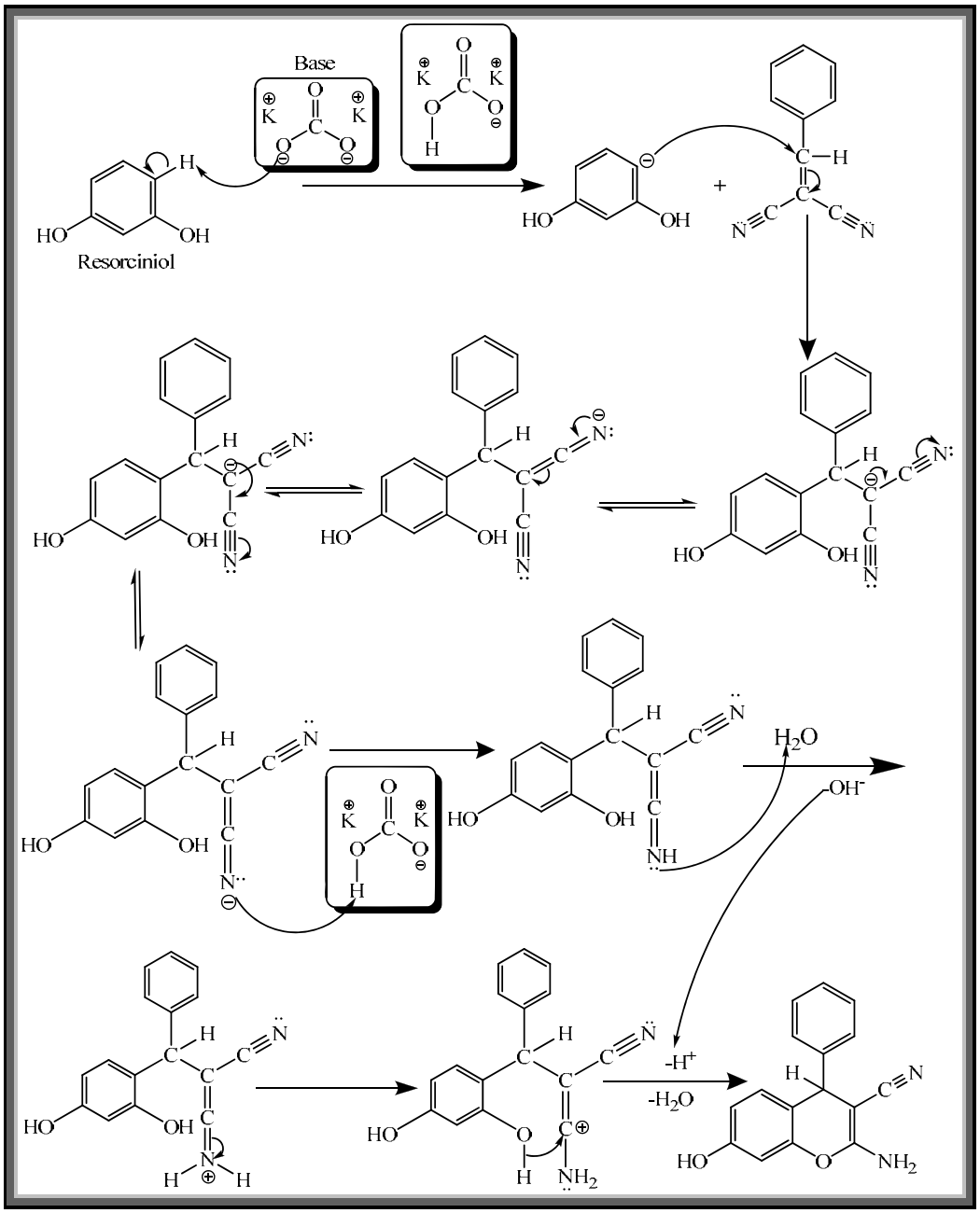
2.4.1 *Step-I: Formation of Benzylidenemalanonitrile*



The reaction mechanism proceeds first via the Knoevenagel Condensation route where in a nucleophilic addition of an active hydrogen compound takes place onto the carbonyl group which when followed by the dehydration reaction and subsequent elimination of water molecule (hence condensation) would afford us the condensate which is often an α, β-conjugated enone. As shown above, first the base molecule, here potassium carbonate would first attack on the active hydrogen compound (malanonitrile) by accepting a proton and thus forming a carbanion, shown herein as the resonating conjugate (2). This carbanion would then attack on the partially positively charged carbon of the carbonyl moiety, forming an unstable intermediate compound with negatively charged oxygen. This would then accept the

hydrogen bonded to the K_2CO_3 and thus the base would then proceed through the anti elimination by cleaving a proton and thus a water molecule to afford the α, β-unsaturated enone, a 2-Benzylidenemalanonitrile in this case.

2.4.2 *Formation of 2-Amino-7-hydroxy-4-(substituted phenyl)-4H-chromene-3-carbonitrile from 2-Benzylidenemalanonitrile*



The base would cleave the acidic proton from resorcinol thus forming a carbanion, this carbanion would then attack on 2-benzylidenemalanonitrile to provide us with resonating conjugates. The negative charge on the nitrogen would abstract the hydrogen out of base and satisfy its valency. The lone pair of that nitrogen would then accept a proton from water molecule and would become an ammonium ion. This is a very unstable moiety and hence the charge displaces on carbon forming a stabilized carbocation. The carbocation is quenched by the elimination of the water molecule by cleaving one hydrogen from the hydroxyl group forming a bond with the carbon containing the positive charge and thus yielding a 2-Amino-7-hydroxy-4-(substituted phenyl)-4H-chromene-3-carbonitrile from 2-Benzylidenemalanonitrile.

2.5 EXPERIMENTAL

2.5.1 *Materials and methods*

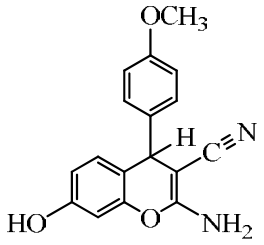
Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. All the reactions were carried out in Samsung MW83Y Microwave Oven which was locally modified for carrying out chemical reactions. IR spectra were recorded in 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 *General Procedure for the synthesis of 2-Amino-7-hydroxy-4-(substituted phenyl)-4H-chromene-3-carbonitriles*

Equimolar amounts of neat reactants, substituted benzaldehyde, malononitrile, and resorcinol were taken in an Erlenmeyer flask, and 10 ml saturated solution of K_2CO_3 in D. M. water was added to it. The reaction mixture was subjected to MWI for a specific time (see Physical data Table) at low power (320 W). The progress of the reaction was monitored by TLC examination at an interval of every 30 seconds. On completion of reaction, the reaction mixture was cooled and was triturated with 2–3 ml of ice cold water to get the solid product, leaving behind K_2CO_3 dissolved in water. The product obtained was filtered, washed with cold water, dried, and recrystallized from ethanol.

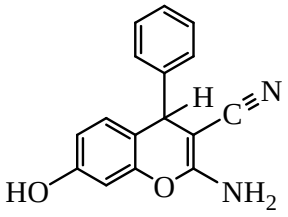
2.6 ANALYTICAL DATA

2.6.1 2-Amino-7-hydroxy-4-(4-methoxyphenyl)-4H-chromene-3-carbonitrile (NB-1)



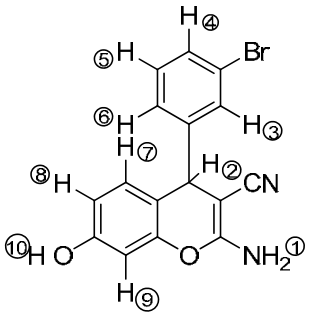
Yield: 92%; M.P.- 210-212 ºC; IR (cm^{-1}): 3610 (O-H stretching of free primary alcohol), 3579, (N-H stretching of free primary amine), 3152 (C-H stretching vibration of aromatic region), 2210 (C≡N stretching of the nitrile group), 1661 (N-H deformation, in plane bending of N-H), 1510-1340 (O-H in plane bending), 1355 (C-N stretching for carbon bonded to amino group), 1265 (Asymmetrical C-O-C stretching found for ethers), 990 (C-H in plane bending of phenyl ring), 685 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 294.10; Anal. Calcd. for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52; O, 16.31 Found: C, 69.25; H, 4.65; N, 9.70; O, 16.12.

2.6.2 2-Amino-7-hydroxy-4-phenyl-4H-chromene-3-carbonitrile (NB-2):



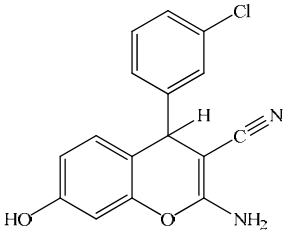
Yield: 84%; M.P.- 142-144 ºC; IR (cm^{-1}): 3615 (O-H stretching of free primary alcohol), 3577 (N-H stretching of free primary amine), 3165 (C-H stretching vibration of aromatic region), 2220 (C≡N stretching of the nitrile group), 1651 (N-H deformation, in plane bending of N-H), 1505-1335 (O-H in plane bending), 1340 (C-N stretching for carbon bonded to amino group), 993 (C-H in plane bending of phenyl ring), 676 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 264.09; Anal. Calcd. for $C_{16}H_{12}N_2O_2$: C, 72.72; H, 4.58; N, 10.60; O, 12.11 Found: C, 71.40; H, 4.35; N, 10.12; O, 11.25.

2.6.3 2-Amino-4-(3-bromophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (NB-3)



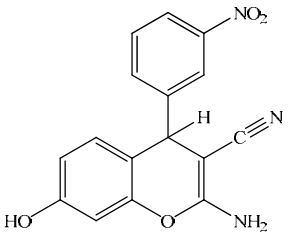
Yield: 86%; M.P.- 152-154 ºC; IR (cm^{-1}): 3630 (O-H stretching of free primary alcohol), 3585, 3321 & 3298, (N-H stretching of free primary amine), 3146 (C-H stretching vibration of aromatic region), 2200 (C≡N stretching of the nitrile group), 1651 (N-H deformation, in plane bending of N-H), 1508-1350 (O-H in plane bending), 1350 (C-N stretching for carbon bonded to amino group), 997 (C-H in plane bending of phenyl ring), 671 (C-H out of plane bending for 1,3-disubstituted phenyl nucleus), 596-528 (C-Br stretching for the halogen group), ^{1}H NMR (DMSO-d_6) δ ppm: 6.25 (s, 2H, H_1), 4.57 (s, 1H, H_2), 7.25 (s, 1H, H_3 J=3.2 Hz), 7.32 (d, 1H, H_4, J=9.2 Hz), 7.15-7.20 (m, 2H, H_5 & H_6, J_{H5}=8 Hz, J_{H6}=*7.2 Hz*), 6.72 (d, 1H, H_7, J= 8.4 Hz.), 6.48-6.51 (d, 1H, H_8, J= 10.8 Hz), 6.46 (s, 1H, H_9, J=2.4 Hz.), 9.41 (s, 1H, H_{10}) MS: *m/z:* 342.00; Anal. Calcd. for $C_{16}H_{11}BrN_2O_2$: C, 56.00; H, 3.23; Br, 23.28; N, 8.16; O, 9.32 Found: C, 55.40; H, 3.05; Br, 22.80 N, 8.00; O, 9.25.

2.6.4 2-Amino-4-(3-chlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (NB-4)



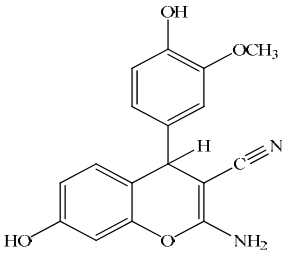
Yield: 87%; M.P.- 178-180 ºC; IR (cm^{-1}): 3602 (O-H stretching of free primary alcohol), 3530 (N-H stretching of free primary amine), 3102 (C-H stretching vibration of aromatic region), 2198 (C≡N stretching of the nitrile group), 1648 (N-H deformation, in plane bending of N-H), 1523-1345 (O-H in plane bending), 1368 (C-N stretching for carbon bonded to amino group), 989 (C-H in plane bending of phenyl ring), 677 (C-H out of plane bending for 1,3-disubstituted phenyl nucleus), 592-520 (C-Cl stretching for the halogen group), MS: *m/z:* 298.05; Anal. Calcd. for $C_{16}H_{11}ClN_2O_2$: C, 64.33; H, 3.71; Cl, 11.87; N, 9.38; O, 10.71 Found: C, 64.15; H, 3.45; Cl, 11.70 N, 9.00; O, 10.25.

2.6.5 2-Amino-7-hydroxy-4-(3-nitrophenyl)-4H-chromene-3-carbonitrile (NB-5)



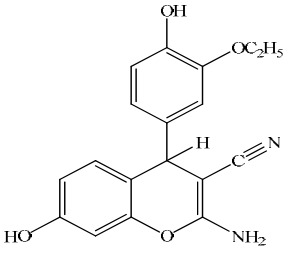
Yield: 94%; M.P.- 158-160 ºC; IR (cm^{-1}): 3612 (O-H stretching of free primary alcohol), 3582, (N-H stretching of free primary amine), 3160 (C-H stretching vibration of aromatic region), 2218 (C≡N stretching of the nitrile group), 1621 (N-H deformation, in plane bending of N-H), 1500-1340 (O-H in plane bending), 1367 (C-N stretching for carbon bonded to amino group), 987 (C-H in plane bending of phenyl ring), 676 (C-H out of plane bending for 1,3-disubstituted phenyl nucleus), 879 and 1545 (C-N stretching for the nitro group), MS: *m/z:* 309.07; Anal. Calcd. for $C_{16}H_{11}N_3O_4$: C, 62.14; H, 3.58; N, 13.59; O, 20.69 Found: C, 62.05; H, 3.45; N, 13.10; O, 20.35.

2.6.6 2-Amino-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-4H-chromene-3 carbonitrile (NB-6)



Yield: 85%; M.P.- 162-164 ºC; IR (cm^{-1}): 3675-3630 (O-H stretching of free primary alcohol), 3560 (N-H stretching of free primary amine), 3171 (C-H stretching vibration of aromatic region), 2240 (C≡N stretching of the nitrile group), 1632 (N-H deformation, in plane bending of N-H), 1525-1375 (O-H in plane bending), 1347 (C-N stretching for carbon bonded to amino group), 988 (C-H in plane bending of phenyl ring), 665 (C-H out of plane bending for phenyl nucleus), 1248 (Asymmetrical C-O-C stretching found for ethers), MS: *m/z:* 310.10; Anal. Calcd. for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03; O, 20.62 Found: C, 65.70; H, 4.45; N, 8.90; O, 20.45.

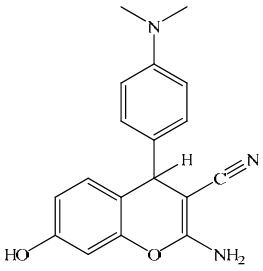
2.6.7 2-Amino-4-(3-ethoxy-4-hydroxyphenyl)-7-hydroxy-4H-chromene-3-carbonitrile (NB-7)



Yield: 84%; M.P.- 176-178 ºC; IR (cm^{-1}): 3680-3650 (O-H stretching of free primary alcohol), 3578 (N-H stretching of free primary amine), 3123 (C-H stretching vibration of aromatic region), 2290 (C≡N stretching of the nitrile group), 1610 (N-H deformation, in plane bending of N-H), 1534-1390 (O-H in plane bending), 1337 (C-N stretching for carbon bonded to amino

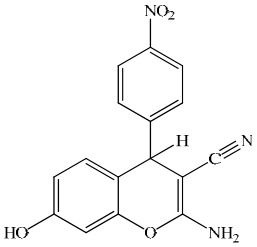
group), 997 (C-H in plane bending of phenyl ring), 648 (C-H out of plane bending for phenyl nucleus), 1288 (Asymmetrical C-O-C stretching found for ethers), MS: *m/z:* 324.11; Anal. Calcd. for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64; O, 19.73 Found: C, 66.50; H, 4.78; N, 8.55; O, 19.61.

2.6.8 2-Amino-4-(4-(dimethylamino)phenyl)-7-hydroxy-4H-chromene-3-carbonitrile (NB-8)



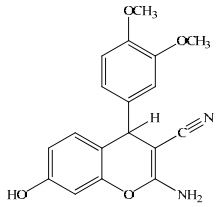
Yield: 88%; M.P.- 156-158 ºC; IR (cm^{-1}): 3620 (O-H stretching of free primary alcohol), 3573, 3325 & 3299, (N-H stretching of free primary amine), 3146 (C-H stretching vibration of aromatic region), 2210 (C≡N stretching of the nitrile group), 1642 (N-H deformation, in plane bending of N-H), 1520-1380 (O-H in plane bending), 1365 (C-N stretching for carbon bonded to amino group), 1301 (C-N stretching for tertiary amines), 997 (C-H in plane bending of phenyl ring), 671 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 307.13; Anal. Calcd. for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67; O, 10.41Found: C, 70.25; H, 5.43; N, 13.55; O, 10.32.

2.6.9 2-Amino-7-hydroxy-4-(4-nitrophenyl)-4H-chromene-3-carbonitrile (NB-9):



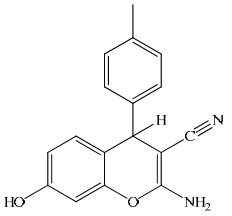
Yield: 92%; M.P.- 172-174 ºC; IR (cm^{-1}): 3620 (O-H stretching of free primary alcohol), 3590, (N-H stretching of free primary amine), 3180 (C-H stretching vibration of aromatic region), 2233 (C≡N stretching of the nitrile group), 1651 (N-H deformation, in plane bending of N-H), 1603 (Asymmetrical stretching for the N-O of the nitrate group),1510-1326 (O-H in plane bending), 1344 (C-N stretching for carbon bonded to amino group), 945 (C-H in plane bending of phenyl ring), 690 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 309.07; Anal. Calcd. for $C_{16}H_{11}N_3O_4$: C, 62.14; H, 3.58; N, 13.59; O, 20.69 Found: C, 62.10; H, 3.43; N, 13.45; O, 20.52.

2.6.10 2-Amino-4-(3,4-dimethoxyphenyl)-7-hydroxy-4H-chromene-3-carbonitrile



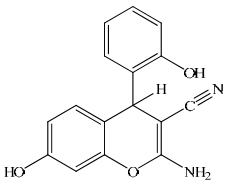
(NB-10) Yield: 95%; M.P.- 204-206 ºC; IR (cm^{-1}): 3601 (O-H stretching of free primary alcohol), 3555 (N-H stretching of free primary amine), 3121 (C-H stretching vibration of aromatic region), 2199 (C≡N stretching of the nitrile group), 1656 (N-H deformation, in plane bending of N-H), 1512-1375 (O-H in plane bending), 1323 (C-N stretching for carbon bonded to amino group), 1251 (Asymmetrical C-O-C stretching found for ethers), 997 (C-H in plane bending of phenyl ring), 671 (C-H out of plane bending for 1,3-disubstituted phenyl nucleus), MS: *m/z:* 324.11; Anal. Calcd. for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64; O, 19.73 Found: C, 66.40; H, 4.83; N, 8.45; O, 19.64.

2.6.11 2-Amino-7-hydroxy-4-p-tolyl-4H-chromene-3-carbonitrile (NB-11)



Yield: 86%; M.P.- 172-174 ºC; IR (cm^{-1}): 3675 (O-H stretching of free primary alcohol), 3551 (N-H stretching of free primary amine), 3133 (C-H stretching vibration of aromatic region), 2218 (C≡N stretching of the nitrile group), 1621 (N-H deformation, in plane bending of N-H), 1528-1358 (O-H in plane bending), 1345 (C-N stretching for carbon bonded to amino group), 985 (C-H in plane bending of phenyl ring), 682 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 278.11; Anal. Calcd. for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07; O, 11.50 Found: C, 73.14; H, 4.93; N, 9.95; O, 11.42.

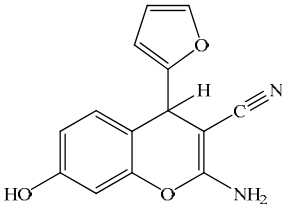
2.6.12 2-Amino-7-hydroxy-4-(2-hydroxyphenyl)-4H-chromene-3-carbonitrile (NB-12)



Yield: 83%; M.P.- 166-168 ºC; IR (cm^{-1}): 3645, 3680 (O-H stretching of free primary alcohol), 3554 (N-H stretching of free primary amine), 3120 (C-H stretching vibration of aromatic region), 2230 (C≡N stretching of the nitrile group), 1649 (N-H deformation, in plane bending of N-H), 1512-1330 (O-H in plane bending), 1365 (C-N stretching for carbon bonded to amino group), 992 (C-H in plane bending of phenyl ring), 691 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 280.08; Anal. Calcd. for

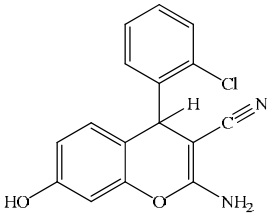
$C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32; N, 9.99; O, 17.13 Found: C, 68.34; H, 4.13; N, 9.85; O, 16.92.

2.6.13 2-Amino-4-(furan-2-yl)-7-hydroxy-4H-chromene-3-carbonitrile (NB-13)



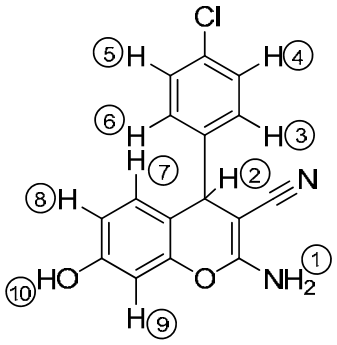
Yield: 80%; M.P.- 154-156 ºC; IR (cm^{-1}): 3650 (O-H stretching of free primary alcohol), 3585 (N-H stretching of free primary amine), 3150 (C-H stretching vibration of aromatic region), 2201 (C≡N stretching of the nitrile group), 1625 (N-H deformation, in plane bending of N-H), 1521-1375 (O-H in plane bending), 1360 (C-N stretching for carbon bonded to amino group), 980 (C-H in plane bending of phenyl ring), MS: *m/z:* 254.07; Anal. Calcd. for $C_{14}H_{10}N_2O_3$: C, 66.14; H, 3.96; N, 11.02; O, 18.88 Found: C, 66.02; H, 3.80; N, 10.85; O, 18.62.

2.6.14 2-Amino-4-(2-chlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (NB-14)



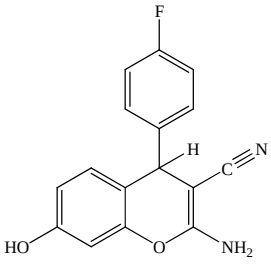
Yield: 92%; M.P.- 182-184 ºC; IR (cm^{-1}): 3648 (O-H stretching of free primary alcohol), 3567 (N-H stretching of free primary amine), 3150 (C-H stretching vibration of aromatic region), 2215 (C≡N stretching of the nitrile group), 1640 (N-H deformation, in plane bending of N-H), 1512-1345 (O-H in plane bending), 1365 (C-N stretching for carbon bonded to amino group), 985 (C-H in plane bending of phenyl ring), 675 (C-H out of plane bending for phenyl nucleus), 595-510 (C-Cl stretching for the halogen group), MS: *m/z:* 298.05; Anal. Calcd. for $C_{16}H_{11}ClN_2O_2$: C, 64.33; H, 3.71; Cl, 11.87; N, 9.38; O, 10.71 Found: C, 64.12; H, 3.60; Cl, 11.74; N, 9.21; O, 10.62.

2.6.15 2-Amino-4-(4-chlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (NB-15)



Yield: 91%; M.P.- 194-196 ºC; IR (cm^{-1}): 3686-3672 (O-H stretching of free alcohol), 3477-3317 (N-H stretching of the amino group), 3136-3059 (C-H stretching for the aromatic region), 2191 (C≡N stretching of the nitrile group), 1649-1583 (N-H deformation due to in plane bending), 1242-960 (C-H in plane bending of the phenyl ring), 823 (out of plane benzene ring for 1,4-Disubstituted benzene ring), 852 & 725 (Out of plane bending frequencies for disubstituted phenyl ring), 746 & 704 (C-Cl stretching for monochlorinated aromatic system) ^{1}H NMR (DMSO-d_6) δ ppm: 5.77 (s, 1H, H_1), 4.53 (s, 1H, H_2), 7.04-7.08 (d, 2H, H_3 & H_6 J_{H3} = 4.4 Hz, J_{H6} = 4.4 Hz, $J_{H3\text{-}6}$ = 13.6 Hz.), 7.16-7.19 (d, 2H, H_4 & H_5, H_4 & H_5 J_{H4} = 4.4 Hz, J_{H5} = 4.4 Hz, $J_{H4\text{-}5}$ = 13.6 Hz.), 6.43-6.46 (d, 1H, H_7, J_{H7} = 10.8 Hz.), 6.62-6.64 (d, 1H, H_8, J_{H8} = 8.4 Hz.), 6.41 (s, 1H, H_9, J_{H9} = 3.2 Hz.), 9.22 (s, 1H, H_{10}), MS: *m/z:* 298.05; Anal. Calcd. for $C_{16}H_{11}ClN_2O_2$: C, 64.33; H, 3.71; Cl, 11.87; N, 9.38; O, 10.71 Found: C, 64.09; H, 3.57; Cl, 11.64; N, 9.27; O, 10.58.

2.6.16 2-Amino-4-(4-fluorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (NB-16)



Yield: 84%; M.P.- 148-150 ºC; IR (cm^{-1}): 3630 (O-H stretching of free primary alcohol), 3585 (N-H stretching of free primary amine), 3146 (C-H stretching vibration of aromatic region), 2200 (C≡N stretching of the nitrile group), 1651 (N-H deformation, in plane bending of N-H), 1508-1350 (O-H in plane bending), 1350 (C-N stretching for carbon bonded to amino group), 997 (C-H in plane bending of phenyl ring), 671 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 282.08; Anal. Calcd. for $C_{16}H_{11}FN_2O_2$: C, 68.08; H, 3.93; F, 6.73; N, 9.92; O, 11.34 Found: C, 67.89; H, 3.77; F, 6.64; N, 9.75; O, 11.18.

2.7 SPECTRAL DISCUSSION

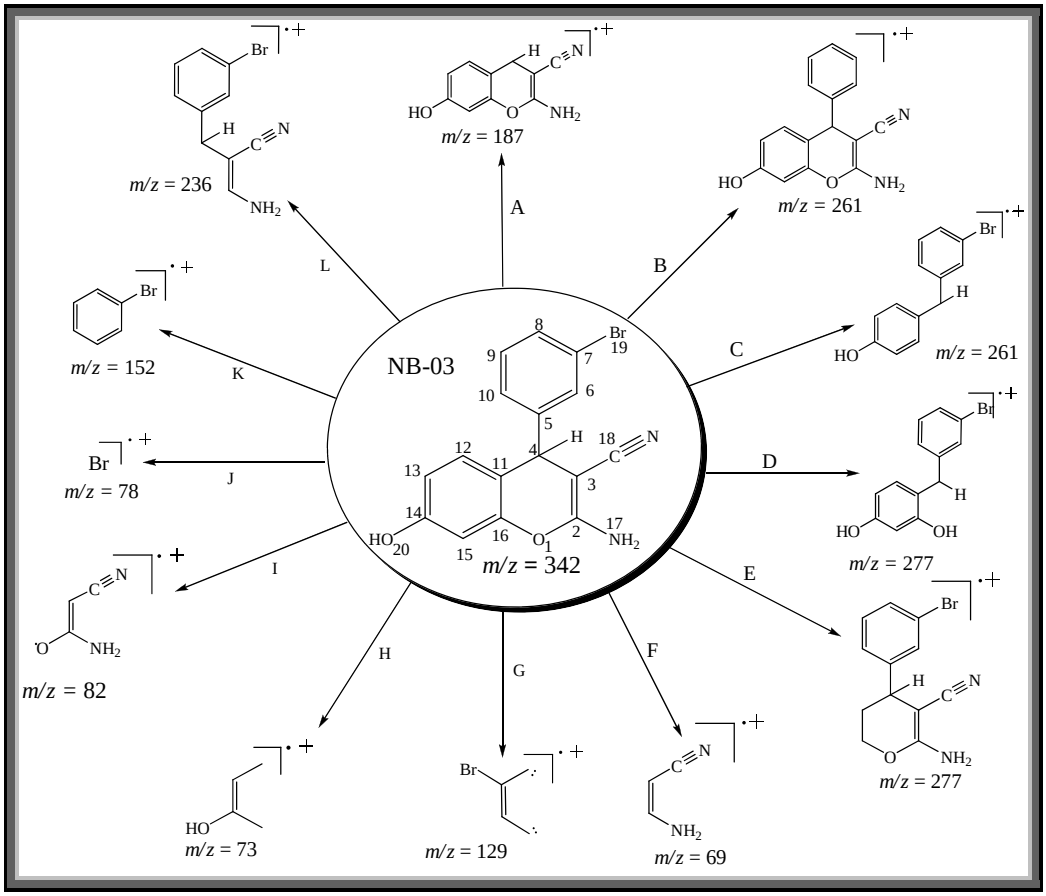
2.7.1 *IR SPECTRAL STUDY*

IR spectra were recorded on **Shimadzu FT-IR-8400** model using KBr pellet method. Various functional groups present were identified by characteristic frequency obtained for them. The characteristic bands of Hydroxyl groups were obtained for stretching at 3640-3600 cm^{-1}, and those for bending were obtained at 1050-1250 cm^{-1}. The characteristic bands of amino group were obtained for stretching at 3500-3400 cm^{-1} with a deformation due to in plane bending at 1650-1580 cm^{-1}. Nitrile group characteristic bands were seen at 2250-2100 cm^{-1}while the general aromatic stretching bands were observed at 3200-3000 cm^{-1}. The characteristic bands for halogen groups like chlorine and bromine were found at 740-700 cm^{-1} & 600-500 cm^{-1}. Also characteristic stretching frequencies of 1,3-Disubstituted and 1,4-Disubstituted phenyl ring were found at 671 cm^{-1} and 823 cm^{-1} respectively suggesting the correct formation of the desired products (NB-1 to NB-16).

2.7.2 *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. The probable Mass fragmentation pattern for the representative compound of each series is discussed below.

2.7.2.1 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF NB-03*

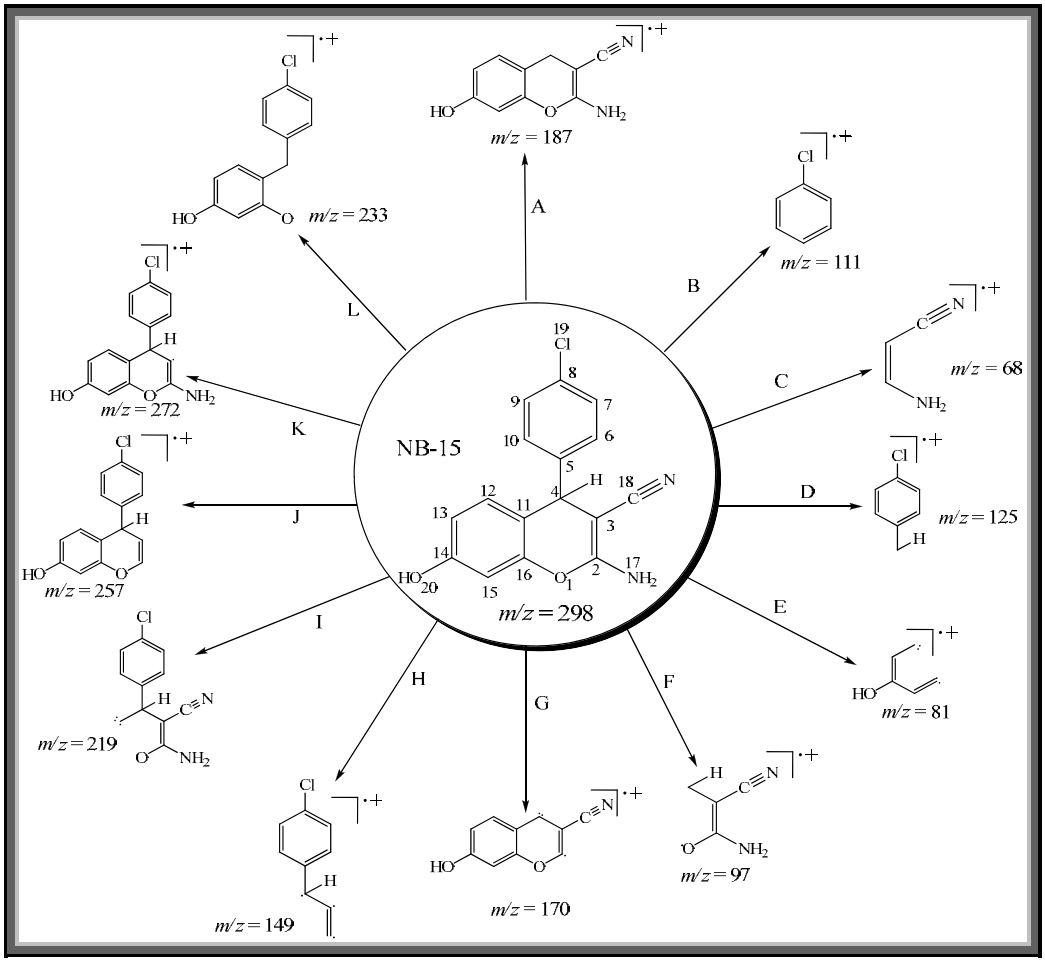


2-Amino-4-(3-bromophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (NB-03)

1. The target compound showed the characteristic molecular ion peak.
2. The bond cleavage between C_4-C_5 generated a molecular ion which corresponds to a characteristic peak at 187 *m/z* **(A).**
3. A bond cleavage between C_7-Br_{19} generated a molecular ion which corresponds to a characteristic peak at 261 *m/z* **(B)**.
4. Bond cleavages between C_3-C_4 as well as O_1-C_{16} generated a molecular ion which corresponds to a characteristic peak at 261 *m/z* **(C)**.
5. Bond cleavage between C_3-C_4 and O_1-C_2 generated a molecular ion which corresponds to a characteristic peak at 277 *m/z* **(D)**.
6. Bond cleavage between C_{11}-C_{12} and C_{15}-C_{16} generated a molecular ion which corresponds to a characteristic peak at 277 *m/z* **(E)**.

7. After the bond cleavage between C_3-C_4 and O_1-C_2 the other molecular ion which was generated corresponded to a characteristic peak at 69 *m/z* **(F)**.
8. After cleaving C_4-C_5, the ion fragment that is generated is further broken down by cleaving bonds between C_8-C_9 and C_5-C_{10} which generated a molecular ion that corresponds to the characteristic peak at 129 *m/z* **(G)**.
9. Bond cleavage between C_{11}-C_{12} and C_{15}-C_{16} generated a molecular ion which corresponds to a characteristic peak at 73 *m/z* **(H)**.
10. Bond cleavage between C_3-C_4 and O_1-C_{16} generated a molecular ion which corresponds to a characteristic peak at 82 *m/z* **(I)**.
11. Bond cleavage between C_7-Br_{19} generated a molecular ion which corresponds to a characteristic peak at 78 *m/z* **(J)**.
12. Another molecular ion which was generated by bond cleavage between C_4-C_5 corresponded to a characteristic peak at 152 *m/z* **(K)**.
13. Bond cleavage between C_4-C_{11} and O_1-C_2 generated a molecular ion which corresponds to a characteristic peak at 236 *m/z* **(L)**.

2.7.2.2 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF NB-15*



2-Amino-4-(4-chlorophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile (NB-15)

1. The target compound shows the desired characteristic molecular ion peak.
2. The bond cleavage between C_4-C_5 generated a molecular ion which corresponds to a characteristic peak at 187 *m/z* **(A)**.
3. The bond cleavage between C_4-C_5 generated another molecular ion which corresponds to a characteristic peak at 111 *m/z* **(B)**.
4. The bond cleavages between C_3-C_4 & O_1-C_2 generated a molecular ion which corresponds to a characteristic peak at 68 *m/z* **(C)**.
5. The bond cleavage between C_3-C_4 & C_4-C_{11} generated a molecular ion which corresponds to a characteristic peak at 125 *m/z* **(D)**.
6. The bond cleavage between C_{11}-C_{12} & C_{11}-C_{16} generated a molecular ion which corresponds to a characteristic peak at 81 *m/z* **(E)**.

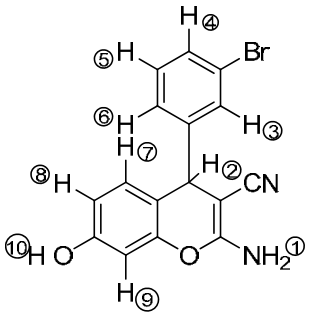
7. After cleaving the bond between C_4-C_5 the subsequent bond cleavage of C_4 - C_{11} & O_1-C_{16} generated a molecular ion which corresponds to a characteristic peak at 97 *m/z* **(F)**.
8. The bond cleavage between C_4-C_5 & C_2-N_{17} generated a molecular ion which corresponds to a characteristic peak at 170 *m/z* **(G)**.
9. The bond cleavages between C_4-C_{11}, C_3-C_{18}, O_1-C_2, & C_2-N_{17} generated a molecular ion which corresponds to a characteristic peak at 149 *m/z* **(H)**.
10. The bond cleavage between C_{11}-C_{12}, C_{11}-C_{16}, & O_1-C_{16} generated a molecular ion which corresponds to a characteristic peak at 219 *m/z* **(I)**.
11. The bond cleavage between C_3-C_{18}, C_2-N_{17} generated a molecular ion which corresponds to a characteristic peak at 257 *m/z* **(J)**.
12. The bond cleavage between C_3-C_{18} generated a molecular ion which corresponds to a characteristic peak at 272 *m/z* **(K)**.
13. The bond cleavage between O_1-C_2, C_2-C_3, C_3-C_4 generated a molecular ion which corresponds to a characteristic peak at 233 *m/z* **(L)**.

Similarly, other mass spectra of other compounds can also be explained through their fragmentation pattern.

2.7.3 *^{1}H-NMR SPECTRAL STUDY*

^{1}H NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in DMSO solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from H NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. The representative spectral interpretation of ^{1}H-NMR can be discussed as under.

2-Amino-4-(3-bromophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (NB-03)

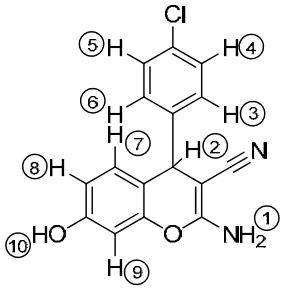


1. The two protons 1 of the amino group gave a characteristic singlet at 6.25 δ ppm.
2. The methine proton 2 on C-4 gave a characteristic singlet 4.57 δ ppm. The assignment of this proton is the most important for the structure elucidation and as it is evident here the successful assignment of this singlet has confirmed the structure. The proton 2 is a bit downfield as compared to a proton singlet in isolation because of the strong electron withdrawing group like –CN present on the adjacent carbon which deshields the proton forcing it to go down field.
3. The proton 3 gave a characteristic singlet in the aromatic region of 7.25 δ ppm with the J value of 3.2 Hz confirming that it is Meta coupled to another proton in the aromatic region.
4. The assignment of proton 4 becomes easier after the assignment of proton 3 as it is Meta to proton 3 hence it was easily seen in the Proton NMR at 7.32 δ ppm with a characteristic doublet. Moreover, the J value of this proton was found to be 9.2 Hz which is in accordance with the rule as it is ortho to Proton no. 5. The J value has come out to be a bit higher as compared to any ortho coupled Proton due to the fact that proton no. 4 is not only ortho coupled to 5 \ but also Meta coupled to proton no. 3.

5. The proton no. 5 as well as proton no. 6 both were seen in the NMR spectrum as a multiplet in the aromatic region between 7.15 δ ppm to 7.20 δ ppm. Their J values were calculated and were found to be 8 Hz and 7.2 Hz respectively which is in accordance with the rule.
6. The proton nos. 3, 4, 5, 6 could be clearly seen in the expanded spectra of the compound and their J values have also been calculated from the same.
7. The proton no. 7 gave a characteristic doublet at 6.72 δ ppm with a J value of 8.4 Hz suggesting that it is ortho coupled with another proton.
8. The proton no. 8 gave a characteristic doublet between 6.48 δ ppm to 6.51 δ ppm the J value was calculated and found out to be 10.8 Hz. This value suggests that it is not only Ortho coupled to another proton but also Meta coupled with another proton hence its assignment as proton no. 8 could be done very lucidly.
9. The proton no. 9 gave a singlet at 6.46 δ ppm with a J value of 2.4 Hz as expected. This is because it is meta coupled with proton no. 8.
10. The proton 10 of the hydroxyl group was seen clearly as a singlet at 9.41 δ ppm and as it is bonded to the electronegative oxygen atom the singlet is shifted to downfield significantly.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton it can be clearly suggested that the proposed structure for compound no. NB-3 has been confirmed.

2-Amino-4-(4-chlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (NB-15)



1. The two protons (proton no. 1) of the amino group were distinctively observed as a broad singlet at 5.77 δ ppm. As the protons are bonded to the electronegative Nitrogen atom they are de-shielded and found at such a downfield region of 5.77 δ ppm.
2. The proton no. 2 or the methine proton which is very important for the structure elucidation is evidently observed as a sharp singlet at 4.53 δ ppm. The reason for it being downfield as compared to other isolated proton normally found at an upfield frequency of around 2 δ ppm is due to the fact that an electronegative nitrile group is attached to itsneighboring carbon atom moreover there it is surrounded phenyl ring which acts as an electron sink thus making the proton significantly de-shielded and hence it is found at 4.53 δ ppm.
3. Now just by looking at the structure its evident that the chemical environment of proton no. 3 and proton no. 6 as well as that of proton no. 4 and 5 are identical, hence their signals should also be the same. This was very clearly seen in the expanded spectra of the compound where it was found that a beautiful double doublet in the aromatic region suggested the presence of 4 environmentally equivalent protons. The sharp doublet assigned for two protons in the aromatic region between 7.04 δ ppm to 7.08 δ ppm are responses for proton no. 3 and proton no. 6. The J values of these protons clearly confirmed their position and gave beautiful values. When the J values of proton no. 3 and proton no. 6 were calculated independently of each other, it came out to be 4.4 Hz for both of them suggesting that they are meta coupled. But when their combined J value was calculated they gave a value of 13.6 Hz which suggests that they are also ortho coupled with other protons.
4. As discussed above, the chemical environment for proton no. 4 as well as proton no. 5 is also similar hence their signals would also similar. This was evidently observed as a doublet between 7.16 δ ppm to 7.19 δ ppm. which accounted for two protons. Moreover, as both these protons have electronegative chlorine atom on their adjacent atom these peaks were observed at a downfield region as compared to the signals for proton no. 3 and

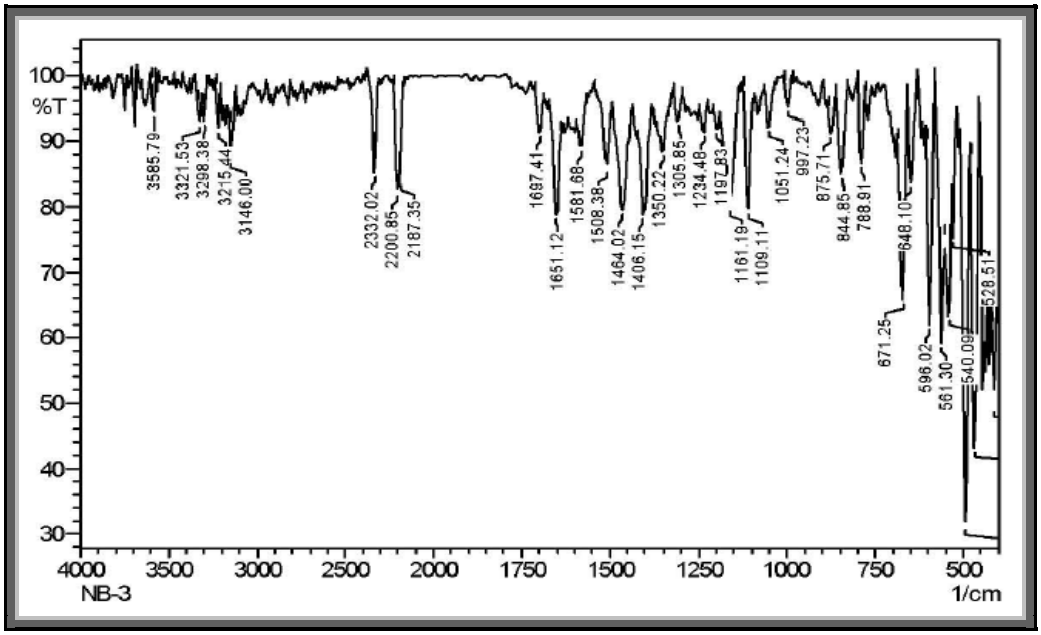
6. Moreover, the study of the J values for both these protons revealed similar results as those of Proton no. 3 and 6. When the J values of each of the proton were calculated independently of each other it gave a result of 4.4 Hz for both proton no. 4 and 5 respectively suggesting that they are meta coupled. Moreover when the J value was calculated keeping both of them together it yielded a value of 13.6 Hz. Suggesting that they are ortho coupled to another set of protons.

5. The J values of all the 4 aromatic protons (proton nos. 3,4,5,6) were found out to be exactly similar. When in isolation, they gave a value of 4.4 Hz and when calculated together as a set they gave a J value of 13.6. Thus this double doublet accounted for 4 protons in the ^{1}H NMR spectrum could be easily assigned to the two sets of chemically equivalent protons no. 3 and 6 and protons no. 4 and 5. Chlorine atom attached to the carbon adjacent to protons 4 and 5 have shifted their signal to a downfield region.
6. The proton no. 7 could be assigned to the doublet shown in the ^{1}H-NMR spectrum between 6.43 δ ppm and 6.46 δ ppm. The J value for this proton was calculated to be 10.8 Hz suggesting that it is ortho coupled to another proton.
7. The proton no. 8 could be easily assigned to the clear doublet found in the ^{1}H NMR spectrum between 6.62 δ ppm and 6.64 δ ppm. The J value for this proton was calculated and found to be 8.4 Hz. Suggesting that it is ortho coupled to proton no. 7.
8. The Singlet observed at 6.41 δ ppm could be assigned to the proton no. 9. Moreover, the J value for this proton was found out to be 3.2 Hz which suggests that it is meta coupled with proton no. 8.
9. The proton bonded to oxygen atom in the hydroxyl group was clearly seen in the NMR spectrum as a broad peak singlet accounting for 1 proton at 9.22 δ ppm. As it is bonded to the electronegative oxygen atom this proton is significantly de-shielded and hence observed at such a downfield region as 9.22 δ ppm.

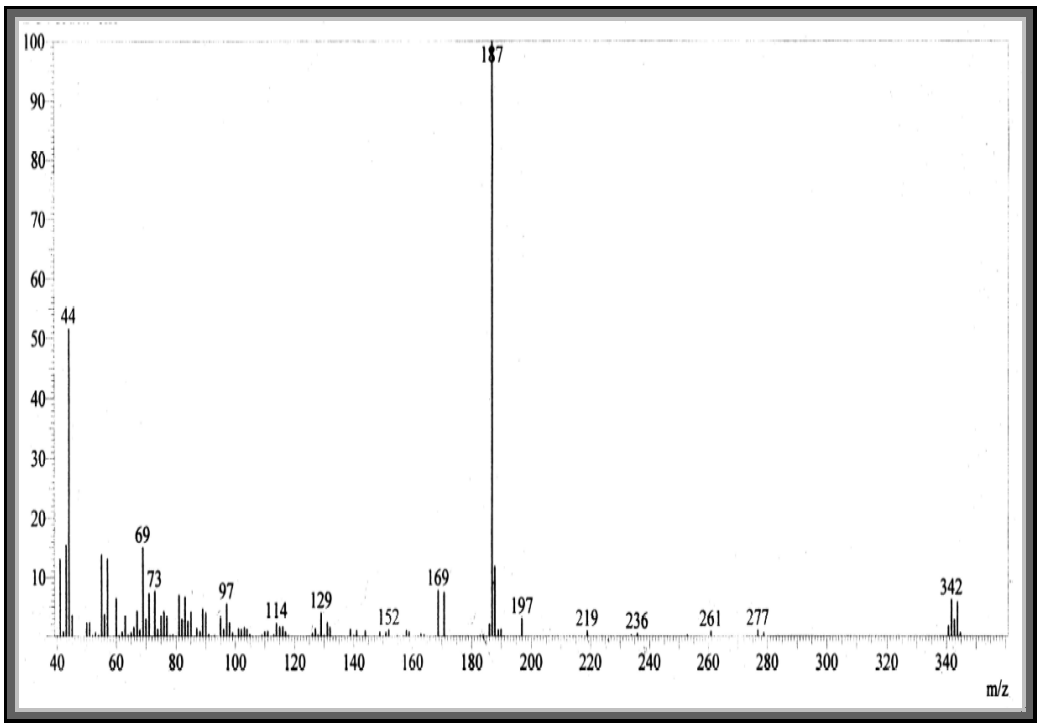
Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton it can be clearly suggested that the proposed structure for compound no. NB-15 has been confirmed.

2.8 SPECTRAL REPRESENTATION OF THE SYNTHESIZED COMPOUNDS

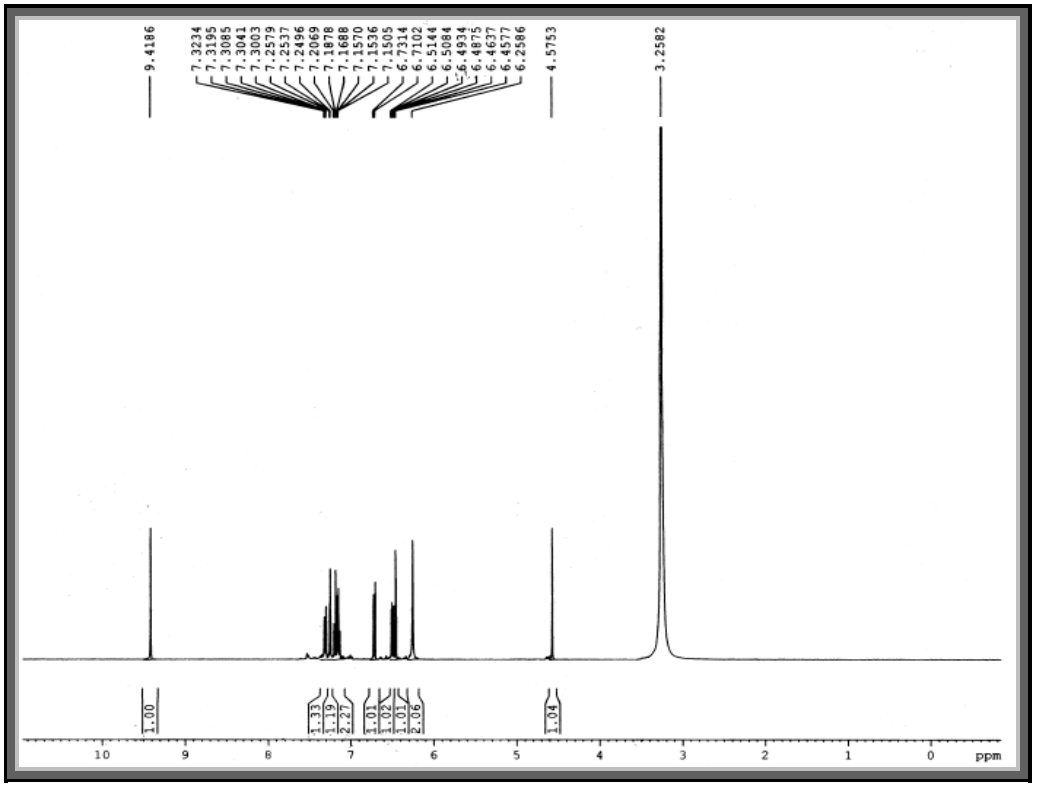
2.8.1 *IR SPECTRUM OF NB-03*



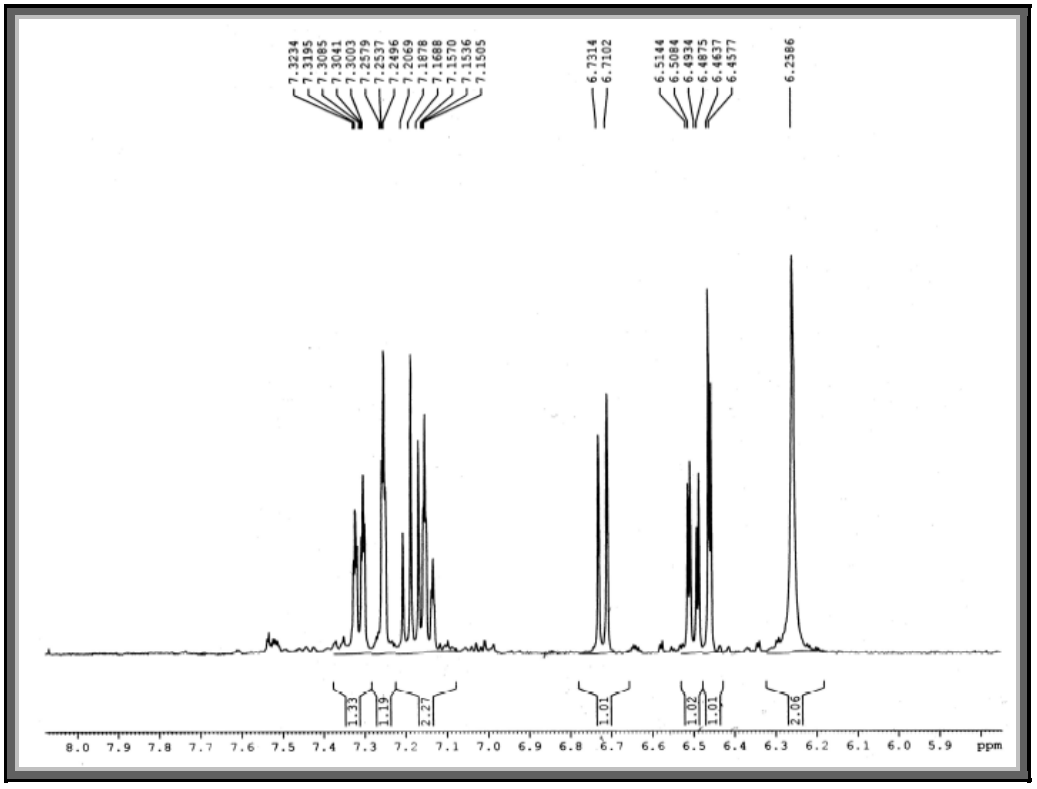
2.8.2 *MASS SPECTRUM OF NB-03*



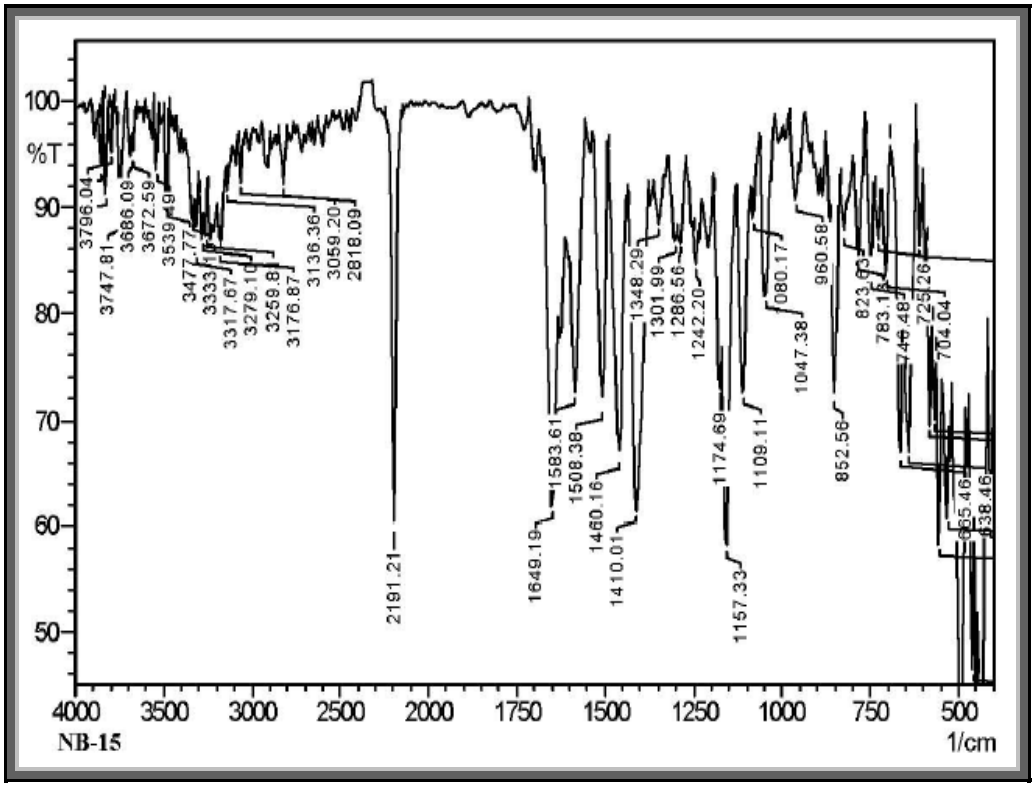
2.8.3 *^{1}H-NMR SPECTRUM OF NB-03*



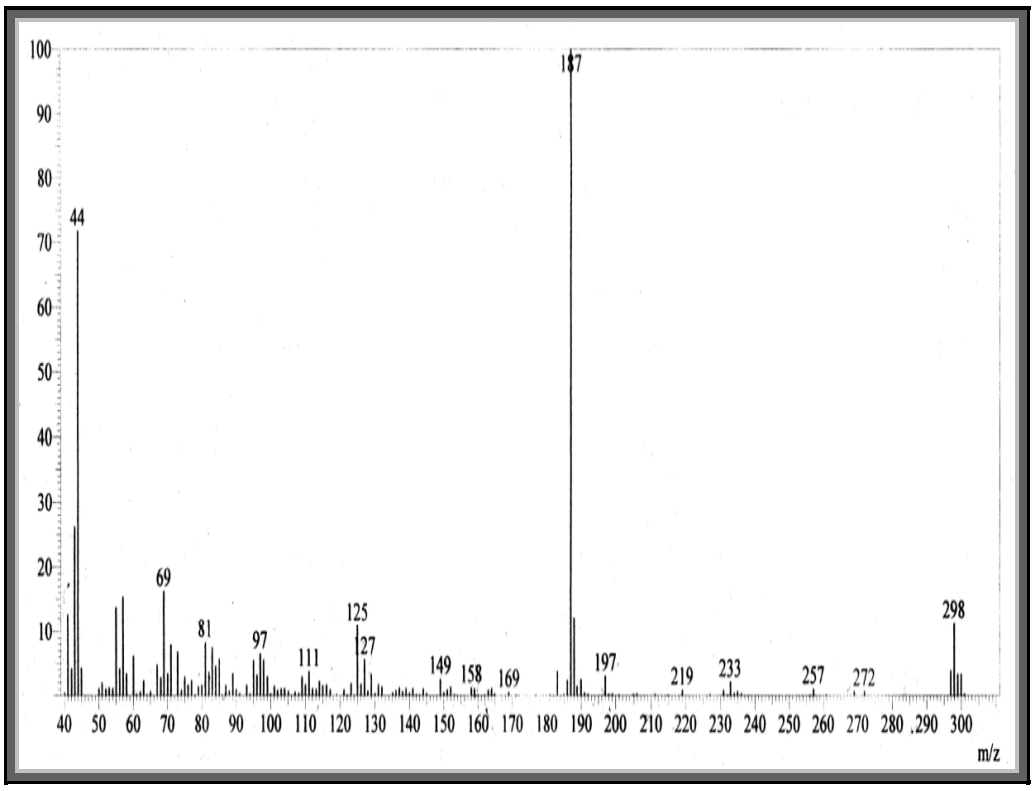
2.8.3.1 *EXPANDED NMR SPECTRUM OF NB-03*



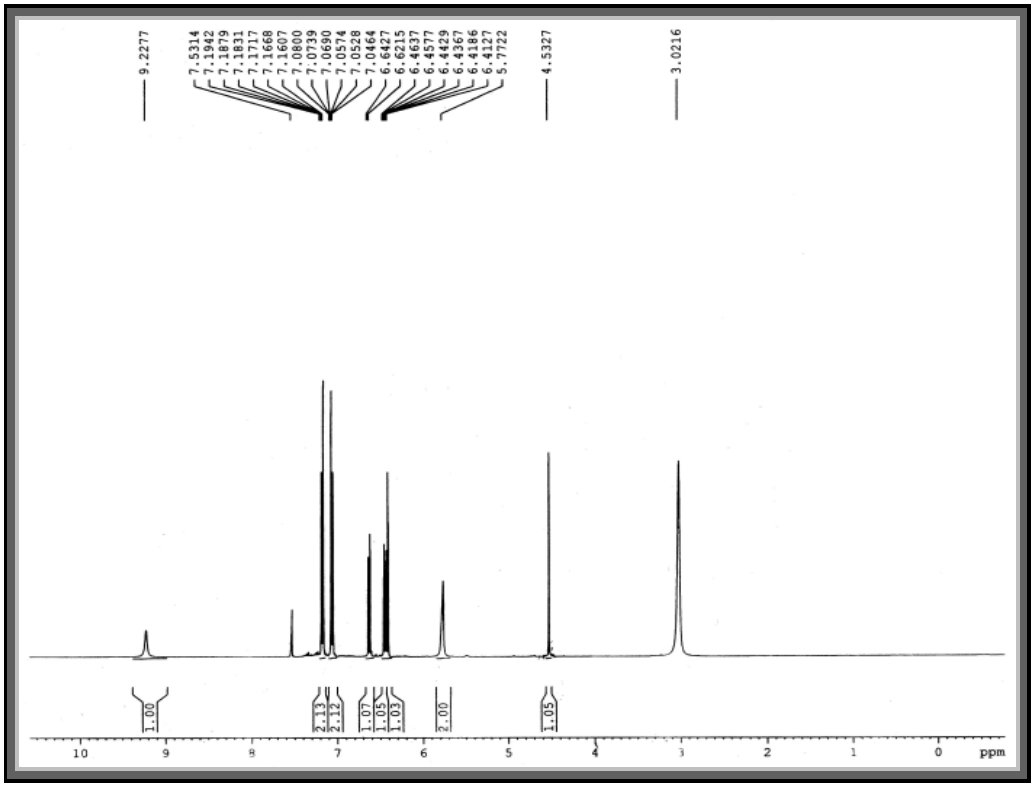
2.8.4 *IR SPECTRUM OF NB-15*



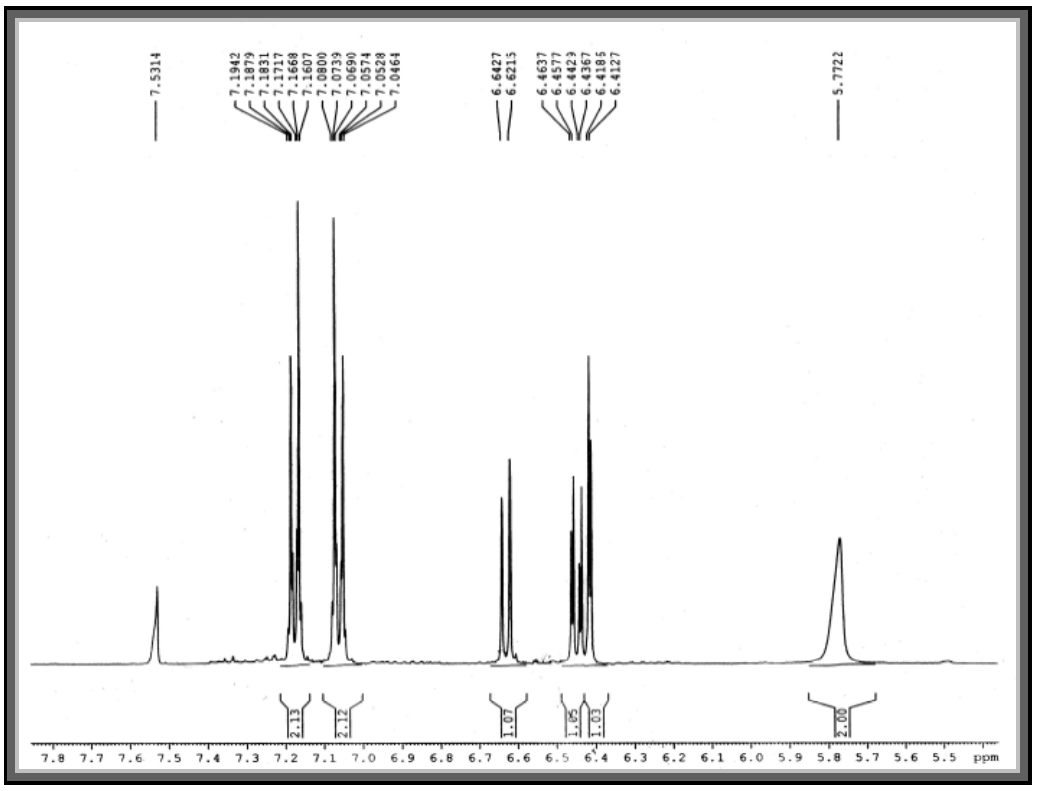
2.8.5 *MASS SPECTRUM OF NB-15*



2.8.6 *^{1}H-NMR SPECTRUM OF NB-15*



2.8.6.1 *EXPANDED ^{1}H-NMR SPECTRUM OF NB-15*



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CHAPTER – 2

Section-B

Aqua mediated and microwave assisted synthesis of *Ethyl-2-Amino-7-hydroxy-4-(substituted phenyl)-4H-chromene-3-carboxylates*

2.9 AIM OF THE CURRENT WORK DONE

The importance of the 2-Amino chromene as a significant chemical entity has already been aptly justified in the previous section of this chapter. Looking at the type of the reaction some new chemical entities could easily be generated by changing any of the reactants. Hence, it was decided to change the active methylene compound for this section. In Section A, Malanonitrile was used which afforded 2-Amino-3-cyano derivative. In current work cyano ethyl acetate, another active methylene compound, generated 2-Amino-3-carboxylate derivative. The literature survey revealed that these kinds of novelty compounds have hardly been reported and its method of synthesis is not very well cited.

Moreover, the literature survey revealed that the Structure activity relationship studies of ethyl 2-Amino-6-cyclopentyl-4-(1-cyano-2-ethoxy-2-oxo)-4*H*-chromene-3-carboxylate (HA 14-1; Fig. 1), an antagonist of the antiapoptotic Bcl-2 proteins, are reported. Bcl-2 and related proteins are key regulators of apoptosis or programmed cell death implicated in human disease including cancer [1]. The cell-permeable Bcl-2 binding peptides could induce apoptosis of human myeloid leukemia *in vitro* and suppress its growth in severe combined immuno deficient mice. *In vitro* binding studies demonstrated the interaction of HA14-1 with this Bcl-2 surface pocket that is essential for Bcl-2 biological function. HA14-1 effectively induced apoptosis of human acute myeloid leukemia (HL-60) cells over expressing Bcl-2 protein that was associated with the decrease in mitochondrial membrane potential and activation of caspase-9 followed by caspase-3. Cytokine response modifier A, a potent inhibitor of Fas-mediated apoptosis, did not block apoptosis induced by HA14-1. Bcl-2 belongs to a growing family of proteins that regulate apoptosis or programmed cell death. The Bcl-2 family includes both death antagonists such as Bcl-2 and Bcl-xL and death agonists such as Bax, Bak, Bid, and Bad.

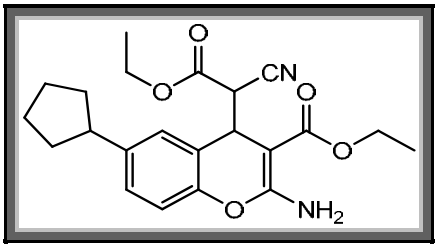


Fig. 1

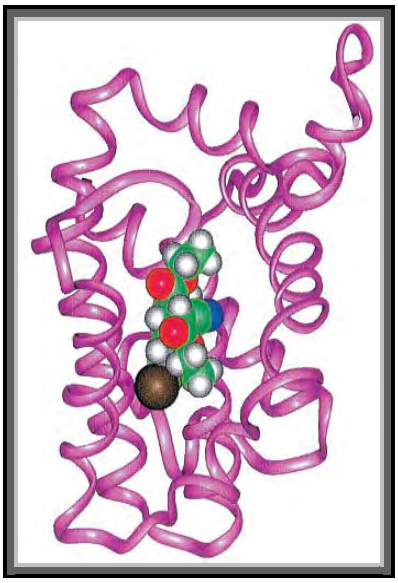


Fig. 2: Structural model for the complex of HA14-1 with the Bcl-2 surface pocket as predicted by computer docking calculation [2].

A series of analogues of Fig-1 with varied functional groups at the 6-position of the chromene ring were synthesized. These candidates were evaluated for their binding interactions with three antiapoptotic proteins: Bcl-2, Bcl-X_L, and Bcl-w. They were also assayed for their in vitro cytotoxicities against a set of Jurkat cells with varied levels of Bcl-2 and Bcl-X_L proteins and a non-small-cell lung carcinoma cell line (NCI-H460). It was found that the 6-bromo of Fig. 1 was not essential for its bioactivity and the 6-position can accommodate a variety of alkyl groups. Fig. 1 and its analogues bind to all of the three antiapoptotic Bcl-2 proteins tested. Positive correlations were observed between the binding affinities of these candidates to the antiapoptotic Bcl-2 proteins and their in vitro cytotoxicities, suggesting that the antiapoptotic Bcl-2 proteins are likely to be the cellular targets of Fig. 1 and its analogues. In this study, the binding interactions of the small molecules to antiapoptotic Bcl-2 proteins were studied by assaying their abilities to compete against a Bak peptide binding to the antiapoptotic Bcl-2 proteins. Inhibitory constants, instead of dissociation constants, were obtained in such assays. The most active compound had a >3-fold increase of binding affinity to the antiapoptotic Bcl-2 proteins and a >13-fold increase of in vitro cytotoxicity over Fig. 1. Though Jurkat cells with transgenic over expression of Bcl-2 or Bcl-X_L protein can develop resistance to standard cancer therapies, such cells failed to develop resistance to Fig. 1

based candidates. Fig. 1 also sensitizes Jurkat cells to cisplatin. These studies provide further support that Fig. 1 and its analogues function as antagonists for antiapoptotic Bcl-2 proteins and that they have the potential, either as a single agent or as a combination therapy with other anticancer agents, to treat cancers with the over expression of antiapoptotic Bcl-2 proteins.

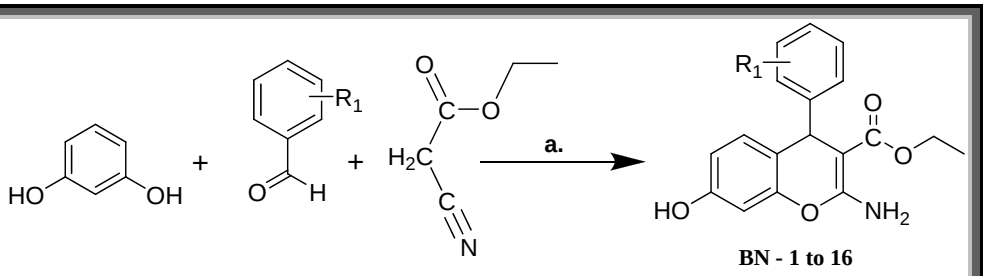
The compounds synthesized in this section are structurally very similar to the compound shown in fig. 1. Moreover, the SAR study in the paper suggested that the substitution on position no. 6 is not an important aspect for the biological activity of this type of compounds.

Several researchers world wide have explored the chemistry as well as biology 2-Amion-4H-chromene derivatives in recent years and some fairly good reviews as well as publications are cited in the references [3-43].

The detailed literature survey on this class of compounds with 2-Amino-4-substitutedphenyl-3-carboxylate derivatives have not been explored and hence synthetic work on this chemical entitity was initiated and evaluated for its biological activity. The route of synthesis is an environmentally friendly green chemistry approach, wherein the reaction is carried out using water as solvent and potassium carbonate as the base catalyst to prepare these compounds under microwave irradiations by which the reaction can be completed in few minutes.

Thus, the opportunity to synthesize some new chemical entities as well as to explore their biological activity was the main rational behind initializing the work included in this chapter.

2.10 REACTION SCHEME



BN - 1 to 16

Reagents & Conditions: a.) K_2CO_3 , H_2O , MWI-320 watts, Open Vessel, 2 to 4 mins.,

Aqua Mediated Synthesis of Ethyl-(2-Amino-7-hydroxy-4-substituted phenyl-4H-chromene-)3-carboxylate

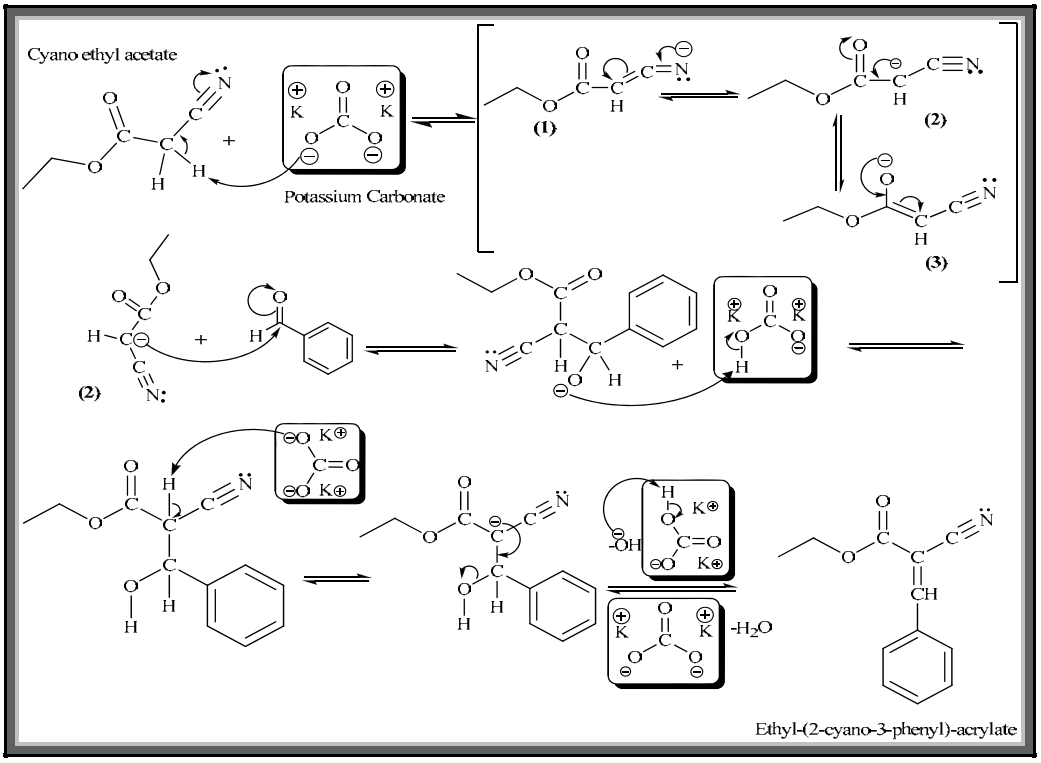
2.10.1 *PHYSICAL DATA TABLE*

Code	R_1	M. F.	M. W.	M. P. ^{0}C	Time (min)	Yield %	R_f
BN-1	H	$C_{18}H_{17}NO_4$	311.33	180-182	2:30	90	0.48
BN-2	4-OCH_3	$C_{19}H_{19}NO_5$	341.35	166-168	2:10	85	0.46
BN-3	3-Br	$C_{18}H_{16}BrNO_4$	390.22	174-176	3:40	88	0.50
BN-4	3-Cl	$C_{18}H_{16}ClNO_4$	345.77	120-122	3:20	80	0.52
BN-5	3-NO_2	$C_{18}H_{16}N_2O_6$	356.32	142-144	3:30	92	0.48
BN-6	3-OCH_3, 4-OH	$C_{19}H_{19}NO_5$	341.35	158-160	3:40	87	0.52
BN-7	3-OC_2H_5, 4-OH	$C_{20}H_{21}NO_6$	371.38	126-128	4:00	85	0.54
BN-8	4-$N(CH_3)_2$	$C_{20}H_{22}N_2O_4$	354.39	132-134	3:40	82	0.58
BN-9	4-NO_2	$C_{18}H_{16}N_2O_6$	356.32	148-150	3:40	90	0.54
BN-10	3,4-OCH_3	$C_{20}H_{21}NO_6$	371.38	174-176	2:40	95	0.45
BN-11	4-CH_3	$C_{19}H_{19}NO_4$	325.35	192-194	2:30	85	0.52
BN-12	2-OH	$C_{18}H_{17}NO_5$	327.33	188-190	3:30	80	0.56
BN-13	Furyl	$C_{16}H_{15}NO_5$	301.29	176-178	4:00	86	0.50
BN-14	2-Cl	$C_{18}H_{16}ClNO_4$	345.77	152-156	3:00	90	0.52
BN-15	4-Cl	$C_{18}H_{16}ClNO_4$	345.77	176-178	3:30	85	0.58
BN-16	4-F	$C_{18}H_{16}FNO_4$	329.32	200-202	3:20	87	0.56

TLC solvent system for R_f = Toluene:Ethyl acetate - 7:3. Microwave Irradiation: 320 Watts

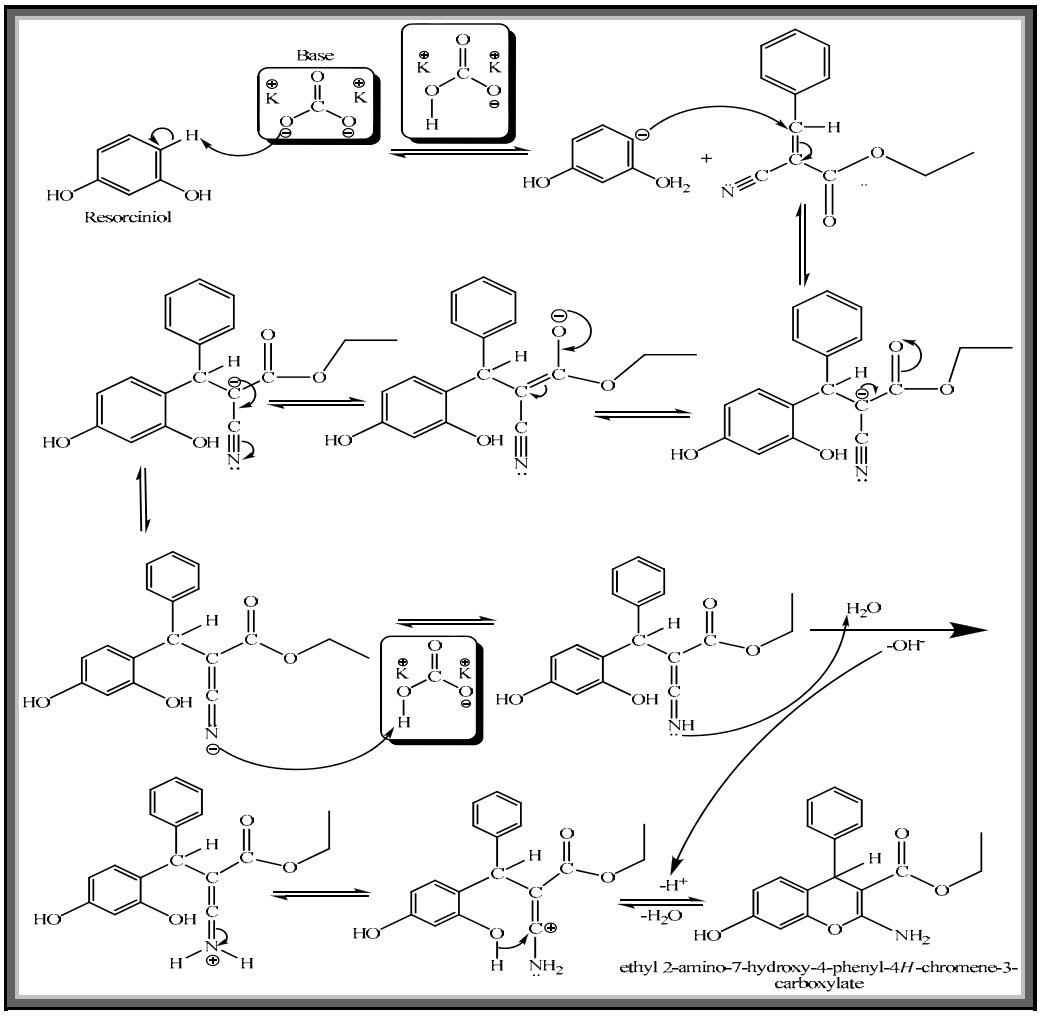
2.11 PLAUSIBLE REACTION MECHANISM

2.11.1 *Formation of Ethyl-(2-cyano-3-phenyl) acrylate Intermediate*



As discussed in Section A the reaction mechanism proceeds first via the Knoevenagel Condensation route where in a nucleophilic addition of an active hydrogen compound takes place onto the carbonyl group which when followed by the dehydration reaction and subsequent elimination of water molecule (hence condensation) would afford us the condensate which is often an α, β-conjugated enone. As shown above, first the base molecule, here potassium carbonate would first attack on the active hydrogen compound (Ethyl cyano acetate) by accepting a proton and thus forming a carbanion, shown herein as the resonating conjugate (2). This carbanion would then attack on the partially positively charged carbon of the carbonyl moiety, forming an unstable intermediate compound with negatively charged oxygen. This would then accept the hydrogen bonded to the K_2CO_3 and thus the base would then proceed through the anti elimination by cleaving a proton and thus a water molecule to afford the α, β-unsaturated enone herein a Ethyl-(2-Cyano-3-phenyl)-acrylate.

2.11.2 *Formation of Ethyl-(2-amino-7-hydroxy-4-substitutedphenyl-4h-chromene)-3-carboxylate From Ethyl-(2-cyano-3-phenyl)-acrylate*



The base would cleave the acidic proton from resorcinol thus forming an carbanion, this carbanion would then attack on Ethyl-(2-Cyano-3-phenyl)-acrylate to provide us with resonating conjugates. The negative charge on the Nitrogen would abstract the hydrogen out of base and satisfy its valency. The lone pair of that nitrogen would then accept a proton from water molecule and would become an ammonium ion. This is a very unstable moiety and hence the charge displaces on carbon forming a stabilized carbocation. The carbocation is quenched by the elimination of the water molecule by cleaving one hydrogen from the hydroxyl group forming a bond with the carbon containing the positive charge and thus giving Ethyl-(2-amino-7-hydroxy-4-substituted phenyl-4*H*-chromene)-3-carboxylate from Ethyl-(2-Cyano-3-phenyl)-acrylate.

2.12 EXPERIMENTAL

2.12.1 *MATERIALS AND METHODS*

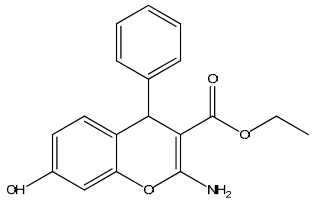
Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. All the reactions were carried out in Samsung MW83Y Microwave Oven which was locally modified for carrying out chemical reactions. IR spectra were recorded in 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.

2.12.2 *General Procedure: Ethyl-(2-amino-7-hydroxy-4 substituted phenyl-4H-chromene)-3-carboxylates*

Equimolar amounts of neat reactants, substituted benzaldehydes, Ethyl cyanoacetate, and resorcinol were taken in an Erlenmeyer flask, and 10 ml saturated solution of K_2CO_3 in demineralized water was added to it. The reaction mixture was subjected to MWI (Micro Wave Irradiations) for a specific time (see Physical data Table) at low power (320 W). The progress of the reaction was monitored by TLC examination at an interval of every 30 seconds. On completion of reaction, the reaction mixture was cooled and was triturated with 2–3 ml of ice cold water to get the solid product, leaving behind K_2CO_3 dissolved in water. The product obtained was filtered, washed with cold water, dried, and recrystallized from ethanol.

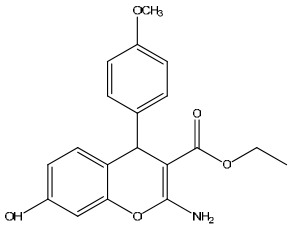
2.13 ANALYTICAL DATA

2.13.1 Ethyl 2-amino-7-hydroxy-4-phenyl-4H-chromene-3-carboxylate (BN-1)



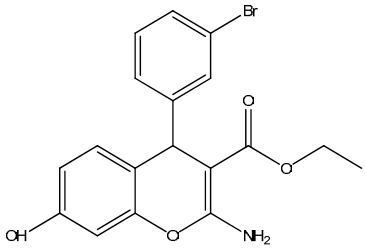
Yield: 90%; M.P.- 180-182 ºC; IR (cm^{-1}): 3620 (O-H stretching of free primary alcohol), 3524-3489, (N-H stretching of free primary amine), 3146 (C-H stretching vibration of aromatic region), 1905-1066 (C-O stretching frequency of esters), 1752 (C=O stretching frequency for esters), 1604 (N-H deformation, in plane bending of N-H), 1558 (O-H in plane bending), 1361 (C-N stretching for carbon bonded to amino group), 952 (C-H in plane bending of phenyl ring), 696 (C-H out of plane bending for phenyl nucleus), ^{1}H NMR (DMSO-d_6) δ ppm: 6.46 (s, 2H, H_1), 4.79 (s, 1H, H_2), 7.14-7.18 (m, 6H, H_3, H_4, H_5, H_6. H_7, H_8, J=3.2 Hz), 7.05-7.06 (s, 1H, H_{10}, J=2.64 Hz), 6.86-6.83 (d, 1H, H_9, J=8.76 *Hz*), 9.25 (s, 1H, H_{11}), 3.95-4.00 (q, 2H, H_{12},), 1.08-1.12 (t, 3H, H_{13}), MS: *m/z:* 311.12; Anal. Calcd. for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50; O, 20.56 Found: C, 69.39; H, 5.41; N, 4.40; O, 20.49.

2.13.2 Ethyl-2-amino-7-hydroxy-4-(4-methoxyphenyl)-4H-chromene-3-carboxylate (BN-2)



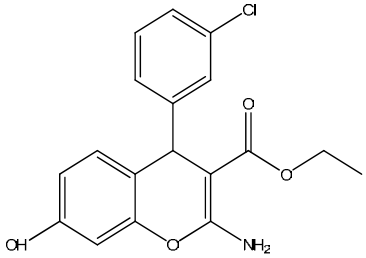
Yield: 85%; M.P.- 166-168 ºC; IR (cm^{-1}): 3614 (O-H stretching of free primary alcohol), 3551-3406 (N-H stretching of free primary amine), 3186 (C-H stretching vibration of aromatic region), 1750 (C=O stretching frequency of esters), 1606 (N-H deformation, in plane bending of N-H), 1514 (O-H in plane bending), 1301 (C-O stretching for aromatic ether group), 1089 (C-H in plane bending of phenyl ring), 692 (C-H out of plane bending for phenyl nucleus), ^{1}H NMR (DMSO-d_6) δ ppm: 6.45 (s, 2H, H_1), 4.74 (s, 1H, H_2), 7.14 (s, 2H, H_3 & H_6), 7.04 (d, 2H, H_4 & H_5), 6.68-6.70 (d, 2H, H_7 & H_8, J_{H5}=8 Hz, J_{H6}=8 *Hz*), 9.22 (s, 1H, H_9), 6.28-6.83 (d, 1H, H_{10}), 3.99-3.99 (s, 2H, H_{11}), 1.12-1.13 (t, 3H, H_{12}), 3.69 (s, 3H, H_{13}), MS: *m/z:* 341.13; Anal. Calcd. for $C_{19}H_{19}NO_5$: C, 66.85; H, 5.61; N, 4.10; O, 23.43 Found: C, 66.79; H, 5.56; N, 4.01; O, 23.36.

2.13.3 Ethyl 2-amino-4-(3-bromophenyl)-7-hydroxy-4H-chromene-3-carboxylate (BN-3)



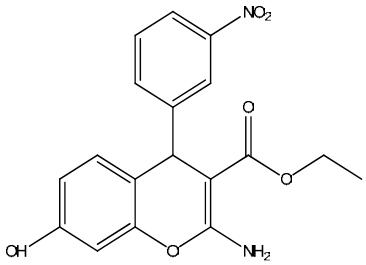
Yield: 88%; M.P.- 174-176 ºC; IR (cm^{-1}): 3610 (O-H stretching of free primary alcohol), 3520-3410, (N-H stretching of free primary amine), 3012 (C-H stretching vibration of aromatic region), 1266 (C-O stretching frequency of esters), 1725 (C=O stretching frequency for esters), 1610 (N-H deformation, in plane bending of N-H), 1553 (O-H in plane bending), 1341 (C-N stretching for carbon bonded to amino group), 1005 (C-H in plane bending of phenyl ring), 650 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 389.03; Anal. Calcd. for $C_{18}H_{16}BrNO_4$: C, 55.40; H, 4.13; Br, 20.48; N, 3.59; O, 16.40 Found: C, 55.29; H, 4.04; Br, 20.39 N, 3.50; O, 16.32.

2.13.4 Ethyl 2-amino-4-(3-chlorophenyl)-7-hydroxy-4H-chromene-3-carboxylate (BN-4)



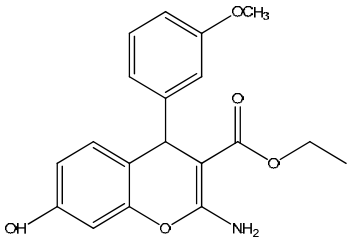
Yield: 80%; M.P.- 120-122 ºC; IR (cm^{-1}): 3632 (O-H stretching of free primary alcohol), 3504-3469, (N-H stretching of free primary amine), 3123 (C-H stretching vibration of aromatic region), 1254 (C-O stretching frequency of esters), 1720 (C=O stretching frequency for esters), 1615 (N-H deformation, in plane bending of N-H), 1523 (O-H in plane bending), 1345 (C-N stretching for carbon bonded to amino group), 1010 (C-H in plane bending of phenyl ring), 697 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 345.08; Anal. Calcd. for $C_{18}H_{16}ClNO_4$: C, 62.52; H, 4.66; Cl, 10.25; N, 4.05; O, 18.51 Found: C, 62.46; H, 4.52; Cl, 10.17 N, 4.00; O, 18.49.

2.13.5 Ethyl 2-amino-7-hydroxy-4-(3-nitrophenyl)-4H-chromene-3-carboxylate (BN-5)



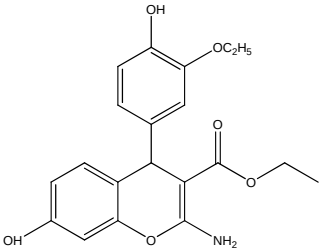
Yield: 92%; M.P.- 142-144 ºC; IR (cm^{-1}): 3642 (O-H stretching of free primary alcohol), 3503-3473, (N-H stretching of free primary amine), 3121 (C-H stretching vibration of aromatic region), 1262 (C-O stretching frequency of esters), 1732 (C=O stretching frequency for esters), 1624 (N-H deformation, in plane bending of N-H), 1528 (O-H in plane bending), 1332 (C-N stretching for carbon bonded to amino group), 978 (C-H in plane bending of phenyl ring), 690 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 356.10; Anal. Calcd. for $C_{18}H_{16}N_2O_6$: C, 60.67; H, 4.53; N, 7.86; O, 26.94 Found: C, 60.63; H, 4.49; N, 7.83; O, 26.88.

2.13.6 Ethyl-2-amino-7-hydroxy-4-(3-methoxyphenyl)-4H-chromene-3 carboxylate (BN-6)



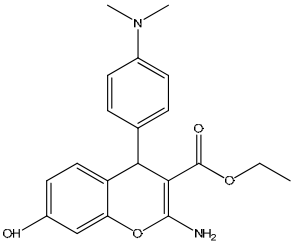
Yield: 87%; M.P.- 158-160 ºC; IR (cm^{-1}): 3614 (O-H stretching of free primary alcohol), 3512-3475, (N-H stretching of free primary amine), 3143 (C-H stretching vibration of aromatic region), 1247 (C-O stretching frequency of esters), 1730 (C=O stretching frequency for esters), 1612 (N-H deformation, in plane bending of N-H), 1562 (O-H in plane bending), 1345 (C-N stretching for carbon bonded to amino group), 964 (C-H in plane bending of phenyl ring), 682 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 341.13; Anal. Calcd. for $C_{19}H_{19}NO_5$: C, 66.85; H, 5.61; N, 4.10; O, 23.43 Found: C, 66.81; H, 5.57; N, 4.05; O, 23.40.

2.13.7 Ethyl 2-amino-4-(3-ethoxy-4-hydroxyphenyl)-7-hydroxy-4H-chromene-3 carboxylate (BN-7)



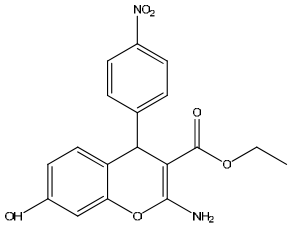
Yield: 85%; M.P.- 126-128 ºC; IR (cm^{-1}): 3623 (O-H stretching of free primary alcohol), 3527-3464, (N-H stretching of free primary amine), 3139 (C-H stretching vibration of aromatic region), 1256 (C-O stretching frequency of esters), 1742 (C=O stretching frequency for esters), 1611 (N-H deformation, in plane bending of N-H), 1548 (O-H in plane bending), 1371 (C-N stretching for carbon bonded to amino group), 958 (C-H in plane bending of phenyl ring), 694 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 371.14; Anal. Calcd. for $C_{20}H_{21}NO_6$: C, 64.68; H, 5.70; N, 3.77; O, 25.85 Found: C, 64.64; H, 5.66; N, 3.71; O, 25.78.

2.13.8 Ethyl 2-amino-4-(4-(dimethylamino)phenyl)-7-hydroxy-4H-chromene-3 carboxylate (BN-8)



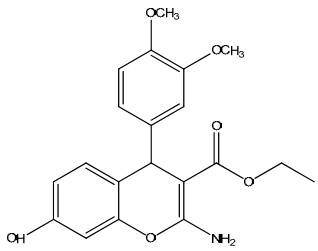
Yield: 82%; M.P.- 132-134 ºC; IR (cm^{-1}): 3615 (O-H stretching of free primary alcohol), 3517-3472 (N-H stretching of free primary amine), 3162 (C-H stretching vibration of aromatic region), 1242 (C-O stretching frequency of esters), 1752 (C=O stretching frequency for esters), 1632 (N-H deformation, in plane bending of N-H), 1498 (O-H in plane bending), 1326 (C-N stretching frequency for aryl tertiary amine), 1351 (C-N stretching for carbon bonded to amino group), 1023 (C-H in plane bending of phenyl ring), 836 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 354.16; Anal. Calcd. for $C_{20}H_{22}N_2O_4$: C, 67.78; H, 6.25; N, 7.90; O, 18.06, Found: C, 67.73; H, 6.21; N, 7.87; O, 18.02.

2.13.9 Ethyl 2-amino-7-hydroxy-4-(4-nitrophenyl)-4H-chromene-3-carboxylate (BN-9)



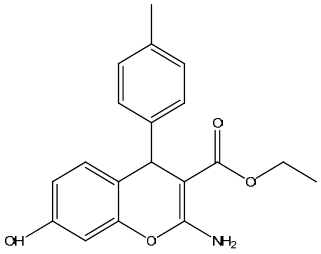
Yield: 90%; M.P.- 148-150 ºC; IR (cm^{-1}): 3632 (O-H stretching of free primary alcohol), 3517-3484, (N-H stretching of free primary amine), 3092 (C-H stretching vibration of aromatic region), 1278 (C-O stretching frequency of esters), 1757 (C=O stretching frequency for esters), 1623 (N-H deformation, in plane bending of N-H), 1498 (O-H in plane bending), 1353 (C-N stretching for carbon bonded to amino group), 977 (C-H in plane bending of phenyl ring), 716 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 356.10; Anal. Calcd. for $C_{18}H_{16}N_2O_6$: C, 60.67; H, 4.53; N, 7.86; O, 26.94 Found: C, 60.63; H, 4.47; N, 7.82; O, 26.89.

2.13.10 Ethyl-2-amino-4-(3,4-dimethoxyphenyl)-7-hydroxy-4H-chromene 3-carboxylate (BN-10)



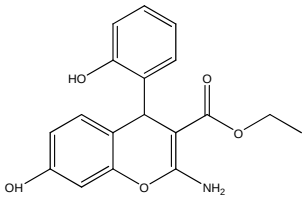
Yield: 95%; M.P.- 174-176 ºC; IR (cm^{-1}): 3624 (O-H stretching of free primary alcohol), 3516-3491 (N-H stretching of free primary amine), 3138 (C-H stretching vibration of aromatic region), 1243 (C-O stretching frequency of esters), 1753 (C=O stretching frequency for esters), 1603 (N-H deformation, in plane bending of N-H), 1573 (O-H in plane bending), 1374 (C-N stretching for carbon bonded to amino group), 974 (C-H in plane bending of phenyl ring), 759 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 371.14; Anal. Calcd. for $C_{20}H_{21}NO_6$: C, 64.68; H, 5.70; N, 3.77; O, 25.85 Found: C, 64.63; H, 7.65; N, 3.72; O, 25.80.

2.13.11 Ethyl-2-amino-7-hydroxy-4-p-tolyl-4H-chromene-3-carboxylate (BN-11)



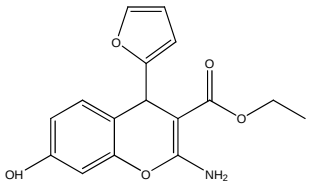
Yield: 85%; M.P.- 192-194 ºC; IR (cm^{-1}): 3612 (O-H stretching of free primary alcohol), 3533-3467, (N-H stretching of free primary amine), 3174 (C-H stretching vibration of aromatic region), 1270 (C-O stretching frequency of esters), 1744 (C=O stretching frequency for esters), 1585 (N-H deformation, in plane bending of N-H), 1542 (O-H in plane bending), 1373 (C-N stretching for carbon bonded to amino group), 964 (C-H in plane bending of phenyl ring), 746 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 325.13; Anal. Calcd. for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31; O, 19.67 Found: C, 70.10; H, 5.73; N, 4.25; O, 19.58.

2.13.12 Ethyl-2-amino-7-hydroxy-4-(2-hydroxyphenyl)-4H-chromene-3-carboxylate (BN-12)



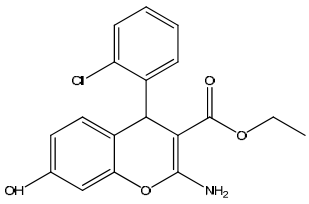
Yield: 80%; M.P.- 188-190 ºC; IR (cm^{-1}): 3617 (O-H stretching of free primary alcohol), 3517-3473, (N-H stretching of free primary amine), 3158 (C-H stretching vibration of aromatic region), 1277 (C-O stretching frequency of esters), 1752 (C=O stretching frequency for esters), 1574 (N-H deformation, in plane bending of N-H), 1568 (O-H in plane bending), 1338 (C-N stretching for carbon bonded to amino group), 1002 (C-H in plane bending of phenyl ring), 710 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 327.11; Anal. Calcd. for $C_{18}H_{17}NO_5$: C, 66.05; H, 5.23; N, 4.28; O, 24.44 Found: C, 65.98; H, 5.19; N, 4.21; O, 24.34.

2.13.13 Ethyl-2-amino-4-(furan-2-yl)-7-hydroxy-4H-chromene-3-carboxylate (BN-13)



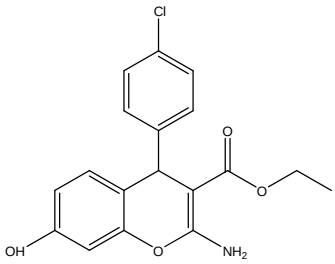
Yield: 86%; M.P.- 176-178 ºC; IR (cm^{-1}): 3616 (O-H stretching of free primary alcohol), 3513-3477, (N-H stretching of free primary amine), 3137 (C-H stretching vibration of furan ring systhem), 1252 (C-O stretching frequency of esters), 1725 (C=O stretching frequency for esters), 1597 (N-H deformation, in plane bending of N-H), 1498 (O-H in plane bending), 1357 (C-N stretching for carbon bonded to amino group), 973 (C-H in plane bending of phenyl ring), 796 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 301.10; Anal. Calcd. for $C_{16}H_{15}NO_5$: C, 63.78; H, 5.02; N, 4.65; O, 26.55 Found: C, 63.69; H, 4.94; N, 4.52; O, 26.48.

2.13.14 Ethyl-2-amino-4-(2-chlorophenyl)-7-hydroxy-4H-chromene-3-carboxylate (BN-14)



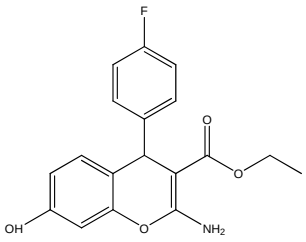
Yield: 90%; M.P.- 152-154 ºC; IR (cm^{-1}): 3622 (O-H stretching of free primary alcohol), 3534-3486, (N-H stretching of free primary amine), 3145 (C-H stretching vibration of aromatic region), 1246 (C-O stretching frequency of esters), 1723 (C=O stretching frequency for esters), 1584 (N-H deformation, in plane bending of N-H), 1548 (O-H in plane bending), 1371 (C-N stretching for carbon bonded to amino group), 1052 (C-H in plane bending of phenyl ring), 754 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 345.08; Anal. Calcd. for $C_{18}H_{16}ClNO_4$: C, 62.52; H, 4.66; Cl, 10.25; N, 4.05; O, 18.51 Found: C, 62.44; H, 4.56; Cl, 10.17; N, 4.01; O, 18.47.

2.13.15 Ethyl-2-amino-4-(4-chlorophenyl)-7-hydroxy-4H-chromene-3-carboxylate (BN-15)



Yield: 85%; M.P.- 176-178 ºC; IR (cm^{-1}): 3625 (O-H stretching of free primary alcohol), 3534-3478, (N-H stretching of free primary amine), 3276 (C-H stretching vibration of aromatic region), 1266 (C-O stretching frequency of esters), 1757 (C=O stretching frequency for esters), 1580 (N-H deformation, in plane bending of N-H), 1508 (O-H in plane bending), 1381 (C-N stretching for carbon bonded to amino group), 987 (C-H in plane bending of phenyl ring), 742 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 345.08; Anal. Calcd. for $C_{18}H_{16}ClNO_4$: C, 62.52; H, 4.66; Cl, 10.25; N, 4.05; O, 18.51 Found: C, 62.45; H, 4.54; Cl, 10.19; N, 4.00; O, 18.45.

2.13.16 Ethyl-2-amino-4-(4-fluorophenyl)-7-hydroxy-4H-chromene-3-carboxylate (BN-16)



Yield: 87%; M.P.- 200-202 ºC; IR (cm^{-1}): 3627 (O-H stretching of free primary alcohol), 3514-3499, (N-H stretching of free primary amine), 3141 (C-H stretching vibration of aromatic region), 1256 (C-O stretching frequency of esters), 1722 (C=O stretching frequency for esters), 1595 (N-H deformation, in plane bending of N-H), 1568 (O-H in plane bending), 1351 (C-N stretching for carbon bonded to amino group), 962 (C-H in plane bending of phenyl ring), 796 (C-H out of plane bending for phenyl nucleus), MS: *m/z:* 329.11; Anal. Calcd. for $C_1H_{16}FNO_4$: C, 65.65; H, 4.90; F, 5.77; N, 4.25; O, 19.43 Found: C, 65.61; H, 4.83; F, 5.59; N, 4.12; O, 19.32.

2.14 SPECTRAL DISCUSSION

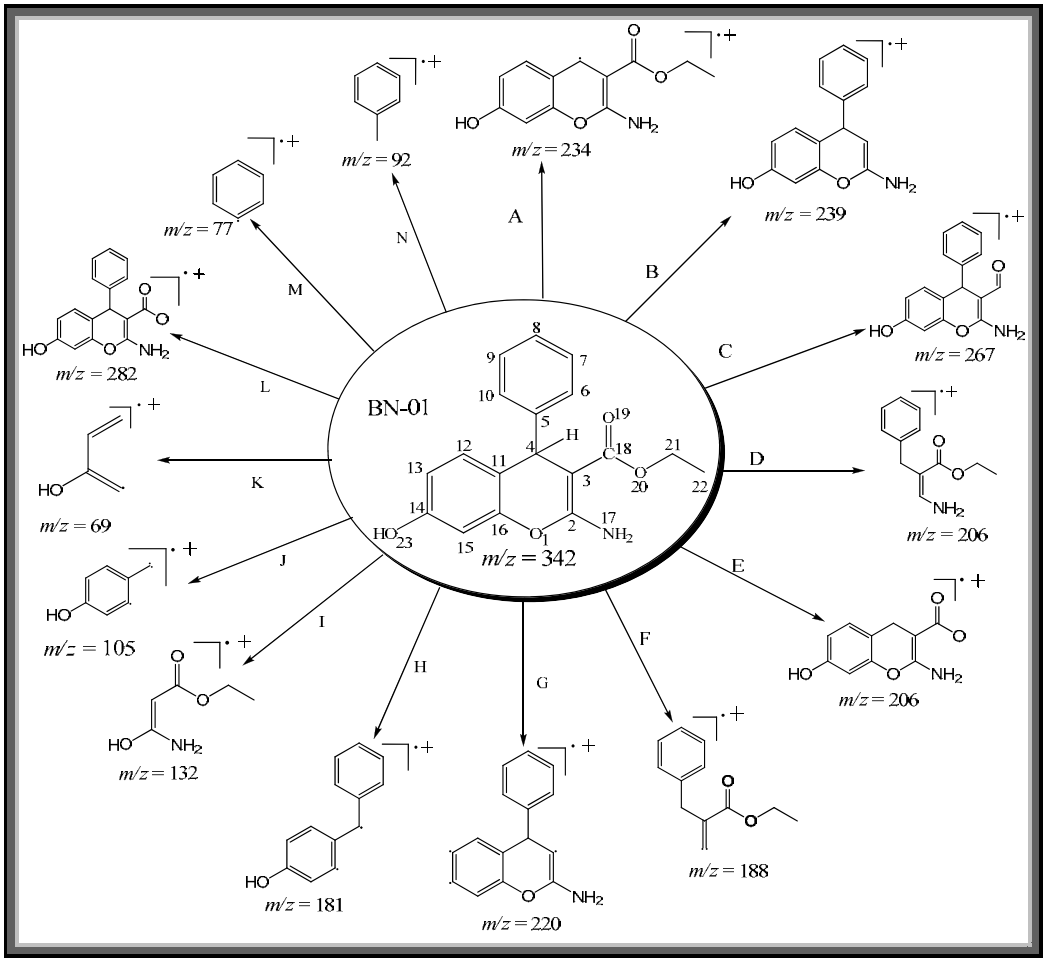
2.14.1 *IR Spectral study*

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. The characteristic bands of Hydroxyl groups were obtained for stretching at 3400-3650 cm^{-1}, and those for bending were obtained at 1050-1250 cm^{-1}. The characteristic bands of amino group were obtained for stretching at 3500-3400 cm^{-1} with a deformation due to in plane bending at 1650-1580 cm^{-1}. The characteristic bands of the Ester group were seen for the Carbonyl function of the group at 2250-2100 cm^{-1} while the C-O stretching frequency was seen at 1095-1066 cm^{-1}, also the C=O stretching frequency was observed around 1710 to 1760 cm^{-1}. The general aromatic C-C stretching bands were observed at 1460-1408 cm^{-1} while the out of plane bending frequency of C-H was seen between 952-696 cm^{-1}. The characteristic bands for halogen groups like chlorine and bromine were found at 740-700 cm^{-1} & 600-500 cm^{-1}. Also characteristic stretching frequencies of 1,3-Disubstituted and 1,4-Disubstituted phenyl ring were found at 671 cm^{-1} and 823 cm^{-1} respectively suggesting the correct formation of the desired products (BN-1 to BN-16).

2.14.2 *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. The probable Mass fragmentation pattern for the representative compound of each series is discussed below.

2.14.2.1 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF BN-01*

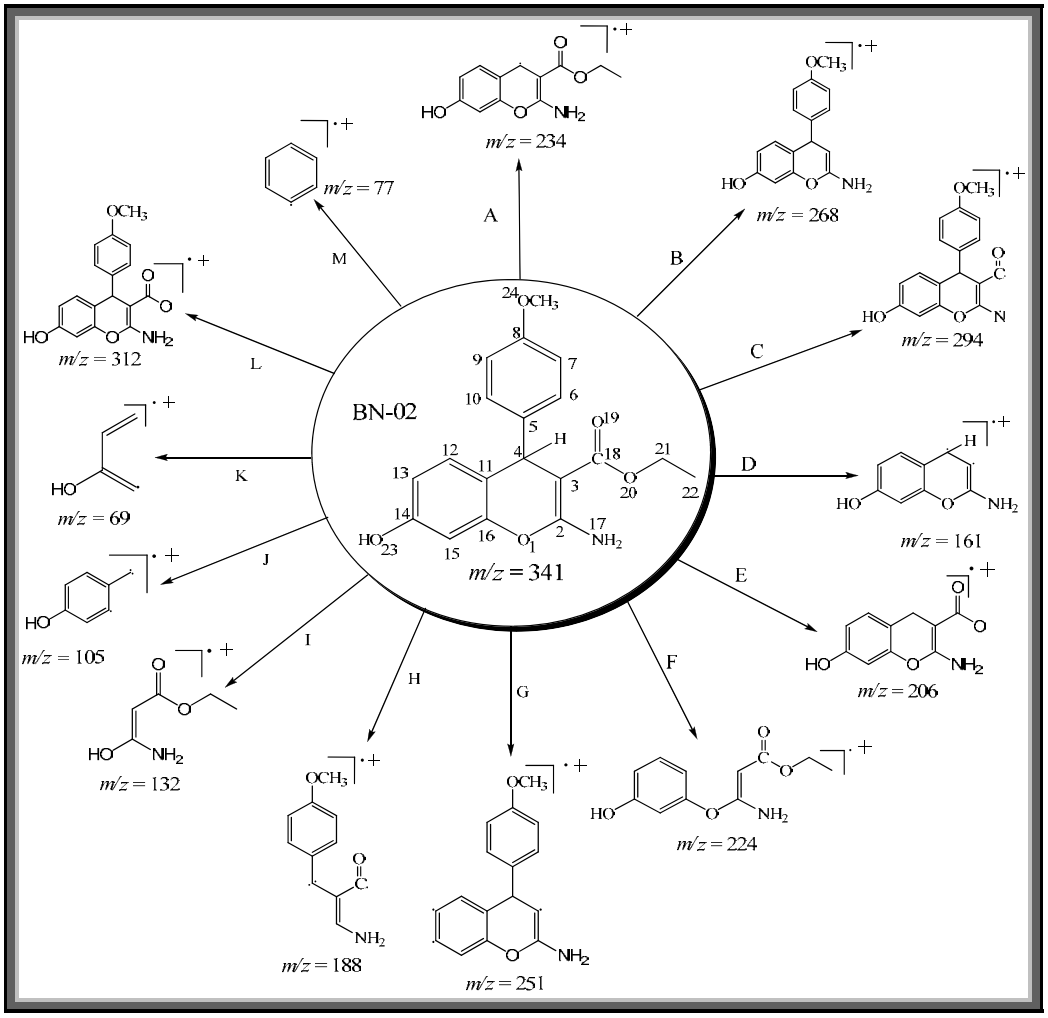


Ethyl 2-amino-7-hydroxy-4-phenyl-4H-chromene-3-carboxylate (BN-1)

1. The target compound showed the characteristic molecular ion peak 311 *m/z*.
2. The bond cleavage between C_4-C_5 generated a molecular ion which corresponds to a characteristic peak at 234 *m/z* **(A).**
3. A bond cleavage between C_3-C_{18} generated a molecular ion which corresponds to a characteristic peak at 239 *m/z* **(B)**.
4. Bond cleavages between C_{18}-O_{20} generated a molecular ion which corresponds to a characteristic peak at 267 *m/z* **(C)**.
5. Bond cleavages between C_4-C_{11} and O_1-C_2 generated a molecular ion which corresponds to a characteristic peak at 206 *m/z* **(D)**.
6. Bond cleavages between C_4-C_5 and O_{20}-C_{21} generated a molecular ion which corresponds to a characteristic peak at 206 *m/z* **(E)**.

7. Bond cleavages between C_2-N_{17}, O_1-C_2 and C_4-C_{11} generated a molecular ion which corresponds to a characteristic peak at 188 *m/z* **(F)**.
8. Bond cleavages between C_3-C_{18}, and C_{14}-O_{23} generated a molecular ion which corresponds to a characteristic peak at 220 *m/z* **(G)**.
9. Bond cleavages between C_3-C_4 and O_1-C_{16} generated a molecular ion which corresponds to a characteristic peak at 181 *m/z* **(H)**.
10. Bond cleavages between C_3-C_4, O_1-C_{16}, C_4-C_5 & C_4-C_{11} generated a molecular ion which corresponds to a characteristic peak at 132 *m/z* **(I)**.
11. Bond cleavages between C_4-C_5, C_3-C_4, & O_1-C_{16} generated a molecular ion which corresponds to a characteristic peak at 105 *m/z* **(J)**.
12. Bond cleavages between C_{11}-C_{12}, C_{15}-C_{16}, generated a molecular ion which corresponds to a characteristic peak at 69 *m/z* **(K)**.
13. Bond cleavage between O_{20}-C_{21} generated a molecular ion which corresponds to a characteristic peak at 282 *m/z* **(L)**.
14. The other fragment caused due to bond cleavage between C_4-C_5, generated a molecular ion which corresponds to a characteristic peak at 77 *m/z* **(M)**.
15. Bond cleavages between C_3-C_4, & C_4-C_{11} generated a molecular ion which corresponds to a characteristic peak at 92 *m/z* **(N)**.

2.14.2.2 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF BN-02*



Ethyl-2-amino-7-hydroxy-4-(4-methoxyphenyl)-4H-chromene-3-carboxylate (BN-02).

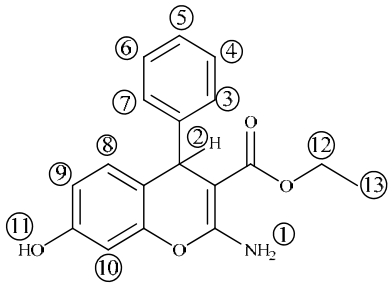
1. The target compound shows the desired characteristic molecular ion peak of 341 *m/z*.
2. The bond cleavage between C_4-C_5 generated a molecular ion which corresponds to a characteristic peak at 234 *m/z* **(A)**.
3. The bond cleavage between C_3-C_{18} generated another molecular ion which corresponds to a characteristic peak at 268 *m/z* **(B)**.
4. The bond cleavage between C_{18}-O_{20} generated a molecular ion which corresponds to a characteristic peak at 294 *m/z* **(C)**.
5. The bond cleavages between C_4-C_5 & C_3-C_{18} generated a molecular ion which corresponds to a characteristic peak at 161 *m/z* **(D)**.

6. The bond cleavages between C_4-C_5 & O_{20}-C_{21} generated a molecular ion which corresponds to a characteristic peak at 206 *m/z* **(E)**.
7. The bond cleavages between C_3-C_4 & C_4-C_{11} generated a molecular ion which corresponds to a characteristic peak at 224 *m/z* **(F)**.
8. The bond cleavages between C_3-C_{18} & C_{14}-O_{23} generated a molecular ion which corresponds to a characteristic peak at 251 *m/z* **(G)**.
9. The bond cleavages between O_1-C_2, O_1-C_{16}, C_{18}-O_{20}, & C_4-C_{11} generated a molecular ion which corresponds to a characteristic peak at 188 *m/z* **(H)**.
10. The bond cleavage between C_3-C_4, C_4-C_5, O_1-C_{16} & C_4-C_{11} generated a molecular ion which corresponds to a characteristic peak at 132 *m/z* **(I)**.
11. Bond cleavages between C_4-C_5, C_3-C_4, & O_1-C_{16} generated a molecular ion which corresponds to a characteristic peak at 105 *m/z* **(J)**.
12. Bond cleavages between C_{11}-C_{12}, C_{15}-C_{16}, generated a molecular ion which corresponds to a characteristic peak at 69 *m/z* **(K)**.
13. Bond cleavage between O_{20}-C_{21} generated a molecular ion which corresponds to a characteristic peak at 312 *m/z* **(L)**.
14. The other fragment caused due to bond cleavage between C_4-C_5, generated a molecular ion which corresponds to a characteristic peak at 77 *m/z* **(M)**.

2.14.3 *^{1}H-NMR SPECTRAL STUDY*

^{1}H-NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in DMSO solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from H-NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. The spectral interpretation can be discussed as under.

Ethyl 2-amino-7-hydroxy-4-phenyl-4H-chromene-3-carboxylate (BN-01)



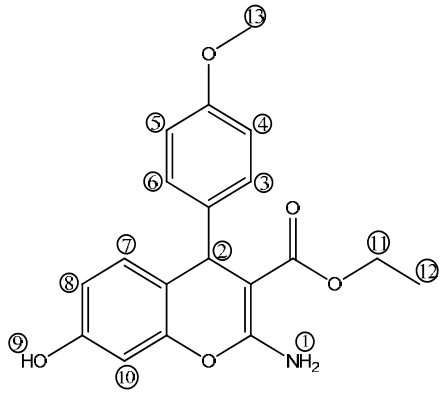
1. The two protons (proton no. 1) of the amino group gave a characteristic singlet at 6.46 δ ppm.
2. The methine proton (proton no. 2) on C-4 gave a characteristic singlet 4.79 δ ppm. The assignment of this proton is the most important for the structure elucidation and as it is evident here the successful assignment of this singlet has confirmed our structure. The proton no. 2 is a bit downfield as compared to a proton singlet in isolation because of the strong electron withdrawing group like $–COOC_2H_5$ present on the adjacent carbon which deshields the proton forcing it to go down field.
3. Now the aromatic protons no. 3, 4, 5, 6, 7, and 8 are all in the aromatic region of the NMR spectrum and gave a characteristic multiplet accounting for six protons between 7.14-7.18 δ ppm.
4. The proton no. 9 gave a characteristic doublet for a single proton between 6.86-6.83 δ ppm with a J value of 8.76 Hz suggesting that it is ortho coupled to another proton in its vicinity.
5. The proton no. 10 gave a characteristic singlet for a single proton in the region of 7.05 δ ppm with the J value of 2.64 Hz which clearly indicates that it is meta coupled with the proton no. 9.
6. The proton of the hydroxyl group gave a characteristic singlet at 9.25 δ ppm. As the proton is bonded directly to Oxygen, an electronegative entity, the

proton gets completely deshielded and the signal shifts to such a downfield region as expected.

7. The two protons of the ethyl group i.e. proton no. 12 is on the carbon directly bonded to the oxygen of the ester moiety thus they would be deshielded due to the electronegetivity of the oxygen atom and hence are seen as the quartet between 3.95-4.00 δ ppm with a very high J value suggesting that it is ortho coupled to the other protons.
8. Proton no 13 of the ethyl group gave a characteristic triplet between 1.08-1.12 δ ppm as expected for a methyl group.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton it can be clearly suggested that the proposed structure for compound no. BN-01 has been confirmed.

Ethyl-2-amino-7-hydroxy-4-(4-methoxyphenyl)-4H-chromene-3-carboxylate (BN-02)



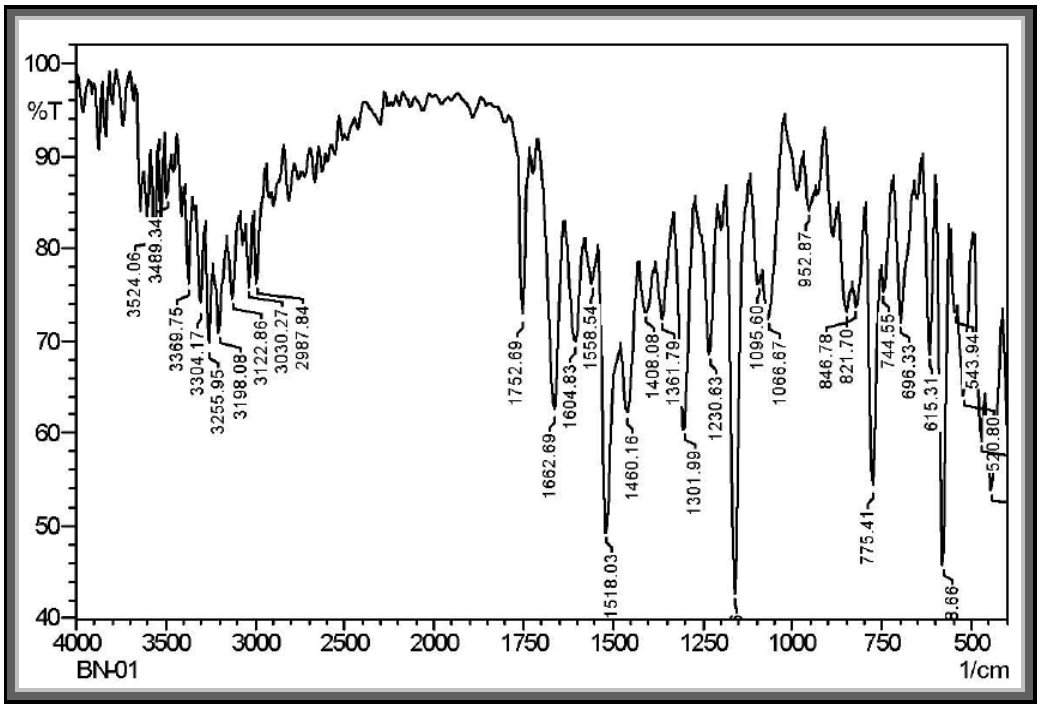
1. The two protons (proton no. 1) of the amino group were distinctively observed as a broad singlet at 6.45 δ ppm. As the protons are bonded to the electronegative Nitrogen atom they are de-shielded and found at such a downfield region of 6.45 δ ppm.
2. The proton no. 2 or the methine proton which is very important for our structure elucidation is evidently observed as a sharp singlet at 4.74 δ ppm. The reason for it being downfield as compared to other isolated proton normally found at an upfield frequency of around 2 δ ppm is due to the fact that an electronegative ester group is attached to its neighboring carbon atom moreover it is surrounded by phenyl ring which acts as an electron sink thus making the proton significantly de-shielded and hence it is found at 4.74 δ ppm.

3. Now just by looking at the structure it is evident that the chemical environment of proton no. 3 and proton no. 6 as well as that of proton no. 4 and 5 are identical, hence their signals should also be the same. The broad singlet assigned for two protons in the aromatic region at 7.14 δ ppm are responses for proton no. 3 and proton no. 6.
4. As discussed above, the chemical environment for proton no. 4 as well as proton no. 5 is also similar hence their signals would also similar. This was evidently observed as a doublet between 7.04 δ ppm to 7.05 δ ppm which accounted for two protons.
5. The proton no. 7 and proton no. 8 could be assigned to the doublet shown in the ^{1}H-NMR spectrum between 6.68 δ ppm and 6.70 δ ppm. The J value for this proton was calculated to be 8 Hz suggesting that it is ortho coupled to another proton.
6. The proton no. 9 here is the hydroxyl proton bonded to the electronegative oxygen atom; hence as it is completely deshielded we observe a sharp singlet at 9.22 δ ppm.
7. The proton no 10 is observed as a doublet between 6.83 δ ppm to 6.82 δ ppm. The splitting occurs due to its proximity to the hydroxyl proton.
8. The two protons assigned as no. 11 here are observed as a singlet at 3.98-3.99 δ ppm and are comparatively downfield due to the oxygen atom directly bonded to it.
9. The proton no. 12 is observed as a characteristic triplet for 3 protons at 1.12 δ ppm to 1.13 δ ppm.
10. The methoxy protons are observed at 3.69 δ ppm as a singlet and are in the downfield region due to their proximity to the oxygen atom.
11. The methine proton, the Methoxy protons as well as all the protons of the aromatic region seen very clearly in the spectra confirms the proposed structure.

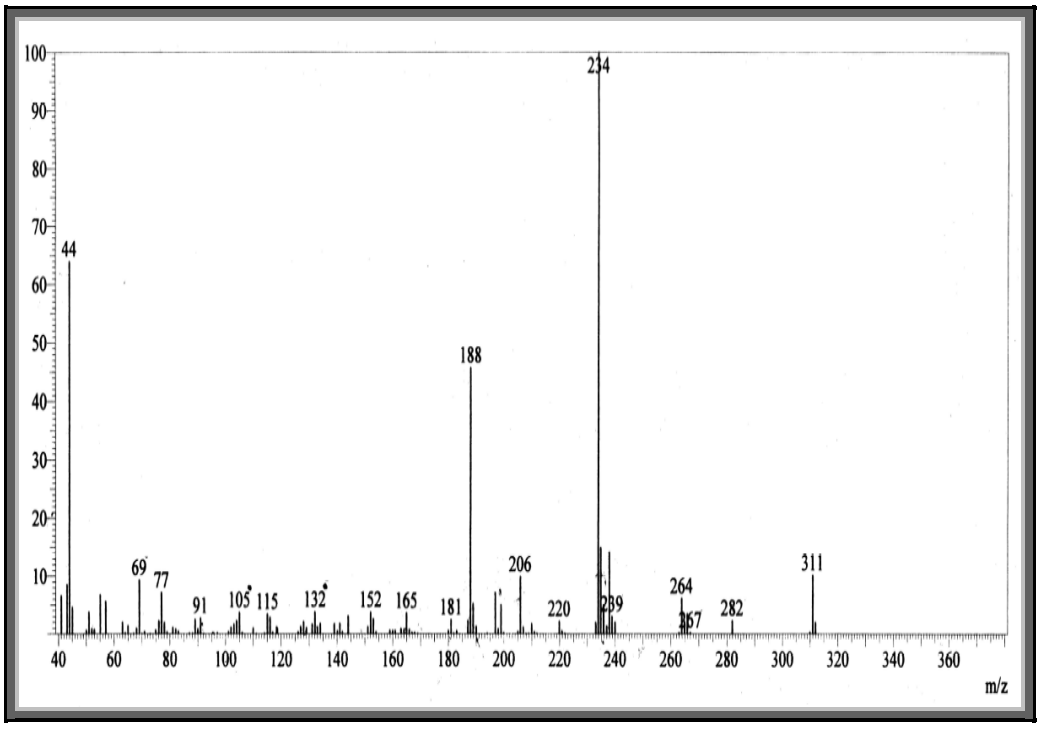
Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton it can clearly be suggested that the proposed structure for compound no. BN-02 has been confirmed.

2.15 SPECTRAL REPRESENTATIONS COMPOUNDS

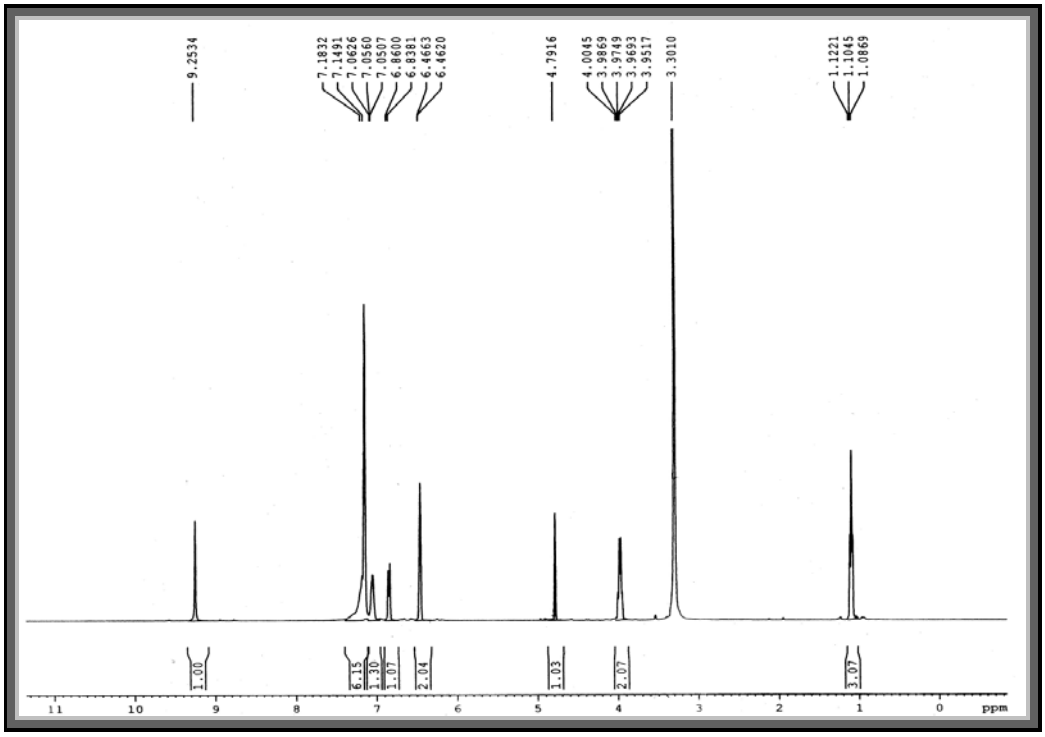
2.15.1 *IR SPECTRUM OF BN-01*



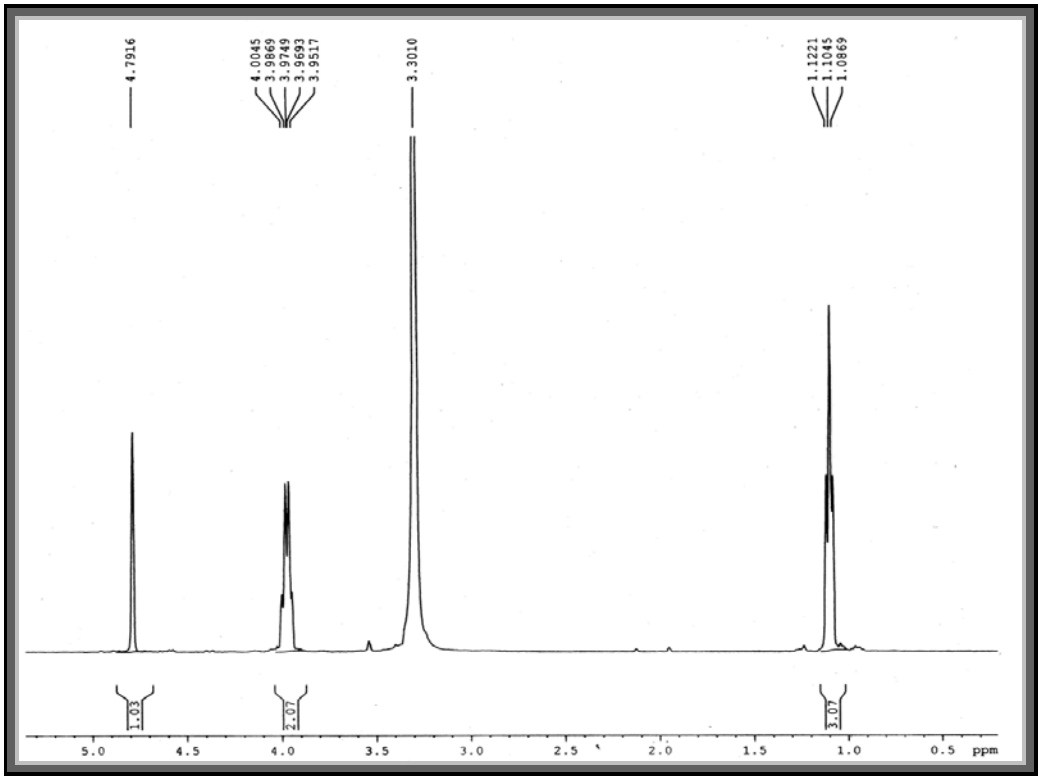
2.15.2 *MASS SPECTRUM OF BN-01*



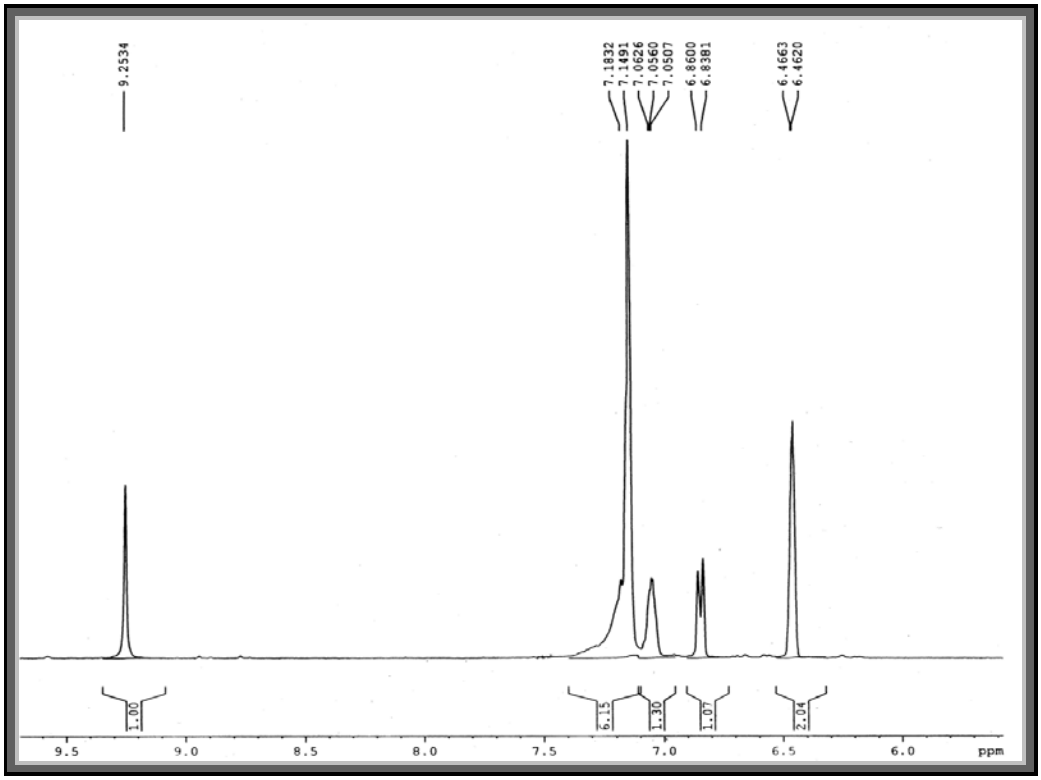
2.15.3 *^{1}H-NMR SPECTRUM OF BN-01*



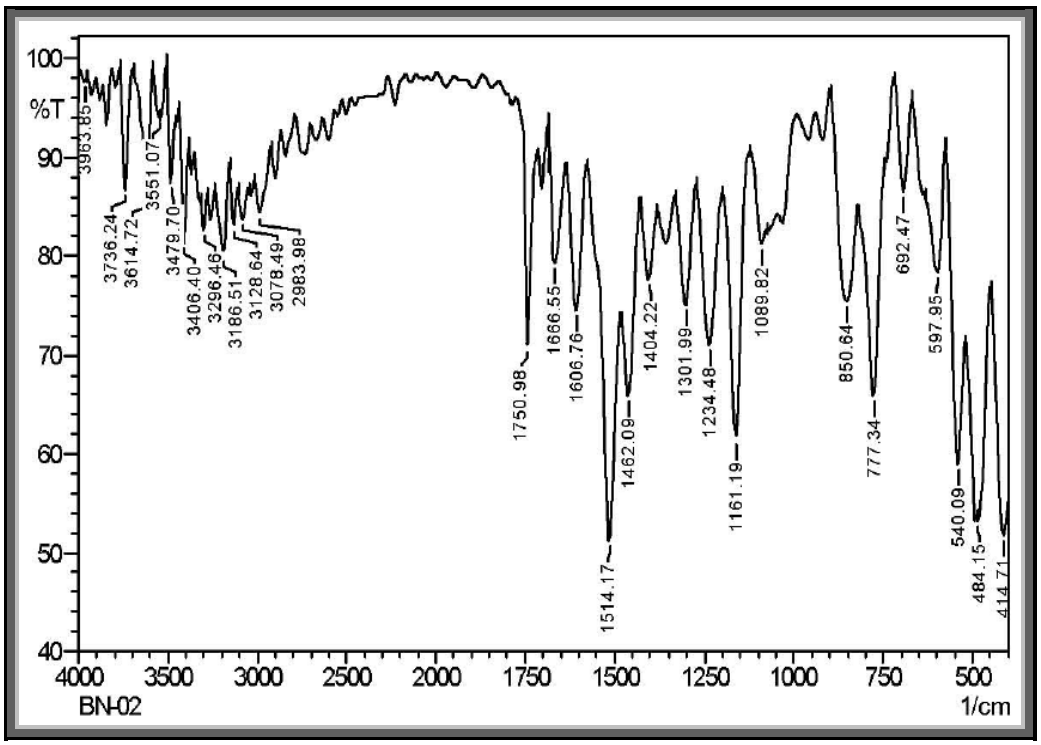
2.15.3.1 *EXPANDED ^{1}H-NMR SPECTRUM OF BN-01*



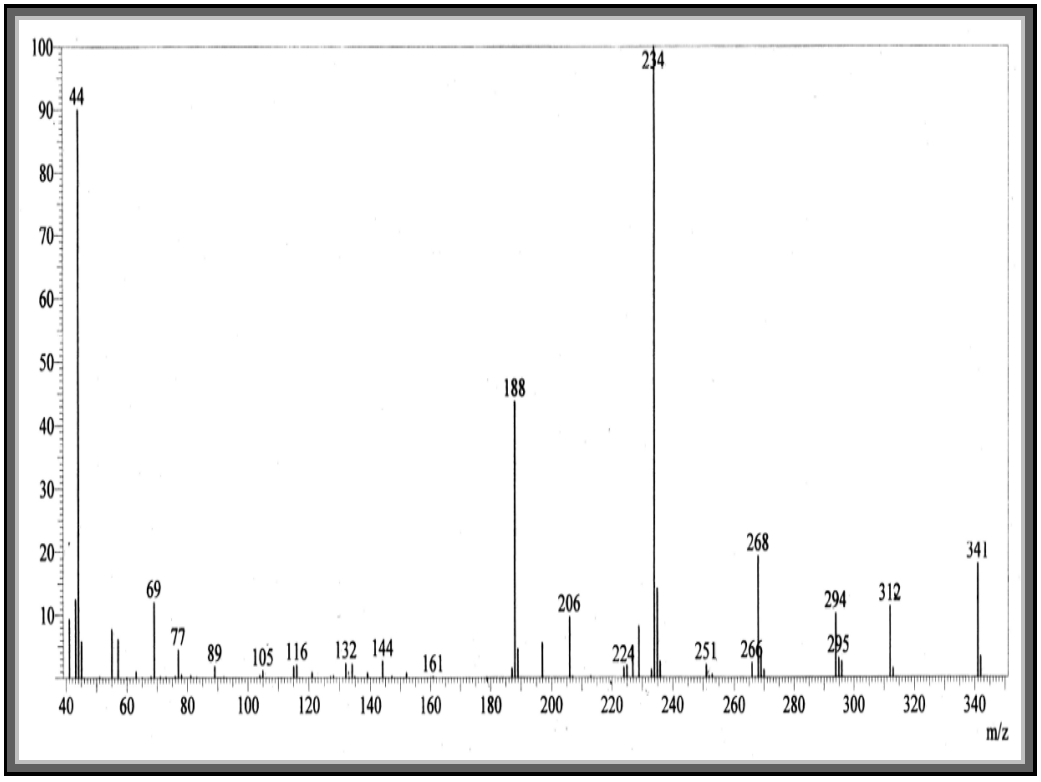
2.15.3.2 *EXPANDED ^{1}H-NMR SPECTRUM OF BN-01*



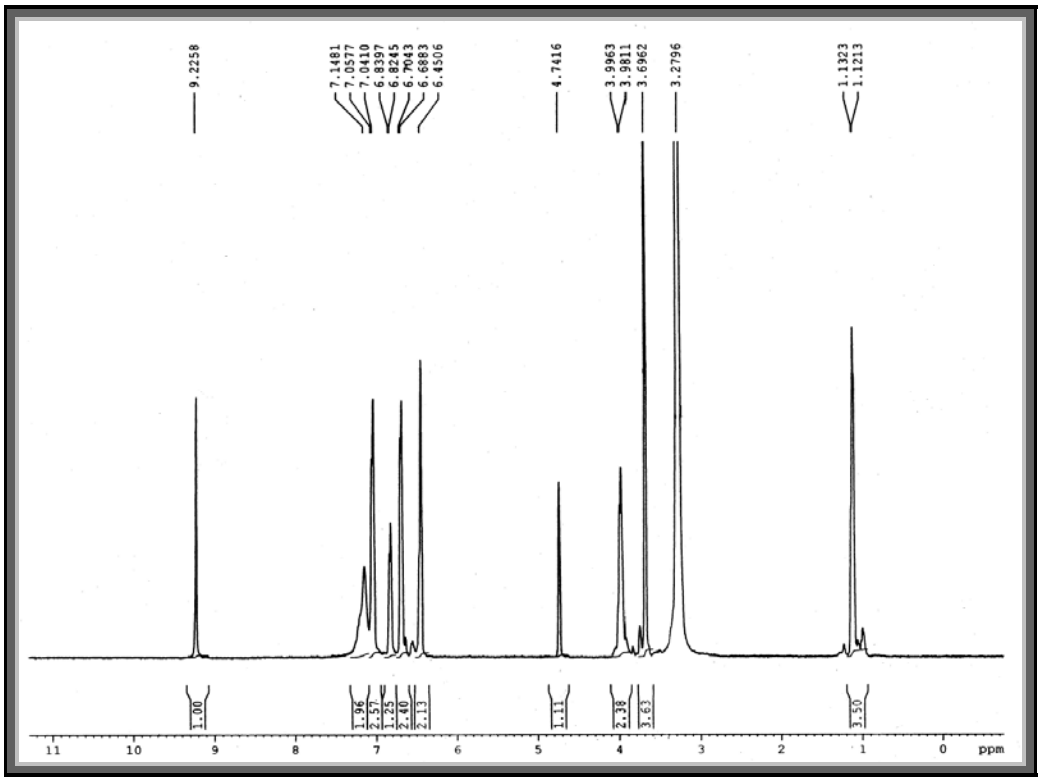
2.15.4 *IR SPECTRUM OF BN-02*



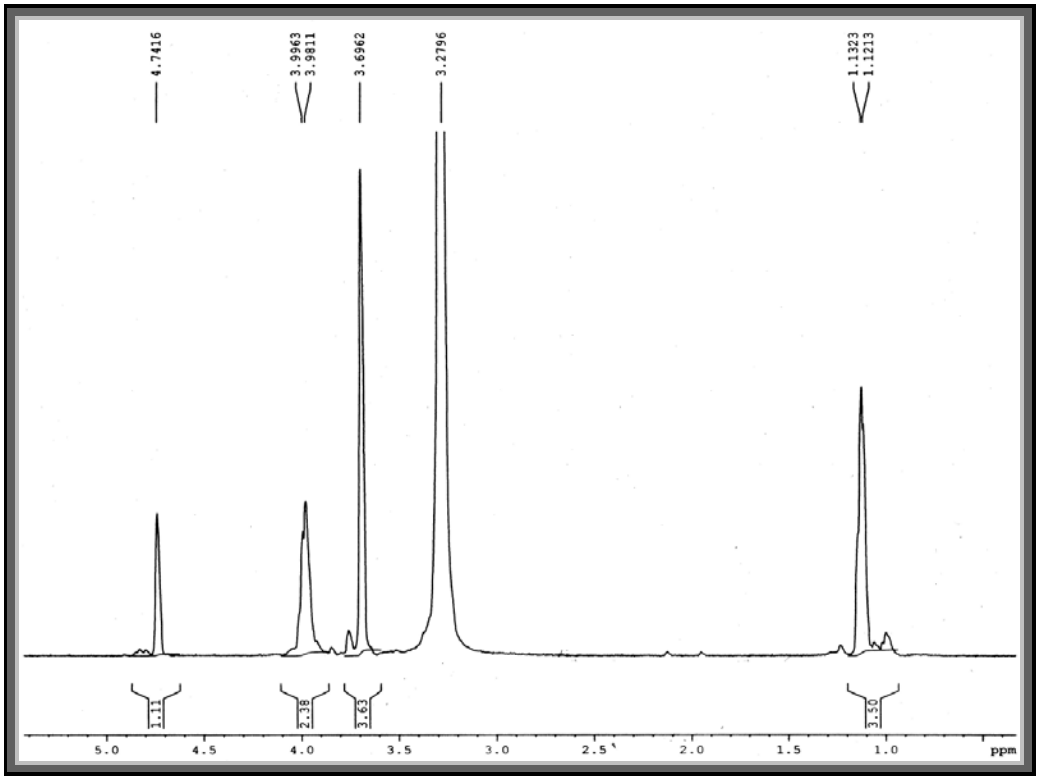
2.15.5 *MASS SPECTRUM OF BN-02*



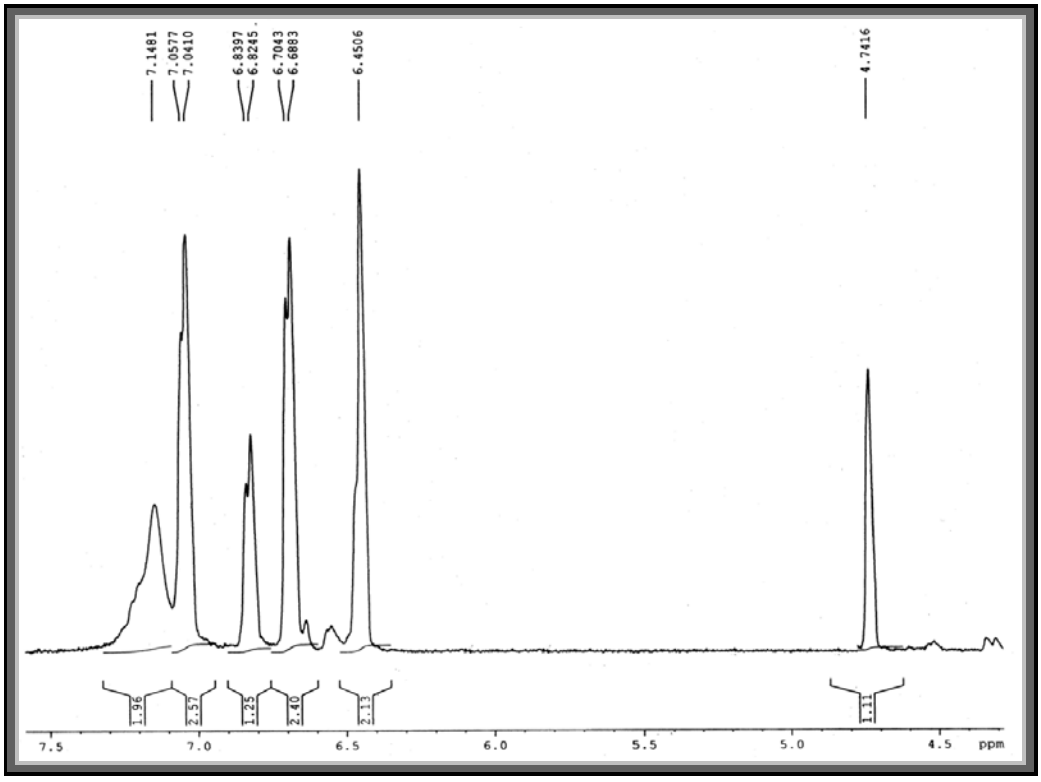
2.15.6 *^{1}H-NMR SPECTRUM OF BN-02*



2.15.6.1 *EXPANDED ^{1}H-NMR SPECTRUM OF BN-02*



2.15.6.2 *EXPANDED ^{1}H-NMR SPECTRUM OF BN-02*



2.16 RESULTS AND DISCUSSIONS

This chapter deals with the 2-Amino-3-cyano / ethyl carboxylate-4-substituted phenyl-benzopyran core structure. The importance of chromene / Benzopyran as a privileged structure was discussed at length in the introduction of this chapter which served as the rational for the synthesis of these kinds of compounds. Moreover, this chapter has quite a lot of interesting features in terms of synthesis methodology as well as biological activity study. First of all the reaction employed in this chapter is a 3 component reaction and as we all know multicomponent reactions have quite a lot of advantages over the normal conventional methodologies. Also, the medium by which the energy was supplied was Microwave irradiation which made this a Microwave assisted Organic Synthesis which again has its own advantage of using optimum time and resources. And last but not the least, none of the hazardous organic volatile solvents were employed in the reaction; instead we did this reaction using the universal solvent i.e. water which made our process appreciably green. The bioactivity of these compounds is all together another interesting aspect of the novel compounds enlisted in this chapter.

2.17 CONCLUSION

This chapter involves some interesting chemical aspects as far as organic chemical synthesis is concerned. The New chemical entities synthesized in this chapter were screened for Anti HIV study. The results of which are discussed separately.

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CHAPTER – 3

Section-A

Microwave assisted synthesis of *8-Hydroxy-5-substituted phenyl-3H-chromeno-[2, 3-d]pyrimidine-4(5H)-ones*

3.1 THE ROLE OF FUSED HETEROCYCLES IN THE DRUG DISCOVERY PARADIGM

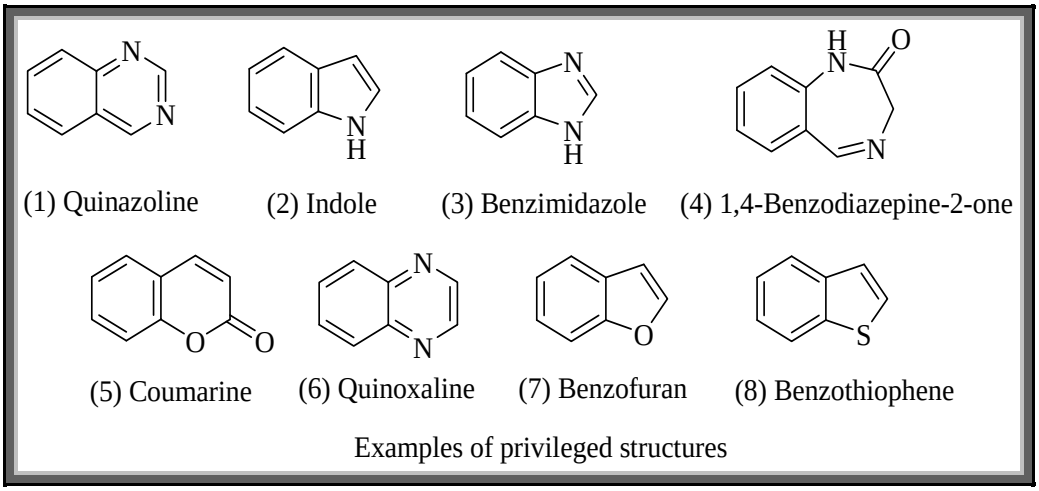
The entire pharmaceutical industry is faced with the challenge of increasing productivity and innovation. The major hurdles are the increasing costs of research and development and a simultaneous stagnating number of new chemical entities (NCE's).

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 NCE's 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].

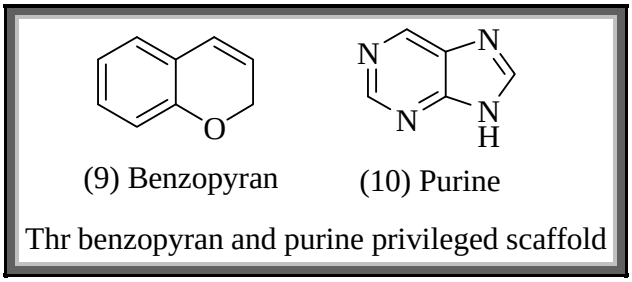
In order to start a new drug discovery project and to find biologically active compounds, different options are available. Hits can be obtained *via* a virtual screening approach or can be copied from scientific or patent literature. Very often, drug discovery projects start with a high-throughput screening 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 bicyclic aromatic heterocycles, we performed a literature survey of commercially available combinatorial libraries from three major vendors (Interbioscreen, ChemBridge and ChemDiv). This search revealed that the number of available bicyclic 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

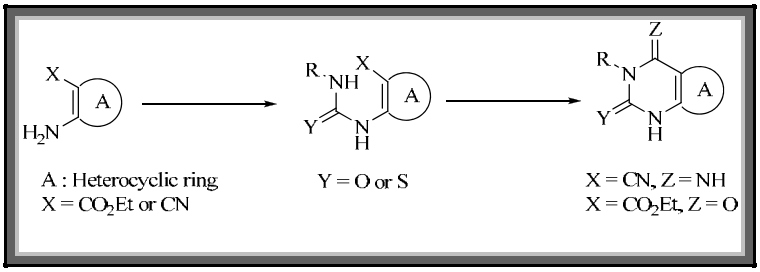
Thr benzopyran and purine privileged scaffold

Fused pyrimidines continue to attract considerable attention of researchers in different countries because of their great practical usefulness, primarily, due to a very wide spectrum of their biological activities. Chromenopyrimidines occupy a special position among these compounds. Also, the important nucleic acid bases like Uracil, thymine and cytosine are all Pyrimidinones.

3.1.1 *SOME REPORTED SYNTHETIC STRATEGIES*

The purine like analogues containing a fused five membered ring system with the pyrimidine nucleus has been vigorously studied world over by various groups working on heterocyclic chemistry. Especially, thieno pyrimidines as well as pyrrolo pyrimidines have been the scaffolds of choice owing to their bioisosterism with the phenyl nucleus which allows a scientist to replace the phenyl group from a bioactive moiety like fused benzopyrimidine and replace it by thiol or pyrrole ring which would lead to new bio active molecules.

There are several strategies to prepare fused pyrimidine ring systems. The construction of a pyrimidine ring system from a 2-amino-five / six membered heterocyclic derivatives follows a typical reaction sequence. One of the most popular approaches to construct the pyrimidine ring is *via* the synthesis of substituted ureas and thio ureas. In a first step, the amino group of any heterocyclic moiety is converted into a urea by treatment with an isocyanate [8], potassium cyanate hydrochloride [9], or chlorosulfonyl isocyanate [10] and into a thiourea by reaction with an isothiocyanate [11], or thiophosgene and an amine [12]. The resulting ureas and thio ureas readily undergo an intramolecular cyclization upon treatment with bases or acids to afford the fused pyrimidine ring systems.



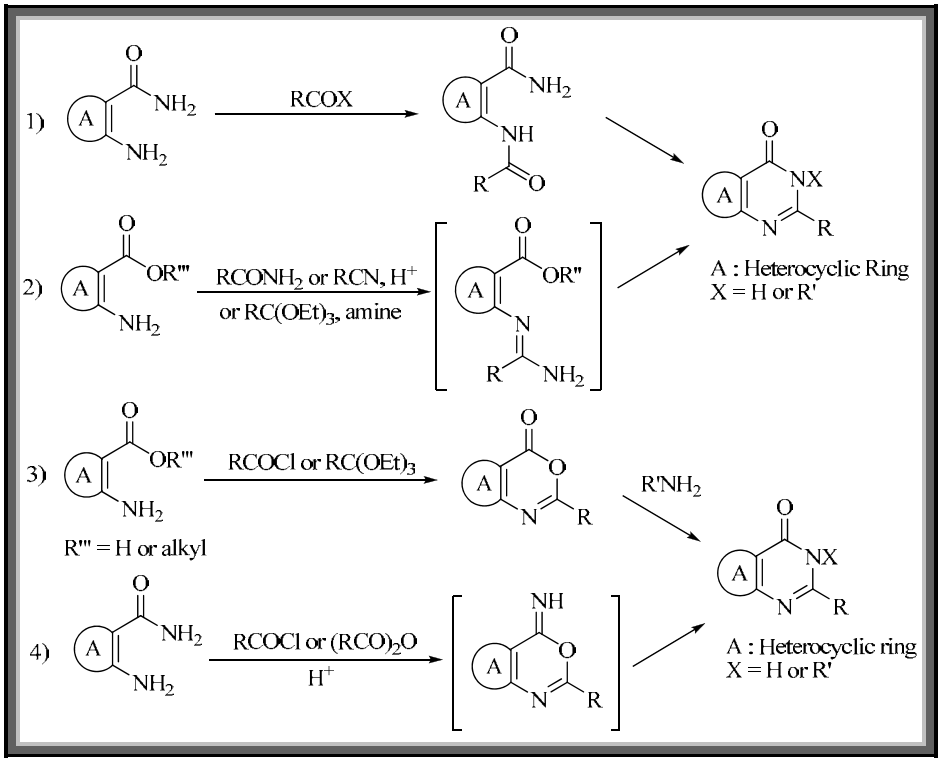
The synthesis of substituted pyrimidin-4-ones is well studied and can be categorized into four groups according to the functional groups on the fused heterocyclic moiety and the structures of the intermediates.

(1) Substituted pyrimidinones can be prepared *via* cyclization of diamides intermediates, which are generated from *vic*-aminocarbamoylbenzopyrans by reaction with acylating agents such as orthoesters [13], acid anhydrides and acid chlorides [14], formic acid [15] and diethyl oxalate [16].

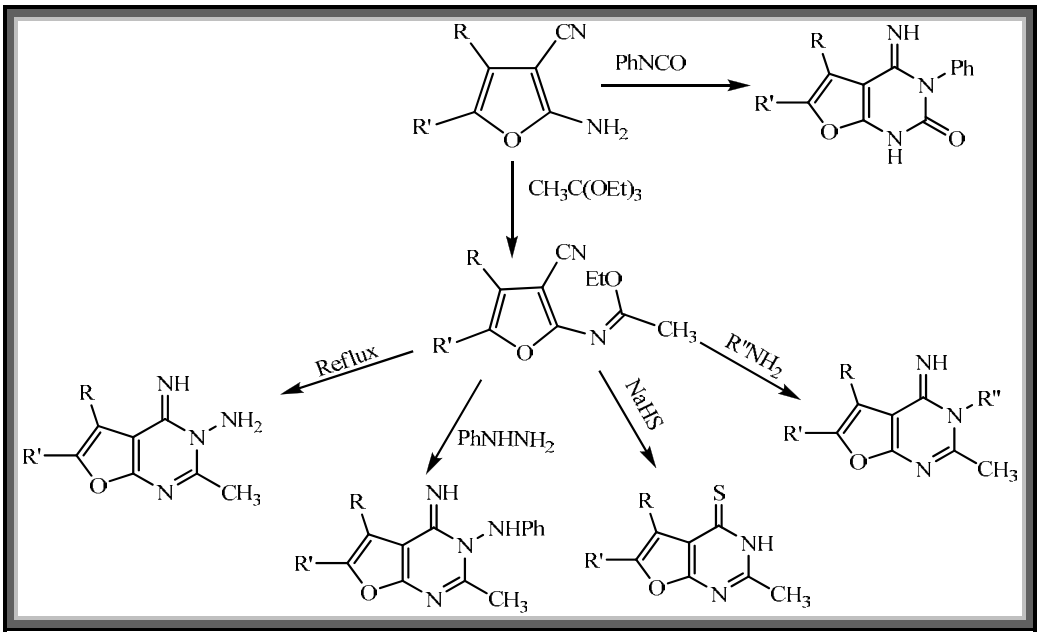
(2) Alternatively, the synthesis of substituted pyrimidinones can be achieved from *vic*-aminoalkoxycarbonylbenzopyrans. Amidine intermediates, formed by the reaction of the fused heterocyclic compound with amides [17], nitrites under acidic conditions [18], orthoesters and amines [19], undergo an intramolecular cyclization to afford chromeno pyrimidinones.

(3) A third procedure is based on the recyclization of substituted oxazinones, which are generated by reaction of *vic*-aminocarboxylic acids or esters with acid chlorides or orthoesters [20]. The recyclization proceeds through the diamide intermediate which is generated upon treatment with amines [21].

(4) *Vic*-aminocyanoheterocyclic compounds also serve as valuable starting materials for the synthesis of substituted pyrimidinones. Initially, the oxazinimine intermediates are generated by the acylation of the amino group and then recyclization in the presence of an acid occurs to afford substituted pyrimidinones [22].

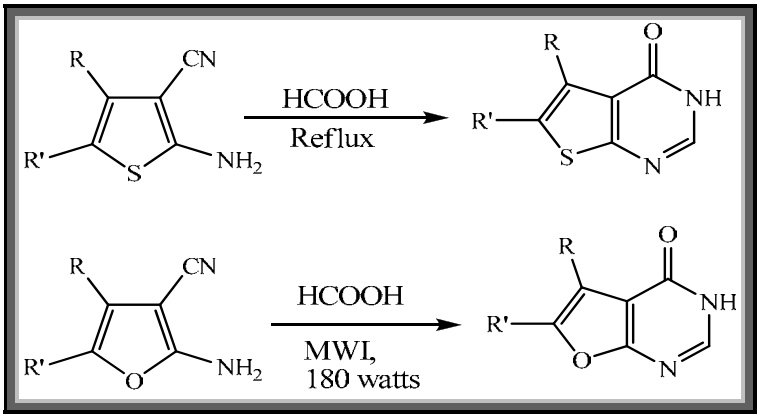


Hassan [23] synthesized various differently substituted furopyrimidine moieties via different sets of reactants and varying reaction parameters and are shown below.



The starting raw material in the above cited scheme is substituted furan moiety. It is also important to mention here that all the above reactions are carried out under the classical reaction conditions.

Ladda and Bhatnagar [24] have described an efficient nimentowski synthesis of novel Pyrimido pyrimidinones via the intermediate preparation of pyrimidin-4-one as shown below. Several other researchers have explored this chemistry in recent years and fairly good reviews and publications are cited in the literature [25-31].



3.2 AIM OF THE CURRENT WORK

This laboratory has been actively involved in exploring the possibility of synthesizing novel Nitrogen containing heterocyclic molecules having significant biological activity. A special mention to a bountiful work done by the team in the class of Dihydropyridine[a] and dihydropyrimidine[b] synthesis under the conditions of atmospheric temperature and pressure as well as studying their biological activities against various different targets has given significant results.

The pyrimidine-4-ones are structural analogues of the naturally occurring nucleic acids. Also the benzopyran moiety has its own importance of being a privileged structure as it is found in various natural products and bioactive compounds derived from the natural products which has already been discussed at length in the previous chapters of this thesis.

As a continuing endeavor to provide and promote the non conventional ways of synthesizing the organic compounds, microwave irradiation as a source of non-conventional energy has been used to synthesize all the compounds of this chapter. This happens to be a greener alternative as it reduces, time, energy as well as the other natural resources.

As evident from the above discussions the inclusion of two bioactive motifs like benzopyran and pyrimidine into a single carbon skeleton has never been achieved before. Hence, the opportunity to synthesize such interesting molecules in an environmentally benign way and exploring its biological activity was the main rational behind the work done under this chapter.

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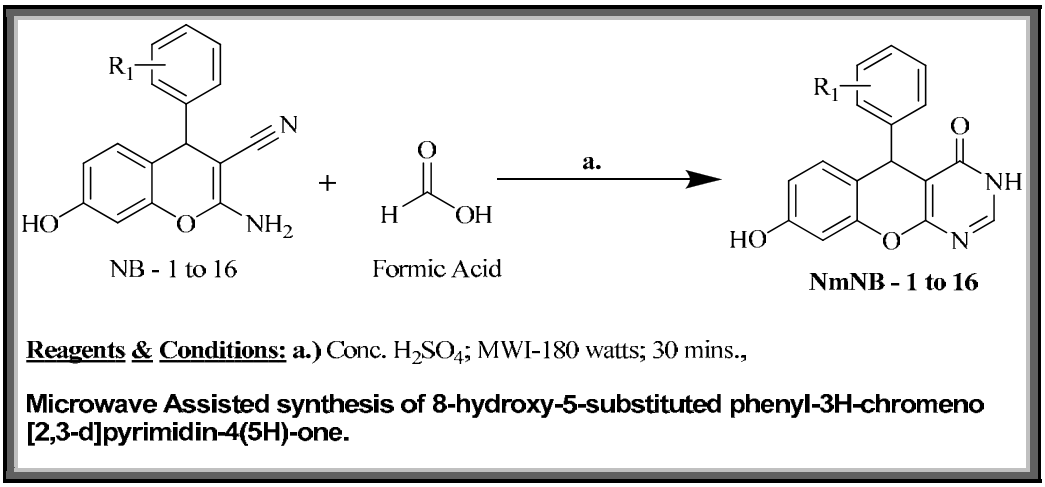
[a] Synthesis and structural conformation studies of a potent unsymmetrical 1,4-Dihydropyridine. Naveen S., Shashidhara Prasad, J.Shashidhara, Manvar Dinesh, Mishra Arun., Anamik Shah, Journal of Chemical Crystallography, 38(4), 315-319, **2008**.

3,5-Dibenzoyl-4-(3-phenoxyphenyl)-1,4-dihydropyridine (DP7): a new multidrug resistance inhibitor devoid of effects on langendorff perfused rat heart Simona Saponara, Antonella Ferrara, Beatrice Gorelli, Anamik Shah, MasamiKawase, Noboru Motohashi, Joseph Molnar, Giampietro Sgaragli, Fabio Fusi, European Journal of Pharmacology, 563, 160-163, **2007**

[b] Synthesis, characterization, crystal and molecular structure analysis of N-(2,4 dimethylphenyl)-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5 carboxamide, Naveen S., Priti Adlakha, Chintan Dholakia, Anamik Shah, Sridhar M.A. and Shashidhara Prasad, J. Structural Chemistry, 17, 569-575, **2006**.

3.3 REACTION SCHEME



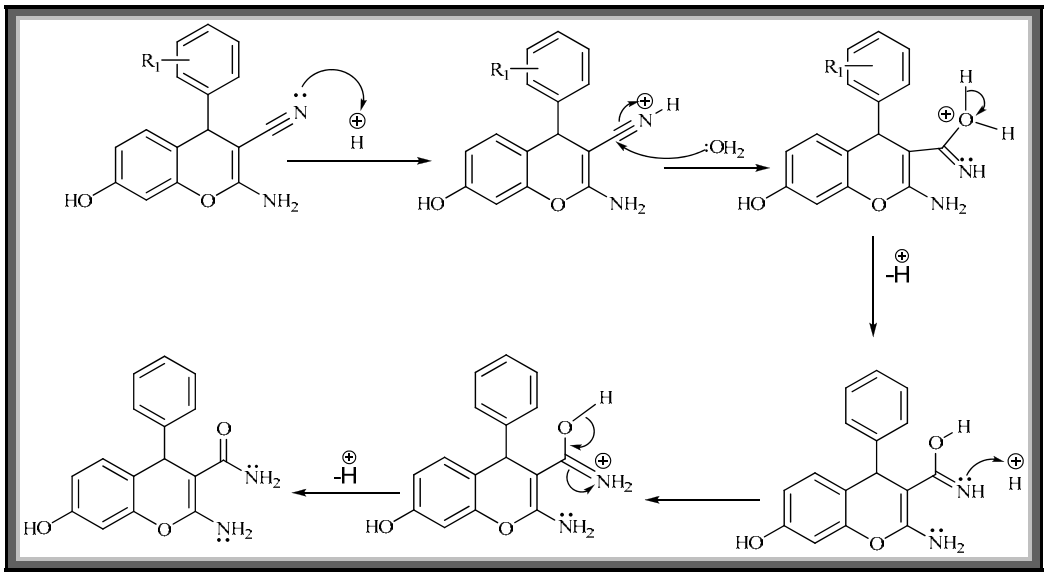
3.3.1 *PHYSICAL DATA TABLE*

Code	R_1	M. F.	M. W.	M. P. ^{0}C	Time (min)	Yield %	R_{f1}
NmNB-1	4-OCH_3	$C_{18}H_{14}N_2O_4$	322.31	215-217	18:00	88	0.48
NmNB- 2	H	$C_{17}H_{12}N_2O_3$	292.28	174-176	15:20	85	0.58
NmNB-3	3-Br	$C_{17}H_{11}BrN_2O_3$	370.18	182-184	21:00	82	0.54
NmNB-4	3-Cl	$C_{17}H_{11}ClN_2O_3$	326.73	186-188	25:20	84	0.56
NmNB-5	3-NO_2	$C_{17}H_{11}N_3O_5$	337.28	178-180	27:30	92	0.46
NmNB-6	3-OCH_3, 4-OH	$C_{18}H_{14}N_2O_5$	338.31	172-174	30:10	86	0.52
NmNB-7	3-OC_2H_5, 4-OH	$C_{19}H_{16}N_2O_5$	352.34	192-194	30:00	88	0.56
NmNB-8	4-$N(CH_3)_2$	$C_{19}H_{16}N_2O_5$	352.34	184-186	28:40	92	0.50
NmNB-9	4-NO_2	$C_{17}H_{11}N_3O_5$	337.29	180-182	26:40	94	0.48
NmNB-10	3,4-OCH_3	$C_{19}H_{16}N_2O_5$	352.34	210-212	29:40	95	0.58
NmNB-11	4-CH_3	$C_{18}H_{14}N_2O_3$	306.32	178-180	25:30	86	0.54
NmNB-12	2-OH	$C_{17}H_{12}N_2O_4$	308.29	180-182	27:30	85	0.58
NmNB-13	Furyl	$C_{15}H_{10}N_2O_4$	282.25	160-162	30:00	82	0.59
NmNB-14	2-Cl	$C_{17}H_{11}ClN_2O_3$	326.73	194-196	25:00	92	0.52
NmNB-15	4-Cl	$C_{17}H_{11}ClN_2O_3$	326.73	204-206	30:00	90	0.48
NmNB-16	4-F	$C_{17}H_{11}FN_2O_3$	310.28	172-174	30:00	85	0.50

TLC solvent system for R_{f1} = Toluene:Ethyl acetate - 7:3. Microwave Irradiation: 180 Watts.

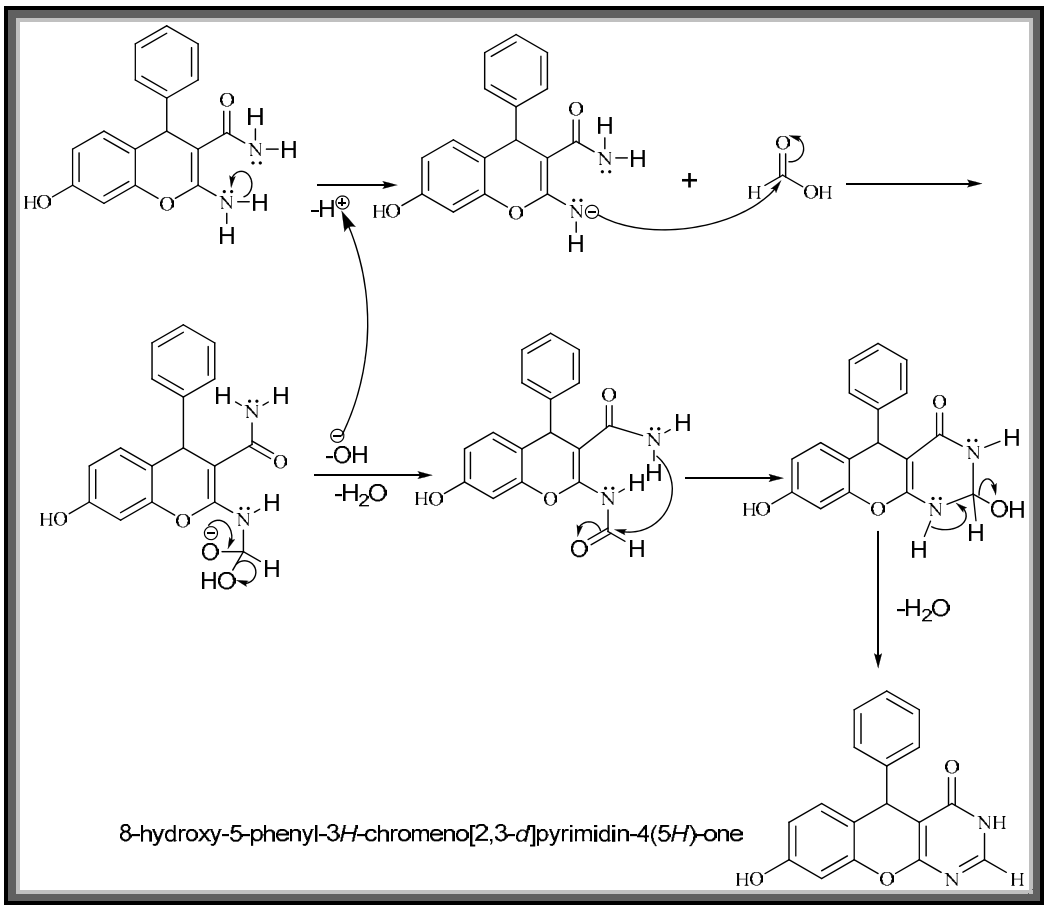
3.4 PLAUSIBLE REACTION MECHANISM

3.4.1 *STEP-I: ACID CATALYZED HYDROLYSIS OF THE NITRILE GROUP*



As evident in the first step, nitrile group is oxidized to amide in the presence of acid. The lone pair of electron first accepts the acidic proton which develops the positive charge on the nitrogen atom. The presence of water molecule in the acid would then quench the positive charge by attacking on the carbon atom of the nitrile moiety. The proton leaves its electron with the oxygen thus quenching the positive charge on it. Further more the lone pair of Nitrogen again accepts the proton thus forming the positively charged nitrogen which is subsequently quenched by the removal of the proton. This will afford the first intermediate 2-amino-7-hydroxy-4-phenyl-4H-chromene-3-carboxamide.

3.4.2 *STEP-2-FORMATION OF 8-Hydroxy-5-substituted phenyl-3H-chromeno [2,3-d]pyrimidin-4(5h)-one*



In this step, a proton from the amino group will leave forming a negatively charged nitrogen atom which is a highly unstable entity. The negative charge would then attack the carbonyl carbon of the acid group and will reach on the oxygen atom which on further displacement would remove the hydroxyl moiety from the acid thus removing a water molecule. After that again a proton, now from the amide group, would get displaced by attacking on the carbonyl carbon. The second water moiety is removed when the hydrogen attached on the amino nitrogen gets displaced to remove the hydroxyl function. This afforded the desired product i.e. 8-Hydroxy-5 -substituted phenyl-3H-chromeno [2,3-d]pyrimidin-4(5H)-one.

3.5 EXPERIMENTAL

3.5.1 *MATERIALS AND METHODS*

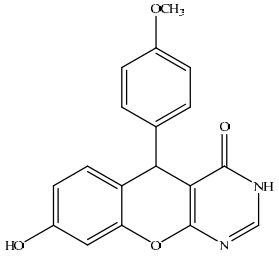
Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. All the reactions were carried out in **Samsung MW83Y Microwave Oven** which was locally modified for carrying out chemical reactions. IR spectra were recorded in **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.

3.5.2 *GENERAL PROCEDURE: 8-Hydroxy-5-substituted-phenyl-3H-chromeno [2,3-d]pyrimidin-4(5H)-ones*

As evident from the scheme, the products of chapter 2 (Section A) i.e. *2-Amino-7-hydroxy-4-(substituted phenyl)-4H-chromene-3-carbonitrile,* were taken as the starting materials. 0.01 M of *2-Amino-7-hydroxy-4-(substituted phenyl)-4H-chromene-3-carbonitrile* was dissolved in 20 ml of Formic acid which was used as a reactant as well as solvent. A few drops of Sulfuric acid were introduced as the acidic catalyst to promote the reaction. The reaction mixture was subjected to MWI for a specific time (see Physical data Table) at low power (180 W). The progress of the reaction was monitored by TLC examination at an interval of every 30 seconds. On completion of the reaction, the reaction mixture was cooled to room temperature and then poured over ice water. The reaction mixture was then neutralized by using sodium bicarbonate and the pH of the mixture was taken to 6. This reaction mixture was then separated using a separating funnel and the organic component was extracted thrice using ethyl acetate. The combined organic extract was washed twice with water and once with brine solution. Sodium sulphate was added to combined organic extract and was left overnight to remove trace amount of moisture. The combined organic layer was vacuum distillated. The product obtained was filtered, washed with cold water, dried, and recrystallized from rectified spirit.

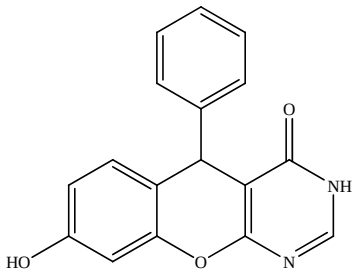
3.6 ANALYTICAL DATA

3.6.1 8-Hydroxy-5-(4-methoxyphenyl)-3H-chromeno-[2,3-d]pyrimidin-4(5H) one (NmNB-01)



Yield: 88%; M.P.- 215-217 ºC; IR (cm^{-1}): 3609 (O-H stretching of free primary alcohol), 1247, (O-H in plane bending frequency), 760 (O-H out of plane bending a broad peak), 3002 (C-H stretching frequency for aryl ethers), 1280 (C-O-C asymmetric stretching frequency for aralkyl ethers), 1668 (C=O stretching frequency for α, β-unsaturated ketone), 3115 (N-H stretching frequency for amides), 1330 (C-N stretching for carbon bonded to amino group in pyrimidine), 2920-2637 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1514 (C-C skeletal stretching of phenyl nucleus), 1199 (C-H in plane bending for the phenyl ring), 821 (C-H out of plane bending for 1,4-disubstituted benzene ring); ^{1}H NMR (DMSO-d_6) δ ppm: 7.57-7.68 (d, 1H, H_1), 9.26 (s, 1H, H_2), 6.59 (s, 1H, H_3), 6.76-6.78 (d, 2H, H_4, H_8, *J*=8 *Hz*), 6.96-6.98 (d, 2H, H_5, H_7, *J*=8 *Hz*), 3.74 (s, 3H, H_6), 7.32-7.38 (m, 3H, H_9, H_{10}, H_{11}), 10.06 (s, 1H, H_{12}); MS: *m/z:* 322.10; Anal. Calcd. for $C_{18}H_{14}N2O_4$: C, 67.07; H, 4.38; N, 8.69; O, 19.86 Found: C, 67.02; H, 4.35; N, 8.62; O, 19.80.

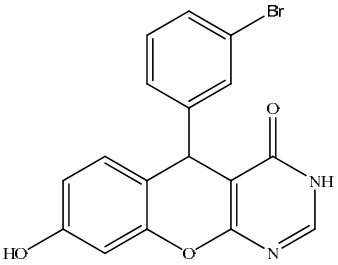
3.6.2 8-Hydroxy-5-phenyl-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-02)



Yield: 85%; M.P.- 174-176 ºC; IR (cm^{-1}): 3610 (O-H stretching of free primary alcohol), 1245, (O-H in plane bending frequency), 765 (O-H out of plane bending a broad peak), 1663 (C=O stretching frequency for α, β-unsaturated ketone), 3118 (N-H stretching frequency for amides), 1332 (C-N stretching for carbon bonded to amino group in pyrimidine), 2924-2630 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1517 (C-C skeletal stretching of phenyl nucleus), 1210 (C-H in plane bending for the phenyl

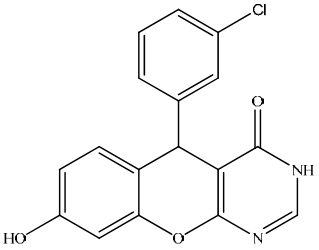
ring), 810 (C-H out of plane bending for 1,4-disubstituted benzene ring); MS: *m/z:* 292.08; Anal. Calcd. for $C_{17}H_{12}N_2O_3$: C, 69.86; H, 4.14; N, 9.58; O, 16.42 Found: C, 69.79; H, 4.06; N, 9.50; O, 16.35.

3.6.3 5-(3-Bromophenyl)-8-hydroxy-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-03)



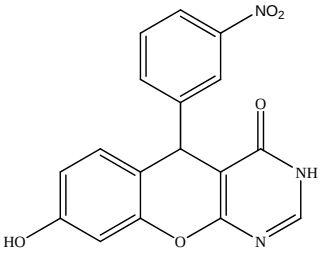
Yield: 82%; M.P.- 182-184 ºC; IR (cm^{-1}): 3248 (O-H stretching of free primary alcohol), 1252, (O-H in plane bending frequency), 713 (O-H out of plane bending a broad peak), 1666 (C=O stretching frequency for α, β-unsaturated ketone), 2985-2927 (N-H stretching frequency for amides), 1334 (C-N stretching for carbon bonded to amino group in pyrimidine), 2926-2662 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1622 (C-C skeletal stretching of phenyl nucleus), 1124 (C-H in plane bending for the phenyl ring), 870 (C-H out of plane bending for 1,4-disubstituted benzene ring), 592 (C-Br stretching for aromatic compounds); MS: *m/z:* $M^{\cdot+}$= 370.00 and M+2=372; Anal. Calcd. for $C_{17}H_{11}BrN_2O_3$: C, 55.01; H, 2.99; Br, 21.53; N, 7.55; O, 12.93 Found: C, 54.93; H, 2.94; Br, 21.45 N, 7.49; O, 12.84.

3.6.4 5-(3-Chlorophenyl)-8-hydroxy-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-04)



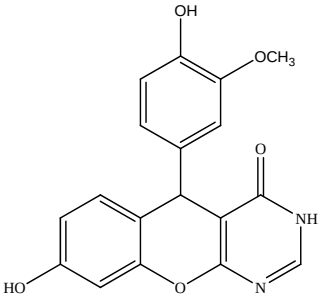
Yield: 84%; M.P.- 186-188 ºC; IR (cm^{-1}): 3245 (O-H stretching of free primary alcohol), 1250, (O-H in plane bending frequency), 700 (O-H out of plane bending a broad peak), 1665 (C=O stretching frequency for α, β-unsaturated ketone), 2985-2920 (N-H stretching frequency for amides), 1330 (C-N stretching for carbon bonded to amino group in pyrimidine), 2925-2660 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1610 (C-C skeletal stretching of phenyl nucleus), 1125 (C-H in plane bending for the phenyl ring), 872 (C-H out of plane bending for 1,4-disubstituted benzene ring), 764 (C-Cl stretching for aromatic compounds); MS: *m/z:* $M^{\cdot+}$= 326.05 and M+2=328.07; Anal. Calcd. for $C_{17}H_{11}ClN_2O_3$: C, 62.49; H, 3.39; Cl, 10.85; N, 8.57; O, 14.69 Found: C, 62.44; H, 3.31; Cl, 10.77; N, 8.50; O, 14.63.

3.6.5 8-Hydroxy-5-(3-nitrophenyl)-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-05)



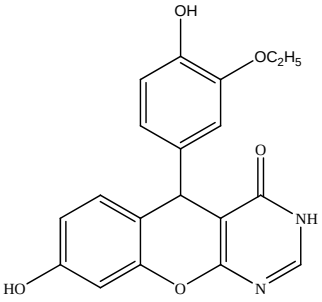
Yield: 92%; M.P.- 178-180 ºC; IR (cm^{-1}): 3612 (O-H stretching of free primary alcohol), 1248, (O-H in plane bending frequency), 763 (O-H out of plane bending a broad peak), 1532 (NO_2 asymmetric stretching), 1345(NO_2 symmetric stretching), 1665 (C=O stretching frequency for α, β-unsaturated ketone), 3112 (N-H stretching frequency for amides), 1337 (C-N stretching for carbon bonded to amino group in pyrimidine), 2926-2635 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1512 (C-C skeletal stretching of phenyl nucleus), 1190 (C-H in plane bending for the phenyl ring), 820 (C-H out of plane bending for 1,4-disubstituted benzene ring); MS: *m/z:* 337.07; Anal. Calcd. for $C_{17}H_{11}N_3O_5$: C, 60.54; H, 3.29; N, 12.46; O, 23.72 Found: C, 60.44; H, 3.25; N, 12.40; O, 23.65.

3.6.6 8-Hydroxy-5-(4-hydroxy-3-methoxyphenyl)-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-06)



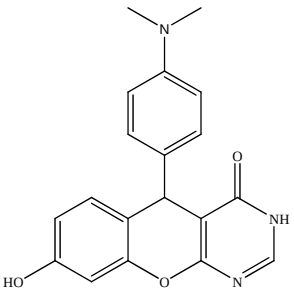
Yield: 86%; M.P.- 172-174 ºC; IR (cm^{-1}): 3623 (O-H stretching of free primary alcohol), 1254, (O-H in plane bending frequency), 782 (O-H out of plane bending a broad peak), 3012 (C-H stretching frequency for aryl ethers), 1283 (C-O-C asymmetric stretching frequency for aralkyl ethers), 1664 (C=O stretching frequency for α, β-unsaturated ketone), 3112 (N-H stretching frequency for amides), 1338 (C-N stretching for carbon bonded to amino group in pyrimidine), 2933-2621 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1512 (C-C skeletal stretching of phenyl nucleus), 1187 (C-H in plane bending for the phenyl ring), 832 (C-H out of plane bending for 1,4-disubstituted benzene ring); MS: *m/z:* 338.09; Anal. Calcd. for $C_{18}H_{14}N_2O_5$: C, 63.90; H, 4.17; N, 8.28; O, 23.65 Found: C, 63.86; H, 4.10; N, 8.22; O, 23.60.

3.6.7 5-(3-Ethoxy-4-hydroxyphenyl)-8-hydroxy-3H-chromeno[2,3-d]pyrimidin 4(5H)-one (NmNB-07)



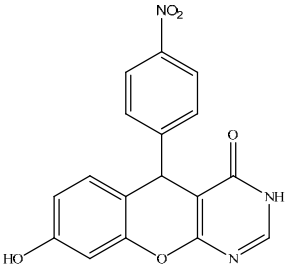
Yield: 88%; M.P.- 192-194 ºC; IR (cm^{-1}): 3618 (O-H stretching of free primary alcohol), 1256, (O-H in plane bending frequency), 764 (O-H out of plane bending a broad peak), 3015 (C-H stretching frequency for aryl ethers), 1287 (C-O-C asymmetric stretching frequency for aralkyl ethers), 1660 (C=O stretching frequency for α, β-unsaturated ketone), 3117 (N-H stretching frequency for amides), 1335 (C-N stretching for carbon bonded to amino group in pyrimidine), 2920-2636 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1519 (C-C skeletal stretching of phenyl nucleus), 1199 (C-H in plane bending for the phenyl ring), 812 (C-H out of plane bending for 1,4-disubstituted benzene ring); MS: *m/z:* 352.11; Anal. Calcd. for $C_{19}H_{16}N_2O_5$: C, 64.77; H, 4.58; N, 7.95; O, 22.70; Found: C, 64.71; H, 4.52; N, 7.87; O, 22.62.

3.6.8 5-(4-(Dimethylamino)phenyl)-8-hydroxy-3H-chromeno[2,3-d]pyrimidin -4(5H)-one (NmNB-08)



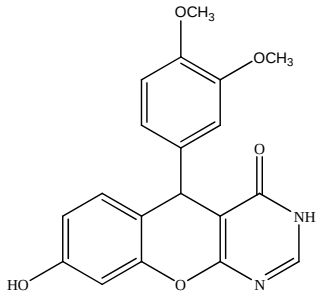
Yield: 92%; M.P.- 184-186 ºC; IR (cm^{-1}): 3610 (O-H stretching of free primary alcohol), 1242, (O-H in plane bending frequency), 769 (O-H out of plane bending a broad peak), 1300 (C-N stretching for aryl tertiary amine), 1663 (C=O stretching frequency for α, β-unsaturated ketone), 3112 (N-H stretching frequency for amides), 1327 (C-N stretching for carbon bonded to amino group in pyrimidine), 2912-2627 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1502 (C-C skeletal stretching of phenyl nucleus), 1179 (C-H in plane bending for the phenyl ring), 813 (C-H out of plane bending for 1,4-disubstituted benzene ring); MS: *m/z:* 335.13; Anal. Calcd. for $C_{19}H_{17}N_3O_3$: C, 68.05; H, 5.11; N, 12.53; O, 14.31, Found: C, 67.98; H, 5.06; N, 12.46; O, 14.25.

3.6.9 8-Hydroxy-5-(4-nitrophenyl)-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-09)



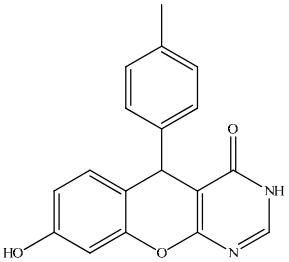
Yield: 94%; M.P.- 180-182 ºC; IR (cm^{-1}): 3608 (O-H stretching of free primary alcohol), 1243, (O-H in plane bending frequency), 767 (O-H out of plane bending a broad peak), 1527 (NO_2 asymmetric stretching), 1348 (NO_2 symmetric stretching), 1669 (C=O stretching frequency for α, β-unsaturated ketone), 3116 (N-H stretching frequency for amides), 1334 (C-N stretching for carbon bonded to amino group in pyrimidine), 2936-2645 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1512 (C-C skeletal stretching of phenyl nucleus), 1190 (C-H in plane bending for the phenyl ring), 820 (C-H out of plane bending for 1,4-disubstituted benzene ring); MS: *m/z:* 337.07; Anal. Calcd. for $C_{17}H_{11}N_3O_5$: C, 60.54; H, 3.29; N, 12.46; O, 23.72 Found: C, 60.44; H, 3.21; N, 12.40; O, 23.65.

3.6.10 5-(3,4-Dimethoxyphenyl)-8-hydroxy-3H-chromeno[2,3-d]pyrimidin-4(5H) one (NmNB-10)



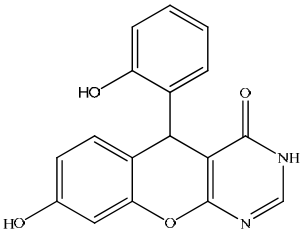
Yield: 95%; M.P.- 210-212 ºC; IR (cm^{-1}): 3609 (O-H stretching of free primary alcohol), 1247, (O-H in plane bending frequency), 760 (O-H out of plane bending a broad peak), 3002 (C-H stretching frequency for aryl ethers), 1280 (C-O-C asymmetric stretching frequency for aralkyl ethers), 1668 (C=O stretching frequency for α, β-unsaturated ketone), 3115 (N-H stretching frequency for amides), 1330 (C-N stretching for carbon bonded to amino group in pyrimidine), 2920-2637 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1514 (C-C skeletal stretching of phenyl nucleus), 1199 (C-H in plane bending for the phenyl ring), 821 (C-H out of plane bending for 1,4-disubstituted benzene ring); MS: *m/z:* 352.11; Anal. Calcd. for $C_{19}H_{16}N_2O_5$: C, 64.77; H, 4.58; N, 7.95; O, 22.70 Found: C, 64.69; H, 4.52; N, 7.90; O, 22.62.

3.6.11 8-Hydroxy-5-p-tolyl-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-11)



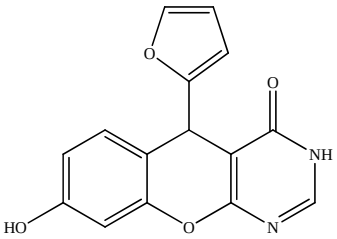
Yield: 86%; M.P.- 178-180 ºC; IR (cm^{-1}): 3617 (O-H stretching of free primary alcohol), 1241 (O-H in plane bending frequency), 761 (O-H out of plane bending a broad peak), 1666 (C=O stretching frequency for α, β-unsaturated ketone), 3111 (N-H stretching frequency for amides), 1335 (C-N stretching for carbon bonded to amino group in pyrimidine), 2921-2639 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1517 (C-C skeletal stretching of phenyl nucleus), 1194 (C-H in plane bending for the phenyl ring), 829 (C-H out of plane bending for 1,4-disubstituted benzene ring); MS: *m/z:* 306.10; Anal. Calcd. for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.61; N, 9.15; O, 15.67 Found: C, 70.54; H, 4.52; N, 9.09; O, 15.63.

3.6.12 8-Hydroxy-5-(2-hydroxyphenyl)-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-12)



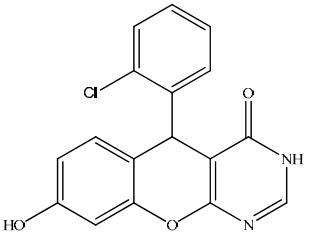
Yield: 85%; M.P.- 180-182 ºC; IR (cm^{-1}): 3605 (O-H stretching of free primary alcohol), 1237, (O-H in plane bending frequency), 749 (O-H out of plane bending a broad peak), 1658 (C=O stretching frequency for α, β-unsaturated ketone), 3123 (N-H stretching frequency for amides), 1320 (C-N stretching for carbon bonded to amino group in pyrimidine), 2910-2617 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1528 (C-C skeletal stretching of phenyl nucleus), 1199 (C-H in plane bending for the phenyl ring), 841 (C-H out of plane bending for 1,4-disubstituted benzene ring);MS: *m/z:* 308.08; Anal. Calcd. for $C_{17}H_{12}N_2O_4$: C, 66.23; H, 3.92; N, 9.09; O, 20.76 Found: C, 66.15; H, 3.85; N, 9.00; O, 20.71.

3.6.13 5-(Furan-2-yl)-8-hydroxy-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-13)



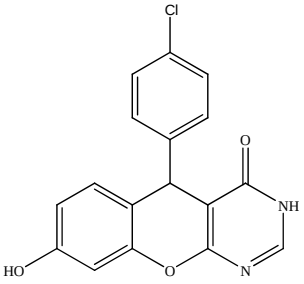
Yield: 82%; M.P.- 160-162 ºC; IR (cm^{-1}): 3606 (O-H stretching of free primary alcohol), 1243 (O-H in plane bending frequency), 768 (O-H out of plane bending a broad peak), 3122 (C-H stretching for the furan ring), 1013 (Ring breathing in furan), 1661 (C=O stretching frequency for α, β-unsaturated ketone), 3115 (N-H stretching frequency for amides), 1339 (C-N stretching for carbon bonded to amino group in pyrimidine), 2912-2637 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1518 (C-C skeletal stretching of phenyl nucleus), 1177 (C-H in plane bending for the phenyl ring); MS: *m/z:* 282.06; Anal. Calcd. for $C_{15}H_{10}N_2O_4$: C, 63.83; H, 3.57; N, 9.92; O, 22.67 Found: C, 63.77; H, 3.51; N, 9.86; O, 22.62.

3.6.14 5-(2-Chlorophenyl)-8-hydroxy-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-14)



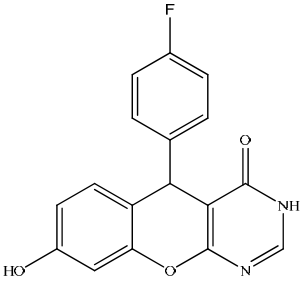
Yield: 92%; M.P.- 194-196 ºC; IR (cm^{-1}): 3236 (O-H stretching of free primary alcohol), 1239, (O-H in plane bending frequency), 698 (O-H out of plane bending a broad peak), 1654 (C=O stretching frequency for α, β-unsaturated ketone), 2973-2914 (N-H stretching frequency for amides), 1320 (C-N stretching for carbon bonded to amino group in pyrimidine), 2914-2657 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1600 (C-C skeletal stretching of phenyl nucleus), 1110 (C-H in plane bending for the phenyl ring), 863 (C-H out of plane bending for 1,4-disubstituted benzene ring), 782 (C-Cl stretching for aromatic compounds); MS: *m/z:* $M^{\cdot+}$= 326.05 and M+2=328.07; Anal. Calcd. for $C_{17}H_{11}ClN_2O_3$: C, 62.49; H, 3.39; Cl, 10.85; N, 8.57; O, 14.69 Found: C, 62.44; H, 3.33; Cl, 10.80; N, 8.53; O, 14.65.

3.6.15 5-(4-Chlorophenyl)-8-hydroxy-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-15)



Yield: 90%; M.P.- 204-206 ºC; IR (cm^{-1}): 3246 (O-H stretching of free primary alcohol), 1249, (O-H in plane bending frequency), 700 (O-H out of plane bending a broad peak), 1664 (C=O stretching frequency for α, β-unsaturated ketone), 2983-2924 (N-H stretching frequency for amides), 1330 (C-N stretching for carbon bonded to amino group in pyrimidine), 2924-2667 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1600 (C-C skeletal stretching of phenyl nucleus), 1120 (C-H in plane bending for the phenyl ring), 873 (C-H out of plane bending for 1,4-disubstituted benzene ring), 792 (C-Cl stretching for aromatic compounds); ^{1}H NMR (DMSO-d_6) δ ppm: 7.56-7.69 (d, 1H, H_1), 9.15 (s, 1H, H_2), 6.52 (s, 1H, H_3), 7.24-7.27 (d, 2H, H_4, H_7, *J*=8.8 *Hz*), 7.36-7.38 (d, 2H, H_5, H_6, *J*=8 *Hz*), 6.75-6.82 (m, 3H, H_8, H_9, H_{10}), 10.02 (s, 1H, H_{11}); MS: *m/z: m/z:* $M^{\cdot+}$= 326.05 and M+2=328.07;; Anal. Calcd. for $C_{17}H_{11}ClN_2O_3$: C, 62.49; H, 3.39; Cl, 10.85; N, 8.57; O, 14.69 Found: C, 62.40; H, 3.33; Cl, 10.80; N, 8.52; O, 14.63.

3.6.16 5-(4-Fluorophenyl)-8-hydroxy-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-16)



Yield: 85%; M.P.- 172-174 ºC; IR (cm^{-1}): 3609 (O-H stretching of free primary alcohol), 1247, (O-H in plane bending frequency), 760 (O-H out of plane bending a broad peak), 3002 (C-H stretching frequency for aryl ethers), 1280 (C-O-C asymmetric stretching frequency for aralkyl ethers), 1668 (C=O stretching frequency for α, β-unsaturated ketone), 3115 (N-H stretching frequency for amides), 1330 (C-N stretching for carbon bonded to amino group in pyrimidine), 2920-2637 (C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system), 1514 (C-C skeletal stretching of phenyl nucleus), 1199 (C-H in plane bending for the phenyl ring), 821 (C-H out of plane bending for 1,4-disubstituted benzene ring), 1084 (C-F stretching frequency for mono fluorinated compounds); MS: *m/z: m/z:* $M^{\cdot+}$= 310.08 and M+2=312.07;; Anal. Calcd. for $C_{17}H_{11}FN_2O_3$: C, 65.81; H, 3.57; F, 6.12; N, 9.03; O, 15.47 Found: C, 65.76; H, 3.50; F, 6.03; N, 8.98; O, 14.35.

3.7 SPECTRAL DISCUSSION

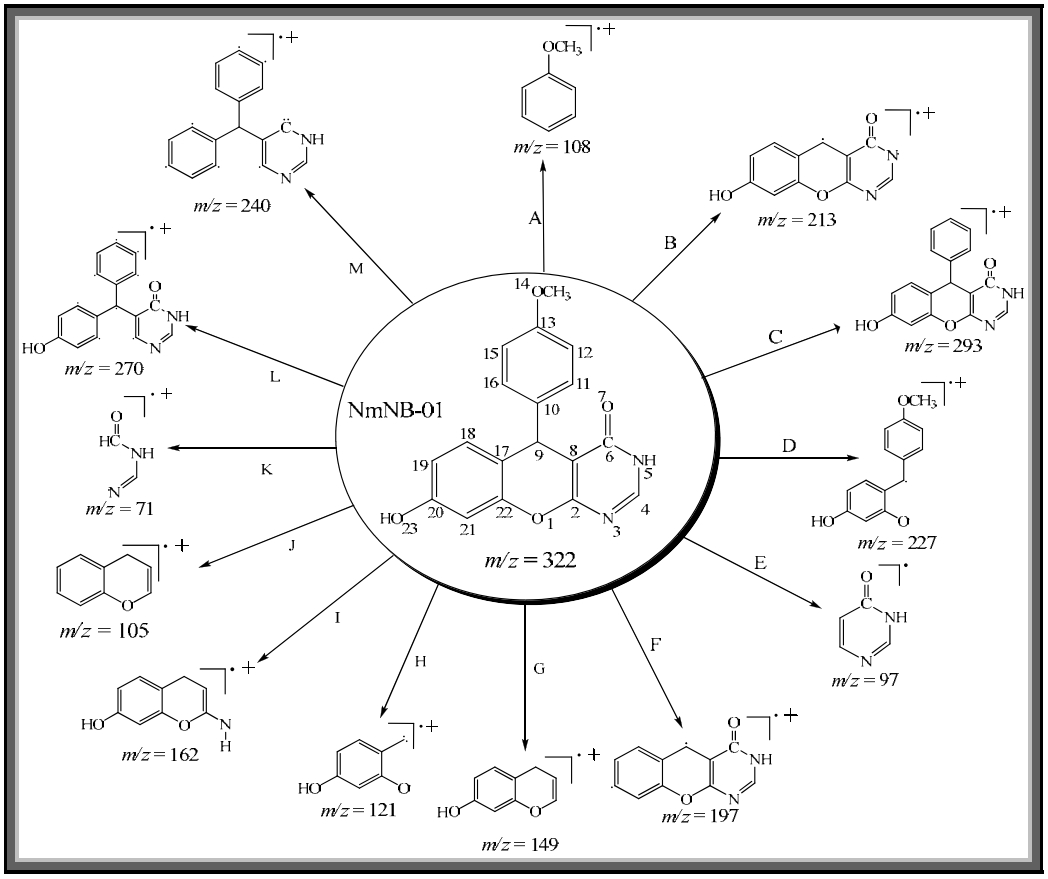
3.7.1 *IR SPECTRAL STUDY*

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. The O-H stretching vibration of free primary alcohol were obtained around 3650-3600 cm^{-1}, The O-H in plane bending frequency was observed between 1235-1255 cm^{-1}, the O-H out of plane bending was obtained as a broad peak around 750 cm^{-1}, the C-H stretching frequency for aryl ethers was obtained around 3000 cm^{-1}, C-O-C asymmetric stretching frequency for aralkyl ethers was obtained around 1260 cm^{-1}, the C=O stretching frequency for α, β-unsaturated ketone was obtained around 1660, the N-H stretching frequency for amides was observed around 3100 cm^{-1}, the C-N stretching for carbon bonded to amino group in pyrimidine was obtained at 1330 cm^{-1}, the C-H stretching vibrations, overtone of C-H out of plane bending giving several combination bands for aromatic system were obtained in the region of 3000-2600 cm^{-1}, the C-C skeletal stretching of the phenyl nucleus was observed around 1500 cm^{-1}, the C-H in plane bending for the phenyl ring was observed around 1150 cm^{-1}, the C-H out of plane bending for 1,4-disubstituted benzene ring was seen around 800 cm^{-1}.

3.7.2 *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. The probable Mass fragmentation pattern for the representative compound of each series is discussed below.

3.7.2.1 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF NmNB-01*

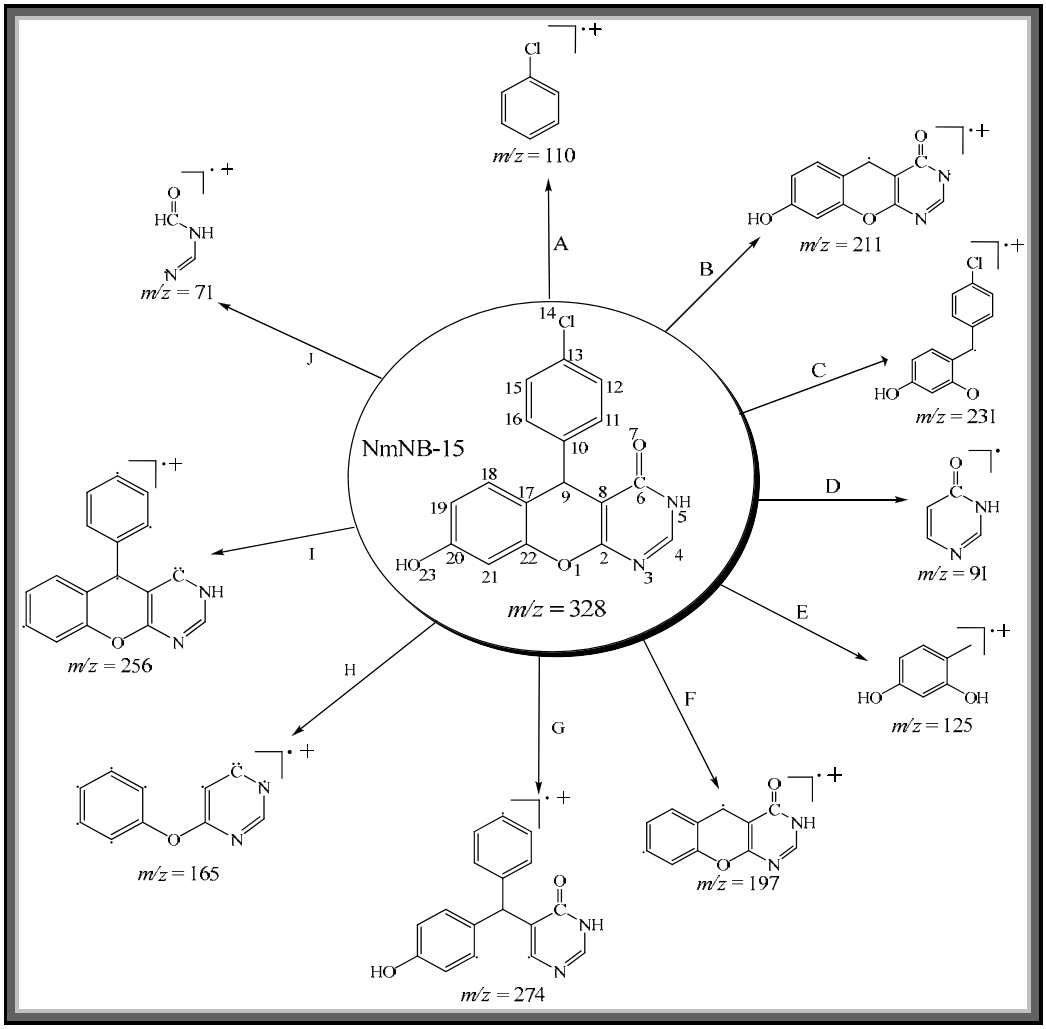


8-Hydroxy-5-(4-methoxyphenyl)-3H-chromeno-[2,3-d]pyrimidin-4(5H)-one (NmNB-01)

1. The target compound showed the characteristic molecular ion peak 322 *m/z*.
2. The bond cleavage between C_9-C_{10} generated a molecular ion which corresponds to a characteristic peak at 108 *m/z* **(A).**
3. A bond cleavage between C_9-C_{10} generated another molecular ion which corresponds to a characteristic peak at 213 *m/z* **(B)**.
4. Bond cleavages between C_{13}-O_{14} generated a molecular ion which corresponds to a characteristic peak at 293 *m/z* **(C)**.
5. Bond cleavages between C_8-C_9 and O_1-C_2 generated a molecular ion which corresponds to a characteristic peak at 227 *m/z* **(D)**.
6. Bond cleavages between C_8-C_9 and O_1-C_2 generated another molecular ion which corresponds to a characteristic peak at 97 *m/z* **(E)**.

7. Bond cleavages between C_9-C_{10}, and C_{20}-O_{23} generated a molecular ion which corresponds to a characteristic peak at 197 *m/z* **(F)**.
8. Bond cleavages between C_9-C_{10}, and C_6-C_8, C_2-N_3 generated a molecular ion which corresponds to a characteristic peak at 149 *m/z* **(G)**.
9. Bond cleavages between C_9-C_{10}, C_8-C_9, O_1-C_2 generated a molecular ion which corresponds to a characteristic peak at 121 *m/z* **(H)**.
10. Bond cleavages between C_9-C_{10}, C_6-C_8 & C_4-N_3 generated a molecular ion which corresponds to a characteristic peak at 162 *m/z* **(I)**.
11. Bond cleavages between C_9-C_{10}, C_6-C_8, C_2-N_3, C_{20}-O_{23} generated a molecular ion which corresponds to a characteristic peak at 133 *m/z* **(J)**.
12. Bond cleavages between C_9-C_{10}, C_6-C_8, C_2-N_3, generated a molecular ion which corresponds to a characteristic peak at 71 *m/z* **(K)**.
13. Bond cleavage between O_1-C_2, O_1-C_{22}, C_{13}-O_{14} generated a molecular ion which corresponds to a characteristic peak at 270 *m/z* **(L)**.
14. The other fragment caused due to bond cleavage between O_1-C_2, O_1-C_{22}, C_{13}-O_{14}, C_6-O_7, C_{20}-O_{23} generated a molecular ion which corresponds to a characteristic peak at 240 *m/z* **(M)**.

3.7.2.2 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF NmNB-15*



5-(4-Chlorophenyl)-8-hydroxy-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (NmNB-15)

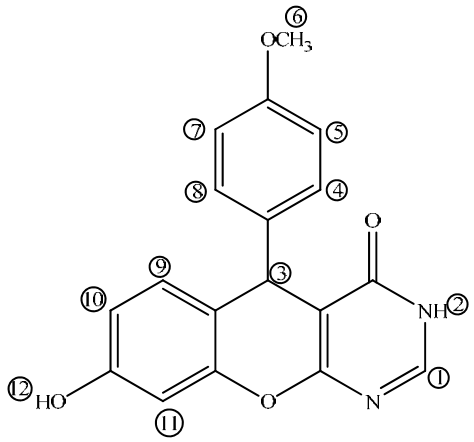
1. The target compound shows the desired characteristic molecular ion peak of 328 *m/z*.
2. The bond cleavage between C_9-C_{10} generated a molecular ion which corresponds to a characteristic peak at 110 *m/z* **(A).**
3. A bond cleavage between C_9-C_{10} generated another molecular ion which corresponds to a characteristic peak at 211 *m/z* **(B)**.
4. Bond cleavages between C_8-C_9 and O_1-C_2 generated a molecular ion which corresponds to a characteristic peak at 231 *m/z* **(C)**.
5. Bond cleavages between C_8-C_9 and O_1-C_2 generated another molecular ion which corresponds to a characteristic peak at 91 *m/z* **(D)**.

6. Bond cleavages between C_9-C_{10} and C_8-C_9, O_1-C_2 generated another molecular ion which corresponds to a characteristic peak at 125 *m/z* **(E)**.
7. Bond cleavages between C_9-C_{10}, and C_{20}-O_{23} generated a molecular ion which corresponds to a characteristic peak at 197 *m/z* **(F)**.
8. Bond cleavages between O_1-C_2, O_1-C_{22} and C_{13}-Cl_{14} generated a molecular ion which corresponds to a characteristic peak at 274 *m/z* **(G)**.
9. Bond cleavages between C_9-C_{10}, C_{20}-O_{23}, O_7-C_6, C_8-C_9, and C_9-C_{17} generated a molecular ion which corresponds to a characteristic peak at 165 *m/z* **(H)**.
10. Bond cleavages between O_7-C_6, C_{13}-Cl_{14} & C_{20}-O_{23} generated a molecular ion which corresponds to a characteristic peak at 256 *m/z* **(I)**.
11. Bond cleavages between C_9-C_{10}, C_6-C_8, C_2-N_3, generated a molecular ion which corresponds to a characteristic peak at 71 *m/z* **(J)**.

3.7.3 *^{1}H-NMR SPECTRAL STUDY*

^{1}H-NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in DMSO solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from H-NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. The spectral interpretation can be discussed as under.

8-Hydroxy-5-(4-methoxyphenyl)-3H-chromeno-[2,3-d]pyrimidin-4(5H)-one (NmNB-01)



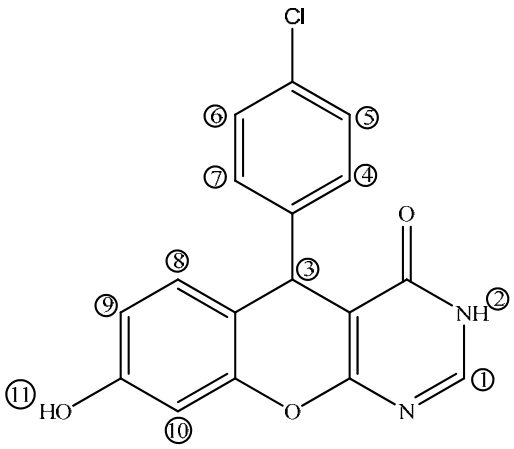
1. The Proton no. 1 is situated between 2 electronegative nitrogen atoms which will deshield the proton to a much greater extent forcing it to give a signal downfield. Moreover as it has a proton on one of its neighboring nitrogen atoms the signal will split into a doublet. On studying the NMR spectrum a doublet between 7.57 δ ppm and 7.68 δ ppm is observed which accounts for a single proton. This signal corresponds to proton no.1.
2. The proton no. 2 is the amido proton which is directly attached to the electro negative nitrogen atom. This will deshield the proton to an even greater extent forcing it to give a signal in the downfield region. A singlet at 9.26 δ ppm is assigned to this amido proton no.2.
3. The Methine proton i.e. proton no.3 will show a singlet at an upfield region as compared to proton no.1 but in the downfield region as compared to an isolated methine proton and it is very clearly seen in the NMR spectrum at 6.59 δ ppm. The reason for it to be at the downfield region is the fact that it is surrounded by a phenyl ring and also has a keto function in its vicinity which will deshield the proton.
4. The proton no. 4 and 8 have similar chemical environment and hence it will give one signal for two protons in the aromatic region. On studying the NMR spectrum a doublet between 6.76 δ ppm and 6.78 δ ppm is observed which is assigned to proton nos. 4 and 8. The J value was calculated to be 8 Hz which suggests that it is ortho coupled to another set of protons and on observing the structure, protons 4 and 8 are ortho to protons 5 and 7.Thus, this doublet is assigned to protons 4 and 8.

5. Similar to protons 4 and 8; the protons 5 and 7 also have similar chemical environments only to be on a bit downfield region due to the methoxy function present in its proximity. In the NMR spectrum a doublet is observed between 6.96 δ ppm and 6.98 δ ppm and its J value was also calculated to be 8 Hz which is in accordance to the structure as they are ortho coupled to protons 4 and 8.
6. The methoxy protons i.e. proton no.6 were observed in the NMR spectrum as a singlet at 3.74 δ ppm and the reason for them being at a downfield region is the bond of their carbon directly with the electronegative oxygen atom which will deshield the protons and force them to give the signal at a downfield region when compared to a proton in isolation.
7. A multiplet in the NMR spectrum was observed between 7.32 δ ppm and 7.38 δ ppm which accounted for 3 protons and we assign this signal to proton nos. 9, 10 and 11.
8. The proton no.12 of the hydroxy function gave a characteristic broad singlet in the NMR spectrum at 10.06 δ ppm and the reason for this proton to be at such a downfield region is its direct bond with the electronegative oxygen atom which will deshield it to a great extent forcing it to give a signal at such a downfield region.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton it can be clearly suggested that the proposed structure for compound no. NmNB-01 has been confirmed.

5-(4-Chlorophenyl)-8-hydroxy-3H-chromeno[2,3-d]pyrimidin-4(5H)-one

(NmNB-15)



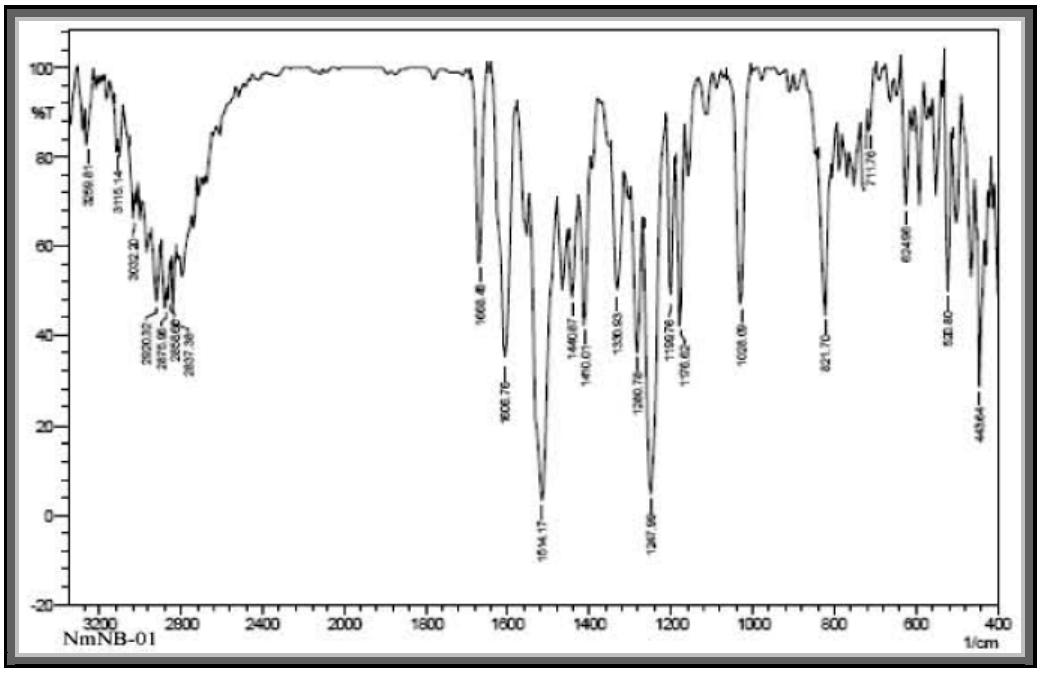
1. The Proton no. 1 is situated between 2 electronegative nitrogen atoms which will deshield the proton to a much greater extent forcing it to give a signal downfield. Moreover as it has a proton on one of its neighboring nitrogen atoms the signal will split into a doublet. On studying the NMR spectrum a doublet between 7.56 δ ppm and 7.69 δ ppm is observed which accounts for a single proton. This signal is assigned to proton no.1.
2. The proton no. 2 is the amido proton which is directly attached to the electro negative nitrogen atom. This will deshield the proton to an even greater extent forcing it to give a signal in the downfield region. A singlet at 9.15 δ ppm is observed which is assigned to this amido proton i.e. proton no.2.
3. The Methine proton i.e. proton no.3 will show a singlet at an upfield region as compared to proton no.1 but in the downfield region as compared to an isolated methine proton and it is very clearly seen in the NMR spectrum at 6.52 δ ppm. The reason for it to be at the downfield region is the fact that it is surrounded by a phenyl ring and also has a keto function in its vicinity which will deshield the proton.
4. The proton no. 4 and 7 have similar chemical environment and hence it will give one signal for two protons in the aromatic region. On studying the NMR spectrum we find a doublet between 7.24 δ ppm and 7.27 δ ppm which we assign to proton nos. 4 and 7. The J value was calculated to be 8.8 Hz which suggests that it is ortho coupled to another set of protons and on observing the structure, its evident that protons 4 and 7 are ortho to protons 5 and 6.Thus, this doublet is assigned to protons 4 and 7.

5. Similar to protons 4 and 7; the protons 5 and 6 also have similar chemical environments only to be on a bit downfield region due to the chloro function present in its proximity. In the NMR spectrum a doublet observed between 7.36 δ ppm and 7.38 δ ppm and its J value was also calculated to be 8 Hz which is in accordance to the structure as they are ortho coupled to protons 4 and 7.
6. A multiplet in the NMR spectrum was observed between 6.75 δ ppm and 6.82 δ ppm which accounted for 3 protons and this signal is assigned to proton nos. 8, 9 and 10.
7. The proton no.11 of the hydroxy function gave a characteristic broad singlet in the NMR spectrum at 10.02 δ ppm and the reason for this proton to be at such a downfield region is its direct bond with the electronegative oxygen atom which will deshield it to a great extent forcing it to give a signal at such a downfield region.

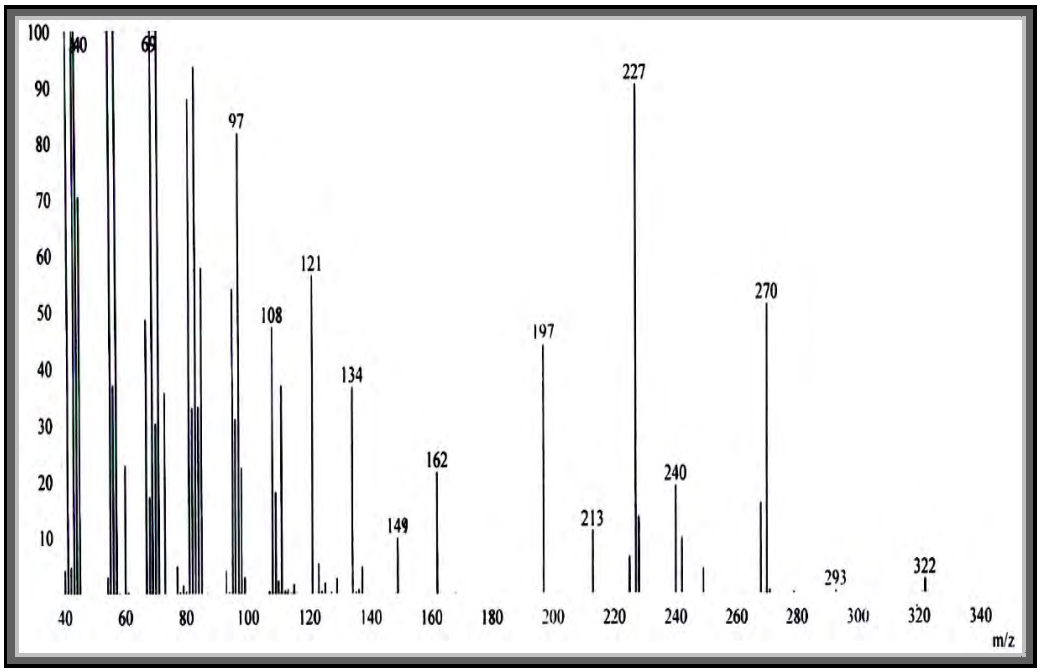
Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton it can be clearly suggested that the proposed structure for compound no. NmNB-15 has been confirmed.

3.8 SPECTRAL REPRESENTATIONS OF SYNTHESIZED COMPOUNDS

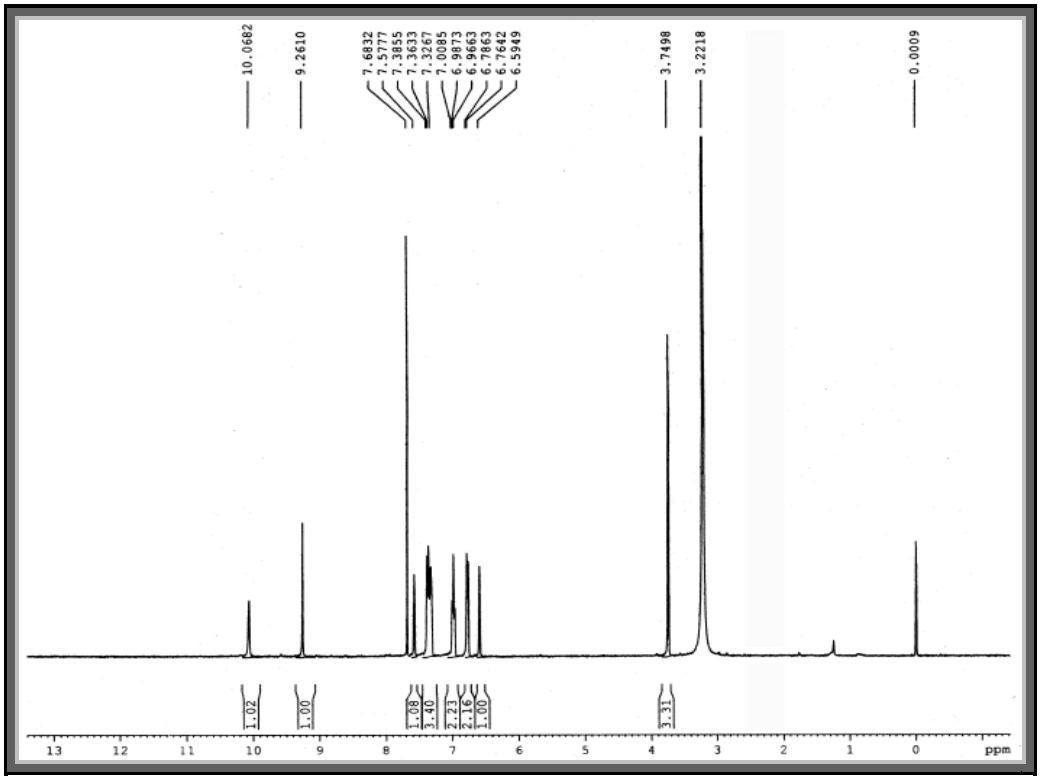
3.8.1 *IR SPECTRUM OF NmNB-01*



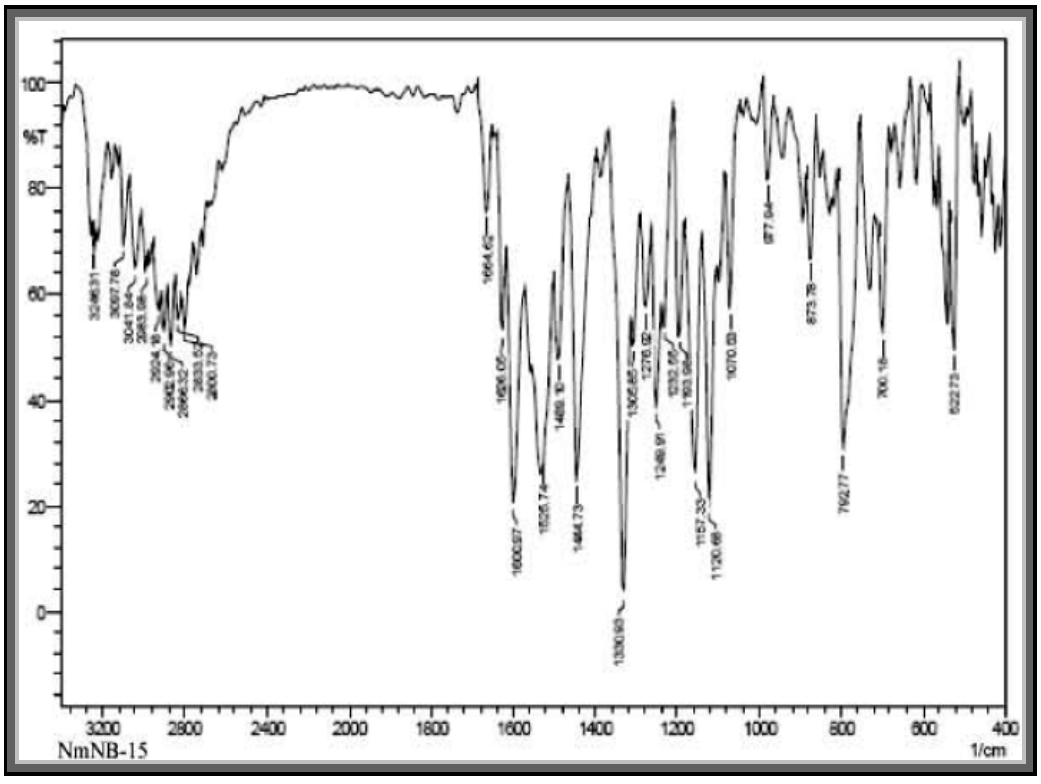
3.8.2 *MASS SPECTRUM OF NmNB-01*



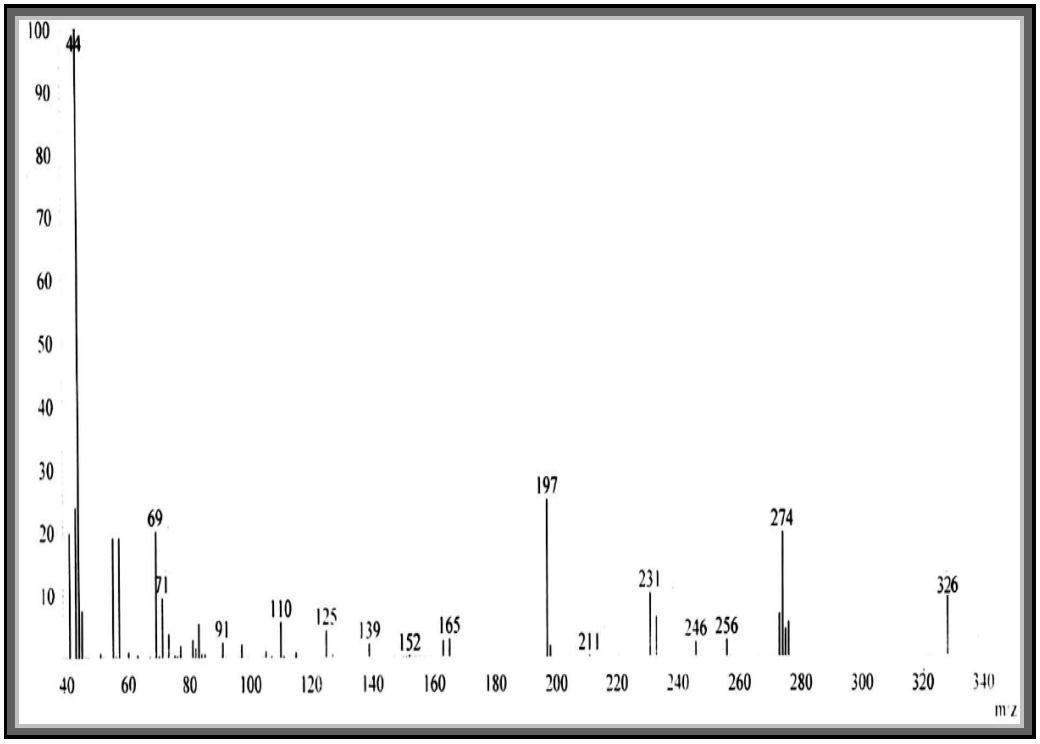
3.8.3 *^{1}H-NMR SPECTRUM OF NmNB-01*



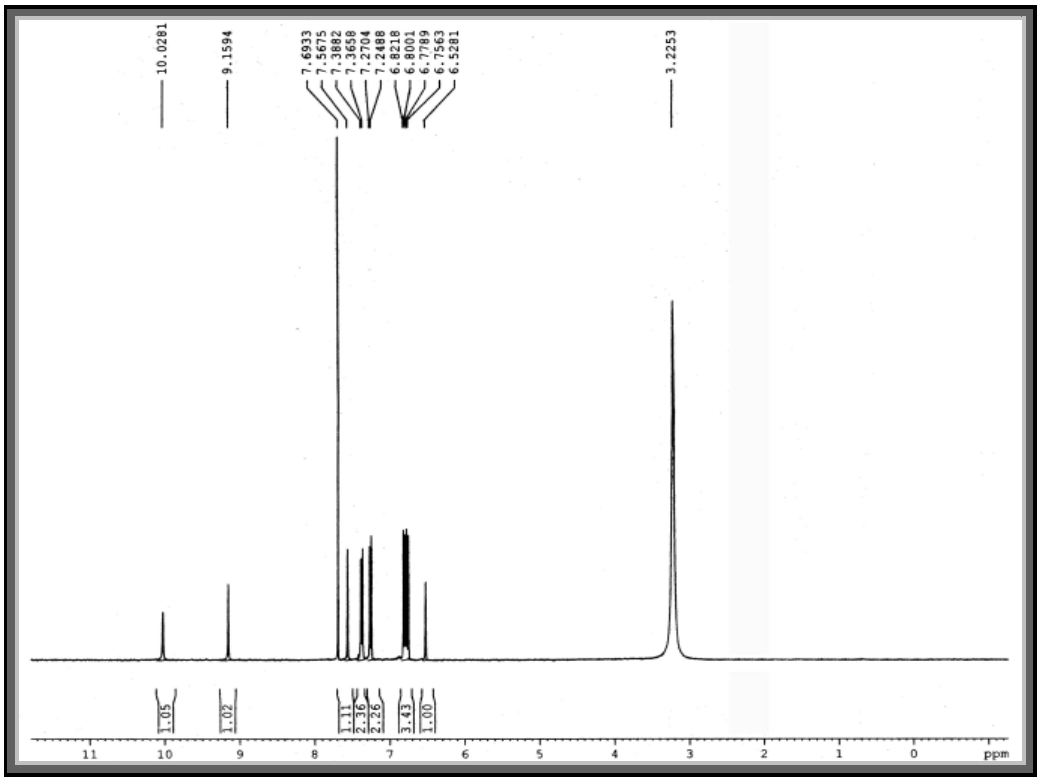
3.8.4 *IR SPECTRUM OF NmNB-15*



3.8.5 *MASS SPECTRUM OF NmNB-15*



3.8.6 *^{1}H-NMR SPECTRUM OF NmNB-15*



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CHAPTER – 3

Section-B

Microwave assisted synthesis of *8-Hydroxy-2-methyl-5-substituted phenyl-3H-chromeno-[2, 3-d] pyrimidine-4(5H)-ones*

3.9 AIM OF THE CURRENT WORK

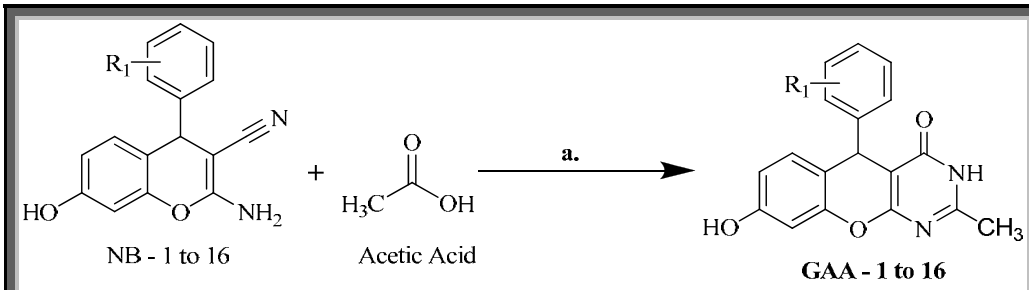
Pyrimidine and thienopyrimidine derivatives have attracted a great deal of interest owing to their medicinal activities [1, 2, 3, 4]. Pyrimidine derivatives and heterocyclic annelated pyrimidines continue to attract great interest due to the wide variety of interesting biological activities observed for these compounds, such as anticancer [4], antiviral [5], antitumor [6], anti-inflammatory [7] and antimicrobial activities [8].

Taking into consideration the methyl substituted pyrimidines, their activity was found out to be Corticotropic Releasing Hormone type-1 (CRH-1) Antagonists. Corticotropin releasing hormone (CRH) is a forty one amino acid peptide implicated in a number of physiological conditions, examples of which include (but are not limited to) anorexia nervosa, Alzheimer's disease, the mediation of stress responses, and being linked to a biological clock as the initiator of the onset of labour. [9] While the hormonal mechanisms that control the onset of parturition and labour are largely unknown, a large body of data suggests that CRH plays a key role in this process. Placental CRH concentrations rise exponentially during pregnancy in maternal circulation. McLean *et al.* have demonstrated that the exponential rise in CRH is linked to a biological clock which determines the length of gestation.[10] Various groups have shown that women in preterm labour have higher CRH concentrations than gestationally matched controls.[11, 12] Smith's group has also demonstrated similar results from different perspectives. [13 - 16]**.** Over the past 5–10 years there has been an up-surge in the number, but unfortunately, not the diversity in the types of small molecules shown to interact specifically with CRH1 receptors. However, such a situation can be advantageous in producing a detailed structure–activity relationship (SAR) study, and thus, a pharmacophore for this receptor was developed.[17] The substituted pyrimidines developed by Whitten *et al.*[18] in producing additional analogues for investigations into pharmacophore development has been taken as the basis for our search of pharmacologically active pyrimidines.[19]

Development of Microwave Assisted Organic Synthesis (MAOS) is of great interests in recent years since MAOS have the advantages such as atom economy, high efficiency and selectivity as well as environmental friendliness.[20-22] On the other hand, microwave (MW) promoted reactions have shown environmentally friendly nature, greater selectivity and enhanced reaction rate.[23-27] Fused pyrimidine derivatives are an important heterocyclic compounds which occur in many biological active products, useful as anticancer [28], antiplatelet [29], antimicrobial[30], antitumor[31], neuroprotective[32], DHFR inhibitory[33], antiviral [34], and antifungal [35], activities. Various methods for the synthesis of pyrimidine derivatives have been reported.[36-46] Most of these methods require prolonged reaction times, isolation of intermediates, complex synthetic pathways or generate moderate yields. Sakurai and coworker have already reported the condensation of hydroxybenzaldehyde and methylene compounds such as benzoylacetonitrile, ethyl cyanoacetate and ethyl acetoacetate in the presence of ammonia or ammonium acetate to give benzoquinazoline derivatives in 28-35% yields.[47] Meyer has reported the synthesis of pyranopyrimidine derivatives by condensation of malonodiamidine dihydrochloride and aromatic aldehyde in 4-51% yields. [48] One of the key areas of green chemistry is the elimination of catalysts and solvents. In today's world, synthetic chemists in both academia and industry have a widespread current debate over the relative "greenness" of different procedures.[49]

In line with all previous work explores, pyrimidines, so widely biologically active compounds attracted our attention to concentrate on the moiety in general and the synthesis of the 2-methyl derivatives of the same in microwave in particular.

3.10 REACTION SCHEME



Reagents & Conditions: **a.)** Conc. H_2SO_4; MWI-180 watts; 30 mins.,

Microwave Assisted synthesis of 8-hydroxy-2-methyl-5-substituted phenyl-3H-chromeno[2,3-d]pyrimidin-4(5H)-one.

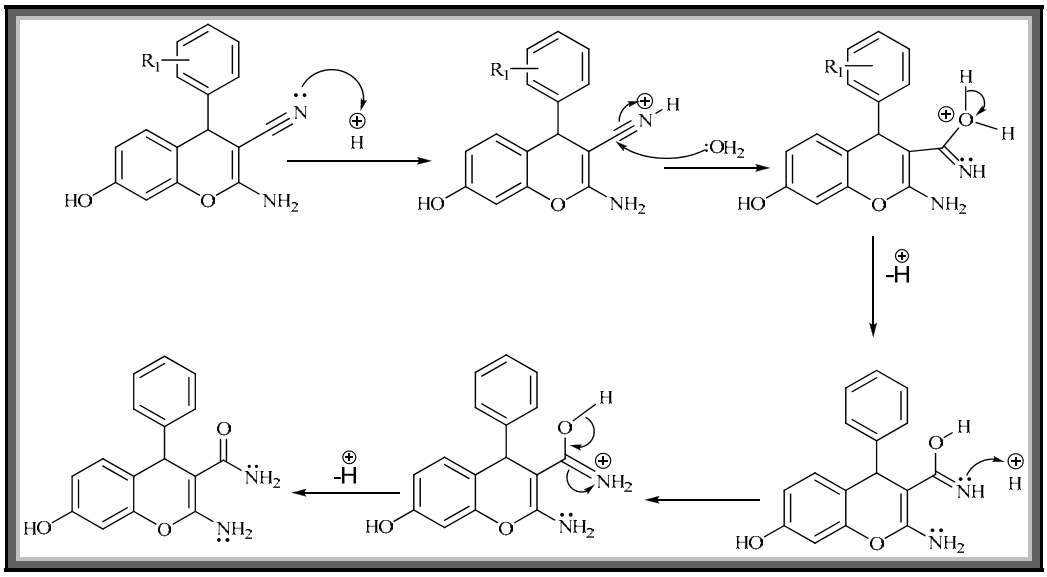
3.10.1 *PHYSICAL DATA TABLE*

Code	R_1	M. F.	M. W.	M. P. ^{0}C	Time (min)	Yield %	R_{f1}
GAA-1	4-OCH_3	$C_{19}H_{16}N_2O_4$	336.34	110-112	28:00	85	0.54
GAA-2	H	$C_{18}H_{14}N_2O_3$	306.32	126-128	25:20	88	0.52
GAA-3	3-Br	$C_{18}H_{13}BrN_2O_3$	385.21	122-124	21:00	82	0.56
GAA-4	3-Cl	$C_{18}H_{13}ClN_2O_3$	351.31	134-136	25:20	85	0.56
GAA-5	3-NO_2	$C_{18}H_{13}N_3O_5$	352.34	130-132	28:30	90	0.46
GAA-6	3-OCH_3, 4-OH	$C_{19}H_{16}N_2O_5$	366.37	142-144	29:10	83	0.58
GAA-7	3-OC_2H_5, 4-OH	$C_{20}H_{18}N_2O_5$	349.38	128-130	30:00	88	0.51
GAA-8	4-$N(CH_3)_2$	$C_{20}H_{19}N_3O_3$	351.31	138-140	28:40	91	0.50
GAA-9	4-NO_2	$C_{18}H_{13}N_3O_5$	366.37	128-130	25:40	90	0.45
GAA-10	3,4-OCH_3	$C_{20}H_{18}N_2O_5$	320.34	108-110	26:40	85	0.52
GAA-11	4-CH_3	$C_{19}H_{16}N_2O_3$	322.31	134-136	25:30	86	0.54
GAA-12	2-OH	$C_{18}H_{14}N_2O_4$	296.28	124-126	27:30	85	0.58
GAA-13	Furyl	$C_{16}H_{12}N_2O_4$	340.76	122-124	30:00	82	0.55
GAA-14	2-Cl	$C_{18}H_{13}ClN_2O_3$	351.31	128-130	27:00	92	0.52
GAA-15	4-Cl	$C_{18}H_{13}ClN_2O_3$	340.76	116-118	30:00	90	0.48
GAA-16	4-F	$C_{18}H_{13}FN_2O_3$	324.31	138-140	28:00	87	0.50

TLC solvent system for R_{f1} = Toluene:Ethyl acetate - 7:3. Microwave Irradiation: 180 Watts.

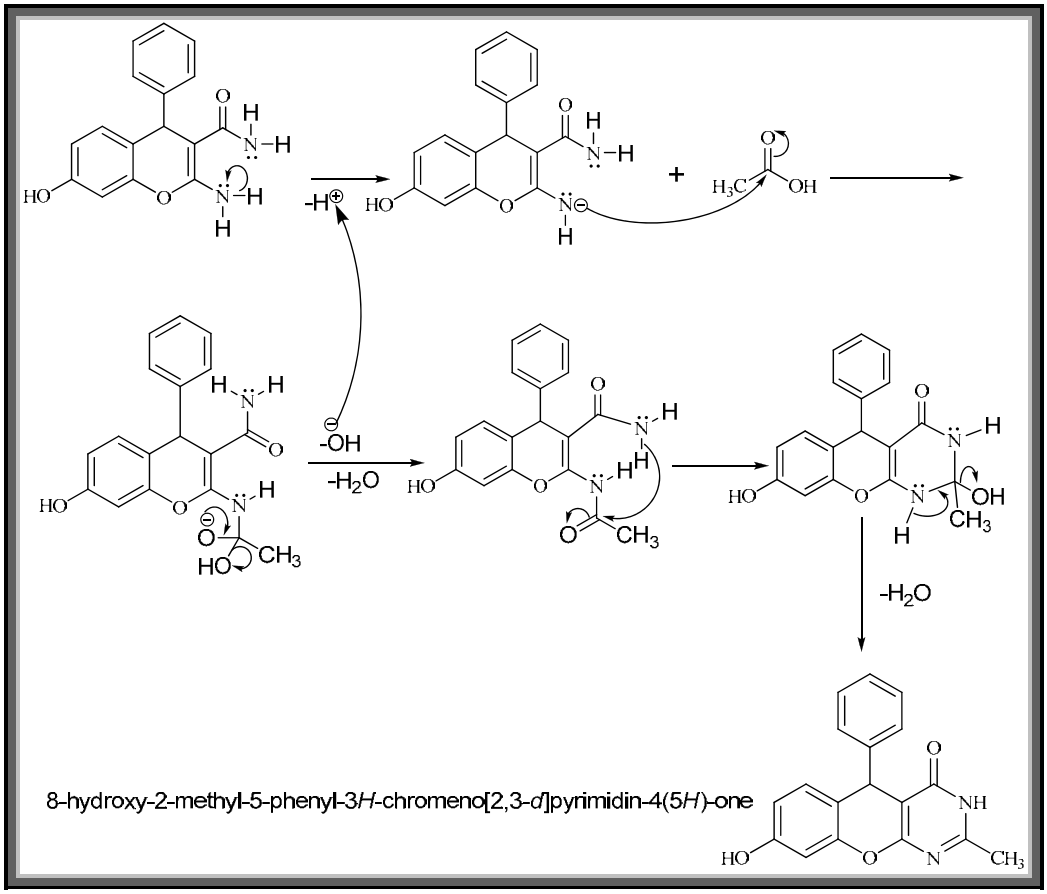
3.11 PLAUSIBLE REACTION MECHANISM

3.11.1 *STEP-I ACID CATALYZED HYDROLYSIS OF THE NITRILE GROUP*



The mechanism is very similar to section A of this chapter. The only difference being the reactant. In this section we have used Acetic acid as the reactant instead of Formic acid as in the case of Section A. The nitrile group is oxidized to amide in the presence of acid catalyst. The lone pair of electron on the nitrogen atom of the nitrile group first accepts the acidic proton which develops the positive charge on the nitrogen atom. The presence of water molecule in the acid would then quench the positive charge by attacking on the carbon atom of the nitrile moiety. The proton leaves its electron with the oxygen thus quenching the positive charge on it. Further more the lone pair of Nitrogen again accepts the proton thus forming the positively charged nitrogen which is subsequently quenched by the removal of the proton. This gave the first intermediate, 2-Amino-7-hydroxy-4-phenyl-4H-chromene-3-carboxamide.

3.11.2 *Step-2-Formation Of 8-Hydroxy-2-methyl- 5-substitutedphenyl-3h-chromeno[2, 3-d]pyrimidin-4(5h)-one*



In this step again, a proton from the amino group will leave forming a negatively charged nitrogen atom which is a highly unstable entity. The negative charge would then attack the carbonyl carbon of the acid group and will reach on the oxygen atom which on further displacement would remove the hydroxyl moiety from the acid thus removing a water molecule. After that again a proton, now from the amide group, would get displaced by attacking on the carbonyl carbon. The second water moiety is removed when the hydrogen attached on the amino nitrogen gets displaced to remove the hydroxyl function. This gave the desired product i.e. 8-Hydroxy-2-methyl-5 -substituted phenyl-3H-chromeno[2,3-d]pyrimidin-4(5H)-one.

3.12 EXPERIMENTAL

3.12.1 *MATERIALS AND METHODS*

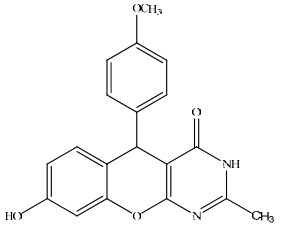
Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. All the reactions were carried out in Samsung MW83Y Microwave Oven which was locally modified for carrying out chemical reactions. IR spectra were recorded in 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.

3.12.2 *GENERAL PROCEDURE: 8-Hydroxy-2-methyl-5-substituted phenyl-3H-chromeno [2,3-d]pyrimidin-4(5H)-ones*

0.01 M of *2-amino-7-hydroxy-4-(substituted phenyl)-4H-chromene-3-carbonitrile* [Chapter-2, Section-A] was dissolved in 20 ml of Glacial acetic acid which was used as a reactant as well as solvent. A few drops of Sulfuric acid were introduced in the reaction mixture as the acidic catalyst to promote the reaction. The reaction mixture was subjected to MWI for a specific time (see Physical data Table) at low power (180 W). The progress of the reaction was monitored by TLC examination at an interval of every 30 seconds. On completion of the reaction, the reaction mixture was cooled to room temperature and then poured over ice water. The reaction mixture was then neutralized by using sodium bicarbonate and the pH of the mixture was taken to 6, then separated using a separating funnel and the organic component was extracted thrice using ethyl acetate. The combined organic extracts were washed twice with water and once with brine solution. Sodium sulphate was added to the combined organic extract and was left overnight to remove any trace amount of moisture. The combined organic layer was vacuum distillated. The product obtained was filtered, washed with cold water, dried, and recrystallized from rectified spirit.

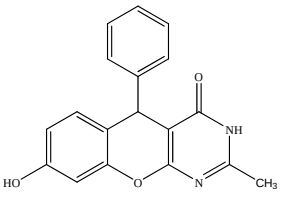
3.13 ANALYTICAL DATA

3.13.1 8-Hydroxy-5-(4-methoxyphenyl)-2-methyl-3H-chromeno[2,3-d]pyrimidin -4(5H)-one (GAA-01)



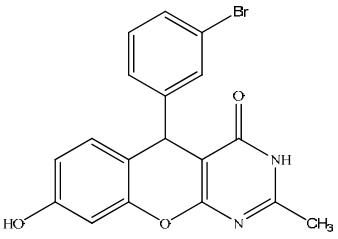
Yield: 85%; M.P.- 110-112 ºC; IR (cm^{-1}): 3302 (O-H stretching of free primary alcohol), 1257, (O-H in plane bending frequency), 704 (O-H out of plane bending a broad peak), 3014 (C-H stretching frequency for aryl ethers), 1264 (C-O-C asymmetric stretching frequency for aralkyl ethers), 1668 (C=O stretching frequency for cyclic amide), 1336 (C-N stretching for carbon bonded to amino group in pyrimidine), 2966 (C-H stretching vibrations for aromatic system), 1516 (C-C skeletal stretching of phenyl nucleus), 1257 (C-H in plane bending for the phenyl ring), 830 (C-H out of plane bending for 1,4-disubstituted benzene ring), 2837-2804 (CH_3 asymmetric stretching for Ar-CH_3), 2767-2706 (Ch_3 symmetric stretching for Ar-CH_3); ^{1}H NMR (DMSO-d_6) δ ppm: 2.29 (s, 1H, H_1), 9.71 (s, 1H, H_2), 6.59 (s, 1H, H_3), 6.78-6.82 (d, 2H, H_4, H_8, *J*=9.38 Hz), 7.24-7.27 (d, 2H, H_5, H_7, *J*=12 *Hz*), 3.73 (s, 3H, H_6), 7.35-7.39 (d, 1H, H_9, *J*=12 *Hz*), 7.72-7.74 (d, 1H, H_{10}, *J*= 8 *Hz*), 7.53 (s, 1H, H_{11}), 10.07 (s, 1H, H_{12}); MS: *m/z:* 336.11; Anal. Calcd. for $C_{19}H_{16}N_2O_4$: C, 67.85; H, 4.79; N, 8.33; O, 19.03 Found: C, 67.79; H, 4.71; N, 8.28; O, 19.01.

3.13.2 8-Hydroxy-2-methyl-5-phenyl-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (GAA-02)



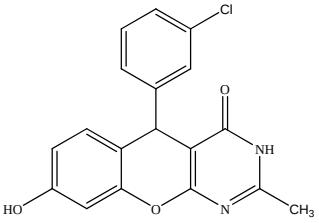
Yield: 88%; M.P.- 126-128 ºC; IR (cm^{-1}): 3312 (O-H stretching of free primary alcohol), 1258, (O-H in plane bending frequency), 706 (O-H out of plane bending a broad peak), 1664 (C=O stretching frequency for for cyclic amide), 1332 (C-N stretching for carbon bonded to amino group in pyrimidine), 2969 (C-H stretching vibrations for aromatic system), 1517 (C-C skeletal stretching of phenyl nucleus), 1254 (C-H in plane bending for the phenyl ring), 830 (C-H out of plane bending for 1,4-disubstituted benzene ring), 2838-2805 (CH_3 asymmetric stretching for Ar-CH_3), 2765-270 (Ch_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 306.10; Anal. Calcd. for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.61; N, 9.15; O, 15.67 Found: C, 70.53; H, 4.59; N, 9.11; O, 15.62.

3.13.3 5-(3-Bromophenyl)-8-hydroxy-2-methyl-3H-chromeno[2,3-d]pyrimidin-4 (5H)-one (GAA-03)



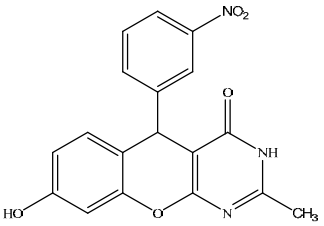
Yield: 82%; M.P.- 122-124 ºC; IR (cm^{-1}): 3269 (O-H stretching of free primary alcohol), 1250, (O-H in plane bending frequency), 710 (O-H out of plane bending a broad peak), 1667 (C=O stretching frequency for cyclic amide), 3103-3010 (N-H stretching frequency for amides), 1327 (C-N stretching for carbon bonded to amino group in pyrimidine), 2615 (C-H stretching vibrations for aromatic system), 1506 (C-C skeletal stretching of phenyl nucleus), 1195 (C-H in plane bending for the phenyl ring), 830 (C-H out of plane bending for 1,4-disubstituted benzene ring), 578 (C-Br stretching for mono brominated aromatic compounds), 2835-2810 (CH_3 asymmetric stretching for Ar-CH_3), 2760-2705 (CH_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 384.01; Anal. Calcd. for $C_{18}H_{13}BrN_2O_3$: C, 56.12; H, 3.40; Br, 20.74; N, 7.27; O, 12.46 Found: C, 56.08; H, 3.36; Br, 20.69 N, 7.21; O, 12.42.

3.13.4 5-(3-Chlorophenyl)-8-hydroxy-2-methyl-3H-chromeno[2,3-d]pyrimidin 4(5H)-one (GAA-04)



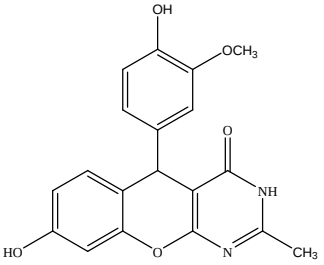
Yield: 85%; M.P.- 134-136 ºC; IR (cm^{-1}): 3260 (O-H stretching of free primary alcohol), 1243, (O-H in plane bending frequency), 730 (O-H out of plane bending a broad peak), 1660 (C=O stretching frequency for cyclic amide), 3105-3020 (N-H stretching frequency for amides), 1323 (C-N stretching for carbon bonded to amino group in pyrimidine), 2614 (C-H stretching vibrations for aromatic system), 1507 (C-C skeletal stretching of phenyl nucleus), 1199 (C-H in plane bending for the phenyl ring), 837 (C-H out of plane bending for 1,4-disubstituted benzene ring), 767 (C-Cl stretching for mono chlorinated aromatic compounds), 2830-2805 (CH_3 asymmetric stretching for Ar-CH_3), 2767-2706 (CH_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 340.06; Anal. Calcd. for $C_{18}H_{13}ClN_2O_3$: C, 63.44; H, 3.85; Cl, 10.40; N, 8.22; O, 14.09 Found: C, 63.40; H, 3.83; Cl, 10.33; N, 8.18; O, 14.02.

3.13.5 8-Hydroxy-2-methyl-5-(3-nitrophenyl)-3H-chromeno[2,3-d]pyrimidin 4(5H)-one (GAA-05)



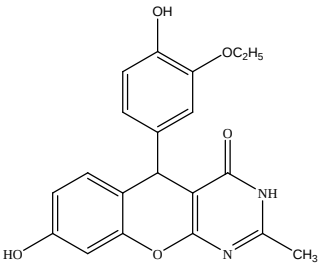
Yield: 90%; M.P.- 130-132 ºC; IR (cm^{-1}): 3305 (O-H stretching of free primary alcohol), 1251, (O-H in plane bending frequency), 708 (O-H out of plane bending a broad peak), 1558 (NO_2 asymmetric stretching in an aromatic compound), 1660 (C=O stretching frequency for cyclic amide), 1332 (C-N stretching for carbon bonded to amino group in pyrimidine), 2968 (C-H stretching vibrations for aromatic system), 1514 (C-C skeletal stretching of phenyl nucleus), 1257 (C-H in plane bending for the phenyl ring), 838 (C-H out of plane bending for 1,3-disubstituted benzene ring), 2833-2812 (CH_3 asymmetric stretching for Ar-CH_3), 2764-2705 (Ch_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 351.09; Anal. Calcd. for $C_{18}H_{13}N_3O_5$: C, 61.54; H, 3.73; N, 11.96; O, 22.77 Found: C, 61.50; H, 3.68; N, 11.92; O, 22.70.

3.13.6 8-Hydroxy-5-(4-hydroxy-3-methoxyphenyl)-2-methyl-3H-chromeno[2,3 d]pyrimidin-4(5H)-one (GAA-06)



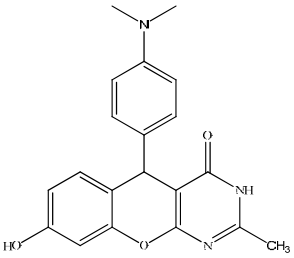
Yield: 83%; M.P.- 142-144 ºC; IR (cm^{-1}): 3314 (O-H stretching of free primary alcohol), 1250, (O-H in plane bending frequency), 712 (O-H out of plane bending a broad peak), 3013 (C-H stretching frequency for aryl ethers), 1265 (C-O-C asymmetric stretching frequency for aralkyl ethers), 1669 (C=O stretching frequency for cyclic amide), 1338 (C-N stretching for carbon bonded to amino group in pyrimidine), 2965 (C-H stretching vibrations for aromatic system), 1517 (C-C skeletal stretching of phenyl nucleus), 1257 (C-H in plane bending for the phenyl ring), 832 (C-H out of plane bending for 1,4-disubstituted benzene ring), 2835-2805 (CH_3 asymmetric stretching for Ar-CH_3), 2765-2700 (Ch_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 352.11; Anal. Calcd. for $C_{19}H_{16}N_2O_5$: C, 64.77; H, 4.58; N, 7.95; O, 22.70 Found: C, 64.71; H, 4.53; N, 7.91; O, 22.64.

3.13.7 5-(3-Ethoxy-4-hydroxyphenyl)-8-hydroxy-2-methyl-3H-chromeno[2,3-d] pyrimidin-4(5H)-one (GAA-07)



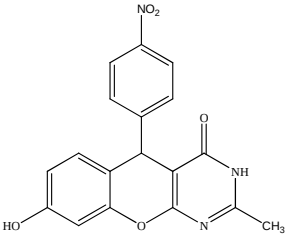
Yield: 88%; M.P.- 128-130 ºC; IR (cm^{-1}): 3302 (O-H stretching of free primary alcohol), 1257, (O-H in plane bending frequency), 704 (O-H out of plane bending a broad peak), 3025 (C-H stretching frequency for aryl ethers), 1273 (C-O-C asymmetric stretching frequency for aralkyl ethers), 1668 (C=O stretching frequency for cyclic amide), 1336 (C-N stretching for carbon bonded to amino group in pyrimidine), 2966 (C-H stretching vibrations for aromatic system), 1516 (C-C skeletal stretching of phenyl nucleus), 1257 (C-H in plane bending for the phenyl ring), 830 (C-H out of plane bending for 1,4-disubstituted benzene ring), 2845-2810 (CH_3 asymmetric stretching for Ar-CH_3), 2770-2702 (Ch_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 366.12; Anal. Calcd. for $C_{20}H_{18}N_2O_5$: C, 65.57; H, 4.95; N, 7.65; O, 21.84; Found: C, 65.53; H, 4.90; N, 7.62; O, 21.80.

3.13.8 5-(4-(Dimethylamino)phenyl)-8-hydroxy-2-methyl-3H-chromeno[2,3-d] pyrimidin-4(5H)-one (GAA-08)



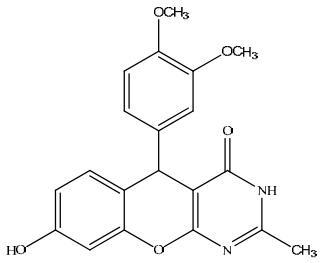
Yield: 91%; M.P.- 138-140 ºC; IR (cm^{-1}): 3308 (O-H stretching of free primary alcohol), 1256, (O-H in plane bending frequency), 711 (O-H out of plane bending a broad peak), 1371 (C-N stretching frequency for aryl tertiary amine), 1664 (C=O stretching frequency for for cyclic amide), 1339 (C-N stretching for carbon bonded to amino group in pyrimidine), 2967 (C-H stretching vibrations for aromatic system), 1518 (C-C skeletal stretching of phenyl nucleus), 1252 (C-H in plane bending for the phenyl ring), 834 (C-H out of plane bending for 1,4-disubstituted benzene ring), 2834-2810 (CH_3 asymmetric stretching for Ar-CH_3), 2767-2706 (Ch_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 349.14; Anal. Calcd. for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.04; O, 13.74, Found: C, 68.69; H, 5.44; N, 12.01; O, 13.68.

3.13.9 8-Hydroxy-2-methyl-5-(4-nitrophenyl)-3H-chromeno[2,3-d]pyrimidin 4(5H)-one (GAA-09)



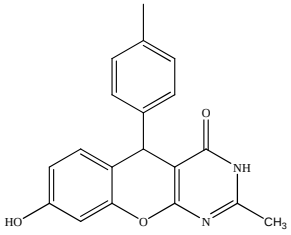
Yield: 90%; M.P.- 128-130 ºC; IR (cm^{-1}): 3305 (O-H stretching of free primary alcohol), 1251, (O-H in plane bending frequency), 708 (O-H out of plane bending a broad peak), 1552 (NO_2 asymmetric stretching in an aromatic compound), 1664 (C=O stretching frequency for cyclic amide), 1337 (C-N stretching for carbon bonded to amino group in pyrimidine), 2962 (C-H stretching vibrations for aromatic system), 1516 (C-C skeletal stretching of phenyl nucleus), 1258 (C-H in plane bending for the phenyl ring), 831 (C-H out of plane bending for 1,4-disubstituted benzene ring), 2834-2809 (CH_3 asymmetric stretching for Ar-CH_3), 2761-2710 (CH_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 351.09; Anal. Calcd. for $C_{18}H_{13}N_3O_5$: C, 61.54; H, 3.73; N, 11.96; O, 22.77 Found: C, 61.69; H, 3.67; N, 11.91; O, 22.73.

3.13.10 5-(3,4-Dimethoxyphenyl)-8-hydroxy-2-methyl-3H-chromeno[2,3-d] pyrimidin -4(5H)-one (GAA-10)



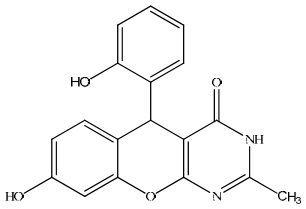
Yield: 85%; M.P.- 108-110 ºC; IR (cm^{-1}): 3302 (O-H stretching of free primary alcohol), 1257, (O-H in plane bending frequency), 704 (O-H out of plane bending a broad peak), 3025 (C-H stretching frequency for aryl ethers), 1278 (C-O-C asymmetric stretching frequency for aralkyl ethers), 1668 (C=O stretching frequency for cyclic amide), 1336 (C-N stretching for carbon bonded to amino group in pyrimidine), 2966 (C-H stretching vibrations for aromatic system), 1516 (C-C skeletal stretching of phenyl nucleus), 1257 (C-H in plane bending for the phenyl ring), 830 (C-H out of plane bending for 1,4-disubstituted benzene ring), 2837-2804 (CH_3 asymmetric stretching for Ar-CH_3), 2767-2706 (Ch_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 366.12; Anal. Calcd. for $C_{20}H_{18}N_2O_5$: C, 65.57; H, 4.95; N, 7.65; O, 21.84 Found: C, 65.53; H, 4.91; N, 7.60; O, 21.79.

3.13.11 8-Hydroxy-2-methyl-5-p-tolyl-3H-chromeno[2,3-d]pyrimidin 4(5H)-one (GAA-11)



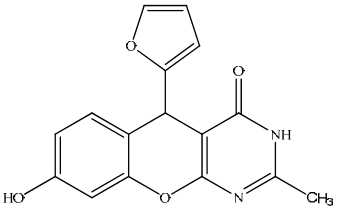
Yield: 86%; M.P.- 134-136 ºC; IR (cm^{-1}): 3301 (O-H stretching of free primary alcohol), 1255, (O-H in plane bending frequency), 705 (O-H out of plane bending a broad peak), 3013 (C-H stretching frequency for aryl ethers), 1269 (C-O-C asymmetric stretching frequency for aralkyl ethers), 1669 (C=O stretching frequency for cyclic amide), 1334 (C-N stretching for carbon bonded to amino group in pyrimidine), 2968 (C-H stretching vibrations for aromatic system), 1517 (C-C skeletal stretching of phenyl nucleus), 1259 (C-H in plane bending for the phenyl ring), 839 (C-H out of plane bending for 1,4-disubstituted benzene ring), 2835-2805 (CH_3 asymmetric stretching for Ar-CH_3), 2760-2700 (CH_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 320.12; Anal. Calcd. for $C_{19}H_{16}N_2O_3$: C, 71.24; H, 5.03; N, 8.74; O, 14.98 Found: C, 71.21; H, 5.01; N, 8.70; O, 14.94.

3.13.12 8-Hydroxy-5-(2-hydroxyphenyl)-2-methyl-3H-chromeno[2,3-d] pyrimidin-4(5H)-one (GAA-12)



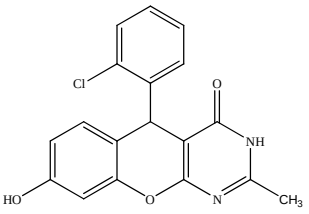
Yield: 85%; M.P.- 124-126 ºC; IR (cm^{-1}): 3324 (O-H stretching of free primary alcohol), 1274, (O-H in plane bending frequency), 712 (O-H out of plane bending a broad peak), 1659 (C=O stretching frequency for cyclic amide), 1328 (C-N stretching for carbon bonded to amino group in pyrimidine), 2956 (C-H stretching vibrations for aromatic system), 1526 (C-C skeletal stretching of phenyl nucleus), 1247 (C-H in plane bending for the phenyl ring), 829 (C-H out of plane bending for 1,4-disubstituted benzene ring), 2827-2798 (CH_3 asymmetric stretching for Ar-CH_3), 2776-2701 (Ch_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 322.10; Anal. Calcd. for $C_{18}H_{14}N_2O_4$: C, 67.07; H, 4.38; N, 8.69; O, 19.86 Found: C, 67.02; H, 4.33; N, 8.63; O, 19.82.

3.13.13 5-(Furan-2-yl)-8-hydroxy-2-methyl-3H-chromeno[2,3-d] pyrimidin-4(5H)-one (GAA-13)



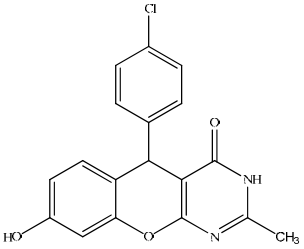
Yield: 82%; M.P.- 122-124 ºC; IR (cm^{-1}): 3312 (O-H stretching of free primary alcohol), 1250, (O-H in plane bending frequency), 700 (O-H out of plane bending a broad peak), 3120 (C-H stretching in furan ring), 1427, 1438, 1444 (Ring stretching, three bands characteristic for furan ring system), 1665 (C=O stretching frequency for cyclic amide), 1330 (C-N stretching for carbon bonded to amino group in pyrimidine), 2964 (C-H stretching vibrations for aromatic system), 1516 (C-C skeletal stretching of phenyl nucleus), 1254 (C-H in plane bending for the phenyl ring), 833 (C-H out of plane bending for 1,4-disubstituted benzene ring), 2830-2805 (CH_3 asymmetric stretching for Ar-CH_3), 2760-2700 (Ch_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 296.08; Anal. Calcd. for $C_{16}H_{12}N_2O_4$: C, 64.86; H, 4.08; N, 9.46; O, 21.60 Found: C, 64.82; H, 4.05; N, 9.41; O, 21.55.

3.13.14 5-(2-Chlorophenyl)-8-hydroxy-2-methyl-3H-chromeno[2,3-d] pyrimidin-4(5H)-one (GAA-14)



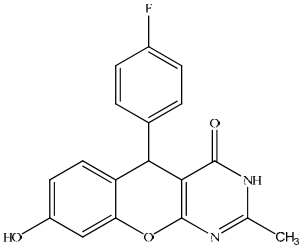
Yield: 92%; M.P.- 128-130 ºC; IR (cm^{-1}): 3263 (O-H stretching of free primary alcohol), 1253, (O-H in plane bending frequency), 700 (O-H out of plane bending a broad peak), 1660 (C=O stretching frequency for cyclic amide), 3100-3014 (N-H stretching frequency for amides), 1325 (C-N stretching for carbon bonded to amino group in pyrimidine), 2617 (C-H stretching vibrations for aromatic system), 1503 (C-C skeletal stretching of phenyl nucleus), 1201 (C-H in plane bending for the phenyl ring), 747 (C-H out of plane bending for 1,2-disubstituted benzene ring), 775 (C-Cl stretching for mono chlorinated aromatic compounds), 2837-2804 (CH_3 asymmetric stretching for Ar-CH_3), 2767-2706 (CH_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 340.06; Anal. Calcd. for $C_{18}H_{13}ClN_2O_3$: C, 63.44; H, 3.85; Cl, 10.40; N, 8.22; O, 14.09 Found: C, 63.41; H, 3.80; Cl, 10.35; N, 8.18; O, 14.04.

3.13.15 5-(4-Chlorophenyl)-8-hydroxy-2-methyl-3H-chromeno[2,3-d] pyrimidin-4(5H)-one (GAA-15)



Yield: 90%; M.P.- 116-118 ºC; IR (cm^{-1}): 3267 (O-H stretching of free primary alcohol), 1249, (O-H in plane bending frequency), 700 (O-H out of plane bending a broad peak), 1662 (C=O stretching frequency for cyclic amide), 3101-3014 (N-H stretching frequency for amides), 1325 (C-N stretching for carbon bonded to amino group in pyrimidine), 2610 (C-H stretching vibrations for aromatic system), 1506 (C-C skeletal stretching of phenyl nucleus), 1196 (C-H in plane bending for the phenyl ring), 825 (C-H out of plane bending for 1,4-disubstituted benzene ring), 770 (C-Cl stretching for mono chlorinated aromatic compounds), 2837-2804 (CH_3 asymmetric stretching for Ar-CH_3), 2767-2706 (CH_3 symmetric stretching for Ar-CH_3); ^{1}H NMR (DMSO-d_6) δ ppm: 2.33 (s, 1H, H_1), 8.86 (s, 1H, H_2), 6.50 (s, 1H, H_3), 6.28-6.84 (d, 2H, H_4, H_7, *J*=8.3 *Hz*), 7.03-7.05 (d, 2H, H_5, H_6, *J*=8 *Hz*), 7.27-7.30 (d, 2H, H_8, H_9), 7.66 (s, 1H, H_{10}), 10.13 (s, 1H, H_{11}); MS: *m/z:* 340.06; Anal. Calcd. for $C_{18}H_{13}ClN_2O_3$: C, 63.44; H, 3.85; Cl, 10.40; N, 8.22; O, 14.09 Found: C, 63.40; H, 3.81; Cl, 10.38; N, 8.17; O, 14.02.

3.13.16 5-(4-Fluorophenyl)-8-hydroxy-2-methyl-3H-chromeno[2,3-d] pyrimidin-4(5H)-one (GAA-16)



Yield: 87%; M.P.- 138-140 ºC; IR (cm^{-1}): 3307 (O-H stretching of free primary alcohol), 1250, (O-H in plane bending frequency), 705 (O-H out of plane bending a broad peak), 1018 (C-F stretching frequency for mono fluorinated compounds), 1679 (C=O stretching frequency for cyclic amide), 1336 (C-N stretching for carbon bonded to amino group in pyrimidine), 2959 (C-H stretching vibrations for aromatic system), 1518 (C-C skeletal stretching of phenyl nucleus), 1262 (C-H in plane bending for the phenyl ring), 832 (C-H out of plane bending for 1,4-disubstituted benzene ring), 2830-2800 (CH_3 asymmetric stretching for Ar-CH_3), 2765-2705 (Ch_3 symmetric stretching for Ar-CH_3); MS: *m/z:* 324.09; Anal. Calcd. for $C_{18}H_{13}FN_2O_3$: C, 66.66; H, 4.04; F, 5.86; N, 8.64; O, 14.80 Found: C, 66.61; H, 4.00; F, 5.83; N, 8.60; O, 14.75.

3.14 SPECTRAL DISCUSSION

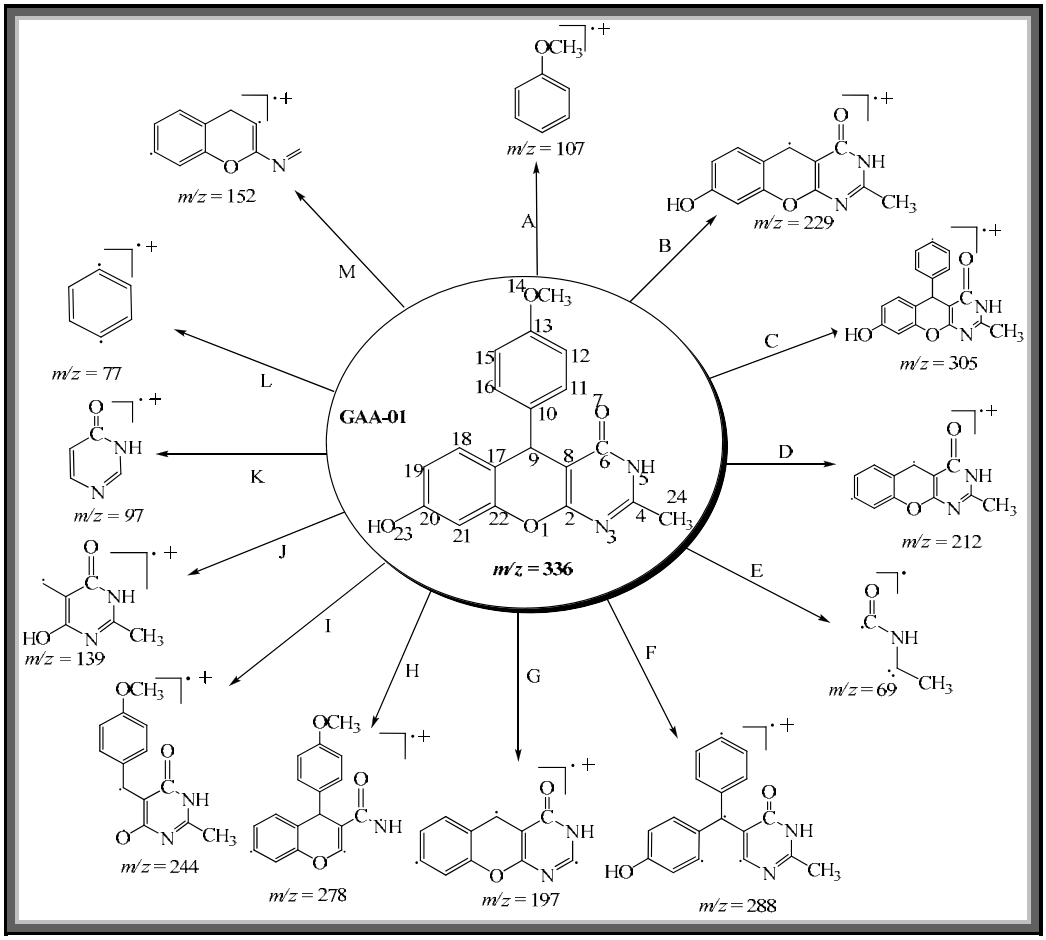
3.14.1 *IR SPECTRAL STUDY*

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. The O-H stretching frequency of free primary alcohol was observed around 3300-3400 cm^{-1}. The O-H in plane bending frequency were observed around 1250 cm^{-1}, where as the O-H out of plane bending vibrations were observed as a broad peak between 700-750 cm^{-1}. The C-H stretching frequency for aryl ethers was seen around 3010 cm^{-1} while the C-O-C asymmetric stretching frequency for aralkyl ethers 1264 cm^{-1}. The C=O stretching frequency for cyclic amide was characteristically seen around 1630-1680 cm^{-1} while the C-N stretching for carbon bonded to amino group in pyrimidine was observed around 1325 cm^{-1}. The C-H stretching vibrations for aromatic system was found a bit low around 2900 cm^{-1} while the C-C skeletal stretching of phenyl nucleus gave the characteristic bands around 1510 cm^{-1}, also the C-H in plane bending vibrations for the phenyl ring gave the characteristic bands around 1250 cm^{-1}. The C-H out of plane bending vibrations for 1,4-disubstituted benzene ring were observed around 800 cm^{-1}. The CH_3 asymmetric stretching for Ar-CH_3 gave the characteristic bands around 2810-2800 cm^{-1}, while the CH_3 symmetric stretching for Ar-CH_3 was seen around 2750-2720 cm^{-1}. The C-Halogen bands were also observed between the ranges of 1100-550 cm^{-1}, thus it suggests the correct formation of the desired products (GAA-01 to GAA-16).

3.14.2 *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. The probable Mass fragmentation pattern for the representative compound of each series is discussed below.

3.14.2.1 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF GAA-01*

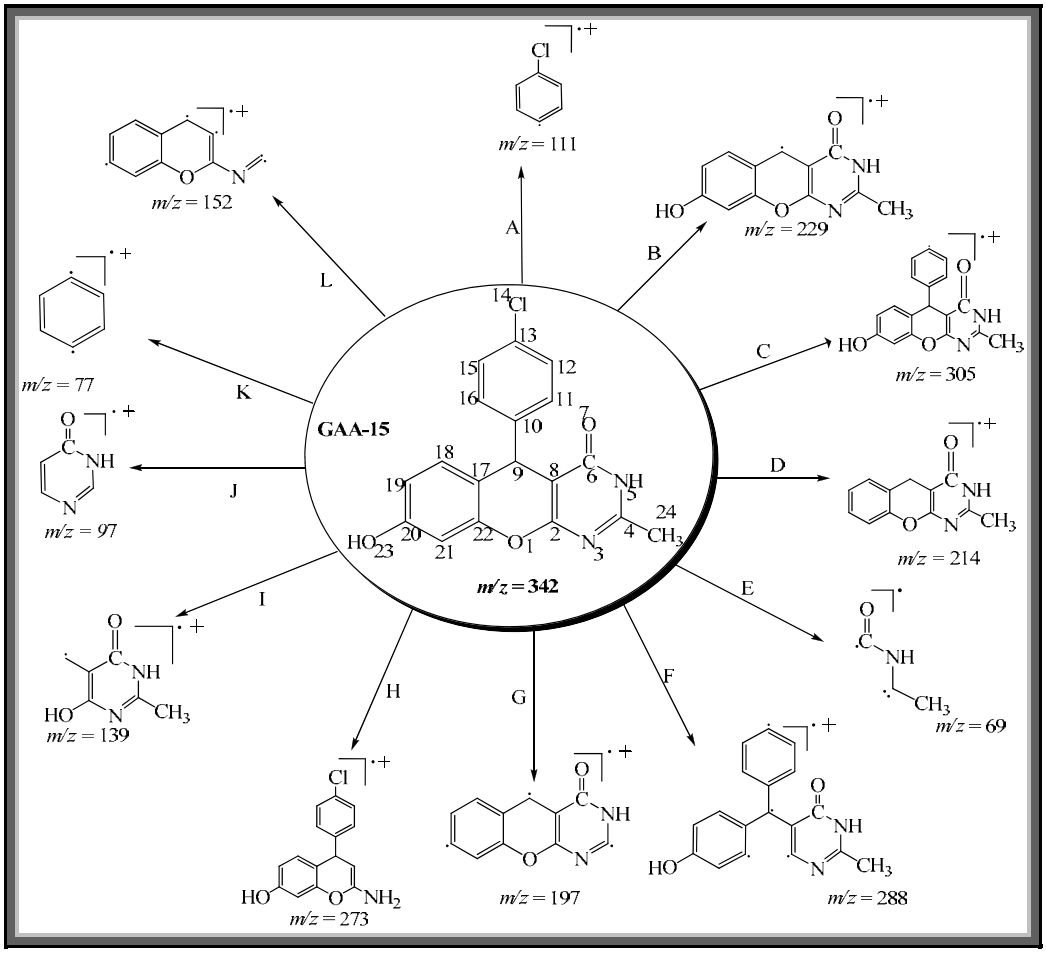


8-Hydroxy-5-(4-methoxyphenyl)-2-methyl-3H-chromeno[2,3-d]pyrimidin -4(5H)-one (GAA-01)

1. The target compound showed the characteristic molecular ion peak 336 *m/z*.
2. The bond cleavage between C_9-C_{10} generated a molecular ion which corresponds to a characteristic peak at 107 *m/z* **(A).**
3. A bond cleavage between C_9-C_{10} generated another molecular ion which corresponds to a characteristic peak at 229 *m/z* **(B)**.
4. Bond cleavages between C_{13}-O_{14} generated a molecular ion which corresponds to a characteristic peak at 305 *m/z* **(C)**.
5. Bond cleavages between C_9-C_{10} and C_{20}-O_{23} generated a molecular ion which corresponds to a characteristic peak at 212 *m/z* **(D)**.
6. Bond cleavages between C_6-C_8 and N_3-C_4 generated a molecular ion which corresponds to a characteristic peak at 69 *m/z* **(E)**.

7. Bond cleavages between O_1-C_{22}, O_1-C_2 and C_{13}-O_{14} generated a molecular ion which corresponds to a characteristic peak at 288 *m/z* **(F)**.
8. Bond cleavages between C_9-C_{10}, and C_{20}-O_{23}, C_4-C_{24} generated a molecular ion which corresponds to a characteristic peak at 197 *m/z* **(G)**.
9. Bond cleavages between C_{20}-O_{23}, C_2-N_3 and C_4-N_5 generated a molecular ion which corresponds to a characteristic peak at 278 *m/z* **(H)**.
10. Bond cleavages between C_9-C_{17}, O_1-C_{22} generated a molecular ion which corresponds to a characteristic peak at 244 *m/z* **(I)**.
11. Bond cleavages between C_9-C_{10}, C_9-C_{17}, & O_1-C_{22} generated a molecular ion which corresponds to a characteristic peak at 139 *m/z* **(J)**.
12. Bond cleavages between C_{20}-O_{23}, O_1-C_2, C_8-C_9 and C_4-C_{24} generated a molecular ion which corresponds to a characteristic peak at 97 *m/z* **(K)**.
13. Bond cleavage between C_9-C_{10}, C_{13}-O_{14} generated a molecular ion which corresponds to a characteristic peak at 77 *m/z* **(L)**.
14. The other fragment caused due to bond cleavage between C_9-C_{10}, C_{20}-O_{23}, C_4-C_{24}, C_6-C_8, and C_4-N_5 generated a molecular ion which corresponds to a characteristic peak at 152 *m/z* **(M)**.

3.14.2.2 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF GAA-15*



5-(4-Chlorophenyl)-8-hydroxy-2-methyl-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (GAA-15)

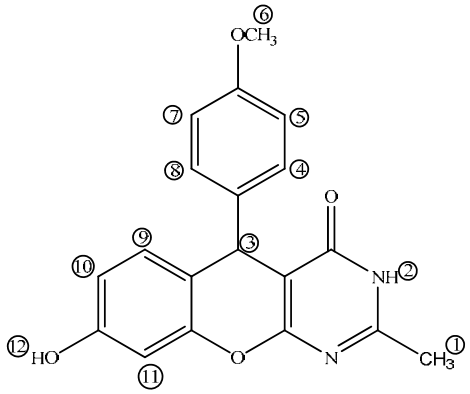
1. The target compound shows the desired characteristic molecular ion peak of 342 *m/z*.
2. The bond cleavage between C_9-C_{10} generated a molecular ion which corresponds to a characteristic peak at 111 *m/z* **(A).**
3. A bond cleavage between C_9-C_{10} generated another molecular ion which corresponds to a characteristic peak at 229 *m/z* **(B)**.
4. Bond cleavages between C_{13}-Cl_{14} generated a molecular ion which corresponds to a characteristic peak at 305 *m/z* **(C)**.
5. Bond cleavages between C_9-C_{10} and C_{20}-O_{23} generated a molecular ion which corresponds to a characteristic peak at 214 *m/z* **(D)**.

6. Bond cleavages between C_6-C_8 and N_3-C_4 generated a molecular ion which corresponds to a characteristic peak at 69 *m/z* **(E)**.
7. Bond cleavages between O_1-C_{22}, O_1-C_2 and C_{13}-Cl_{14} generated a molecular ion which corresponds to a characteristic peak at 288 *m/z* **(F)**.
8. Bond cleavages between C_9-C_{10}, and C_{20}-O_{23}, C_4-C_{24} generated a molecular ion which corresponds to a characteristic peak at 197 *m/z* **(G)**.
9. Bond cleavages between C_6-C_8, N_3-C_4 generated a molecular ion which corresponds to a characteristic peak at 273 *m/z* **(H)**.
10. Bond cleavages between C_9-C_{10}, C_9-C_{17}, & O_1-C_{22} generated a molecular ion which corresponds to a characteristic peak at 139 *m/z* **(I)**.
11. Bond cleavages between C_{20}-O_{23}, O_1-C_2, C_8-C_9 and C_4-C_{24} generated a molecular ion which corresponds to a characteristic peak at 97 *m/z* **(J)**.
12. Bond cleavage between C_9-C_{10}, C_{13}-Cl_{14} generated a molecular ion which corresponds to a characteristic peak at 77 *m/z* **(K)**.
13. The other fragment caused due to bond cleavage between C_9-C_{10}, C_{20}-O_{23}, C_4-C_{24}, C_6-C_8, and C_4-N_5 generated a molecular ion which corresponds to a characteristic peak at 152 *m/z* **(L)**.

3.14.3 *^{1}H-NMR SPECTRAL STUDY*

^{1}H-NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in DMSO solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from H-NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. The spectral interpretation can be discussed as under.

8-Hydroxy-5-(4-methoxyphenyl)-2-methyl-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (GAA-01)



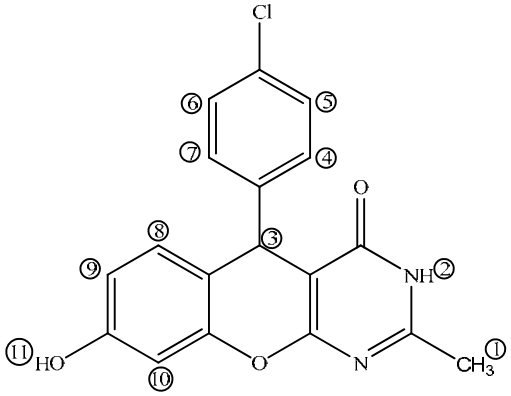
1. The Proton no. 1 i.e. the methyl protons do not have any other protons in their vicinity and hence their signal wont split giving us only a singlet. But as there are two electronegative atoms like nitrogen on both of its sides, the protons should be deshielded and thus found at a downfield region. Upon studying the NMR spectrum a characteristic sharp singlet is observed at 2.29 δ ppm. This signal is assigned to proton no.1 or the methyl protons.
2. The proton no. 2 is the amido proton which is directly attached to the electro negative nitrogen atom. This will deshield the proton to an even greater extent forcing it to give a signal in the downfield region. A singlet observed at 9.71 δ ppm is assigned to this amido proton i.e. proton no.2.
3. The Methine proton i.e. proton no.3 will show a singlet in the downfield region as compared to an isolated methine proton and it is very clearly seen in the NMR spectrum at 6.59 δ ppm. The reason for it to be at the downfield region is the fact that it is surrounded by a phenyl ring and also has a keto function in its vicinity which will deshield the proton.
4. The proton no. 4 and 8 have similar chemical environment and hence it will give one signal for two protons in the aromatic region. On studying the NMR spectrum a doublet between 6.78 δ ppm and 6.82 δ ppm is observed which is assigned to proton nos. 4 and 8. The J value was calculated to be 9.38 Hz which suggests that it is ortho coupled to another set of protons and on observing the structure protons 4 and 8 are ortho to protons 5 and 7.Thus, this doublet is assigned to protons 4 and 8.
5. Similar to protons 4 and 8; the protons 5 and 7 also have similar chemical environments only to be on a bit downfield region due to the methoxy function

present in its proximity. In the NMR spectrum another doublet between 7.24 δ ppm and 7.27 δ ppm is observed and its J value was also calculated to be 12 Hz which is in accordance to the structure as they are ortho coupled to protons 4 and 8.

6. The methoxy protons i.e. proton no.6 were observed in the NMR spectrum as a singlet at 3.73 δ ppm and the reason for them being at a downfield region is the bond of their carbon directly with the electronegative oxygen atom which will deshield the protons and force them to give the signal at a downfield region when compared to a proton in isolation.
7. On studying the NMR spectrum a doublet is observed between 7.35 δ ppm and 7.39 δ ppm accounting for one proton. This signal is assigned to proton no.9 as it will surely give a doublet due to the presence of proton no. 10 which will split its signal. More over a high J value like 12 Hz only shows that it is ortho coupled.
8. The proton no. 10 will go a bit downfield and will give a characteristic doublet due to its coupling with the proton no.9 and thus on studying the NMR spectrum a doublet between 7.72 δ ppm and 7.74 δ ppm is observed whose J value was calculated to be 8 Hz which clearly suggests ortho coupling. Hence without any doubt this characteristic signal of a doublet is assigned to proton no.10.
9. The proton no. 11 would give a singlet as it does not have any proton in its proximity and one such characteristic singlet is observed in the aromatic region at 7.53 δ ppm which is assigned to this proton no. 11.
10. The hydroxy proton gave a characteristic broad singlet at 10.07 δ ppm in the NMR spectrum of our representative compound.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton it can be clearly suggested that the proposed structure for compound no. GAA-01 has been confirmed.

5-(4-Chlorophenyl)-8-hydroxy-2-methyl-3H-chromeno[2,3-d]pyrimidin-4(5H)-one (GAA-15)



1. The Proton no. 1 i.e. the methyl protons do not have any other protons in their vicinity and hence their signal wont split giving us only a singlet. But as there are two electronegative atoms like nitrogen on both of its sides, the protons should be deshielded and thus found at a downfield region. Upon studying the NMR spectrum a characteristic sharp singlet is observed at 2.33 δ ppm. This signal is assigned to proton no.1 or the methyl protons.
2. The proton no. 2 is the amido proton which is directly attached to the electro negative nitrogen atom. This will deshield the proton to an even greater extent forcing it to give a signal in the downfield region. A singlet at 8.86 δ ppm is observed which is assign to this amido proton i.e. proton no.2.
3. The Methine proton i.e. proton no.3 will show a singlet in the downfield region as compared to an isolated methine proton and it is very clearly seen in the NMR spectrum at 6.50 δ ppm. The reason for it to be at the downfield region is the fact that it is surrounded by a phenyl ring and also has a keto function in its vicinity which will deshield the proton.
4. The proton no. 4 and 7 have similar chemical environment and hence it will give one signal for two protons in the aromatic region. On studying the NMR spectrum a doublet between 6.82 δ ppm and 6.84 δ ppm is found which is assigned to proton nos. 4 and 7. The J value was calculated to be 8.3 Hz which suggests that it is ortho coupled to another set of protons and on observing the structure its evident that protons 4 and 7 are ortho to protons 5 and 6.Thus, this doublet is assigned to protons 4 and 7.
5. Similar to protons 4 and 7; the protons 5 and 6 also have similar chemical environments only to be on a bit downfield region due to the chloro function

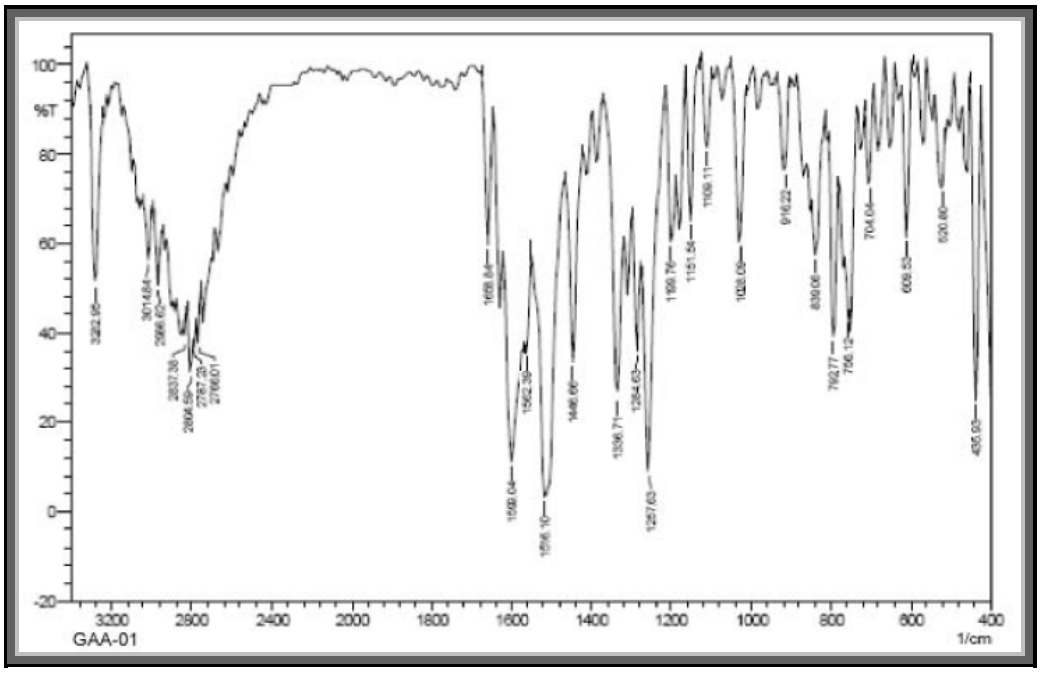
present in its proximity. In the NMR spectrum a doublet is observed between 7.03 δ ppm and 7.05 δ ppm and its J value was also calculated to be 8 Hz which is in accordance to the structure as they are ortho coupled to protons 4 and 7.

6. A doublet in the NMR spectrum was observed between 7.27 δ ppm and 7.30 δ ppm which accounted for 2 protons and this signal is assigned to proton nos. 8 and 9.
7. The proton no. 10 shows a characteristic singlet in the NMR spectrum at 7.66 δ ppm.
8. The proton no.11 of the hydroxy function gave a characteristic broad singlet in the NMR spectrum at 10.13 δ ppm and the reason for this proton to be at such a downfield region is its direct bond with the electronegative oxygen atom which will deshield it to a great extent forcing it to give a signal at such a downfield region.

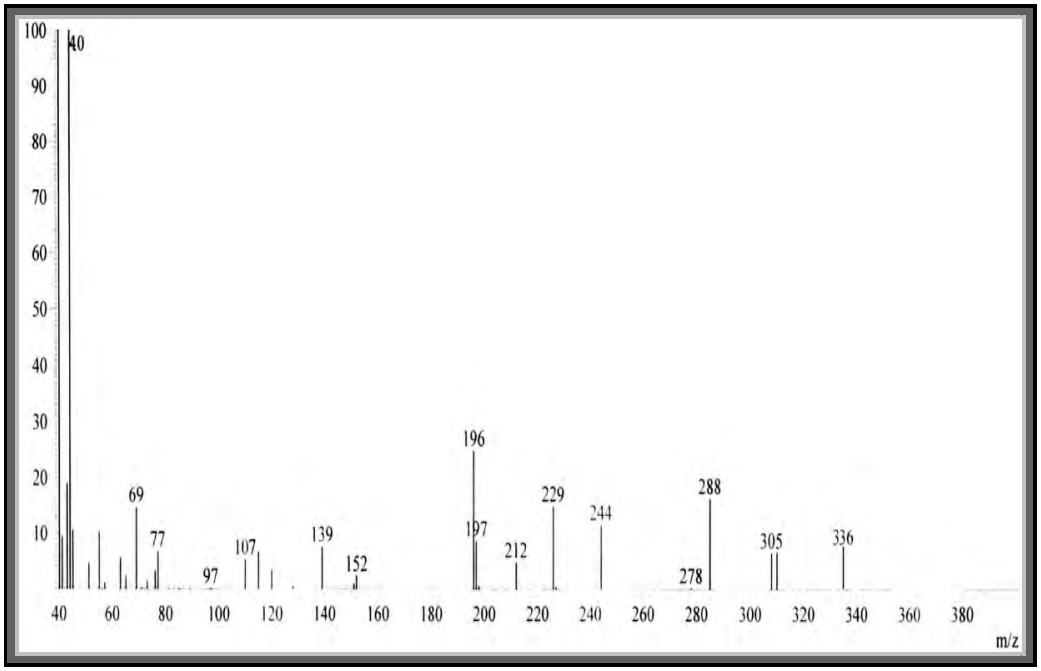
Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton it can be clearly suggested that the proposed structure for compound no. GAA-15 has been confirmed.

3.15 SPECTRAL REPRESENTATIONS OF SYNTHESIZED COMPOUNDS

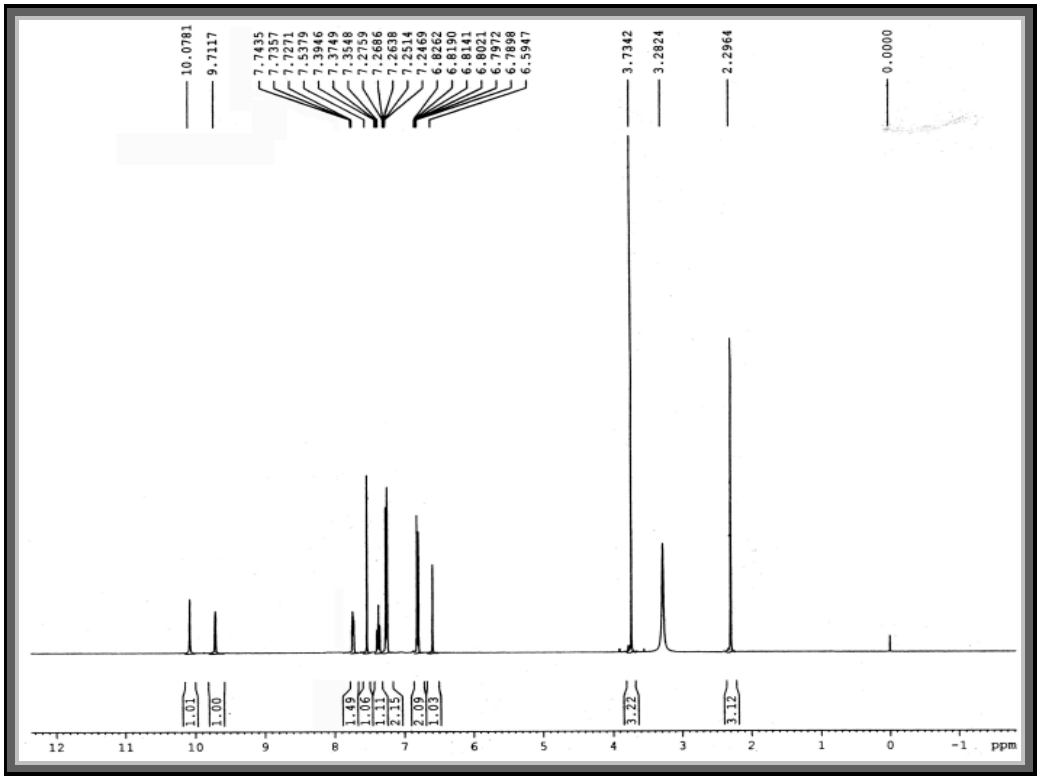
3.15.1 *IR SPECTRUM OF GAA-01*



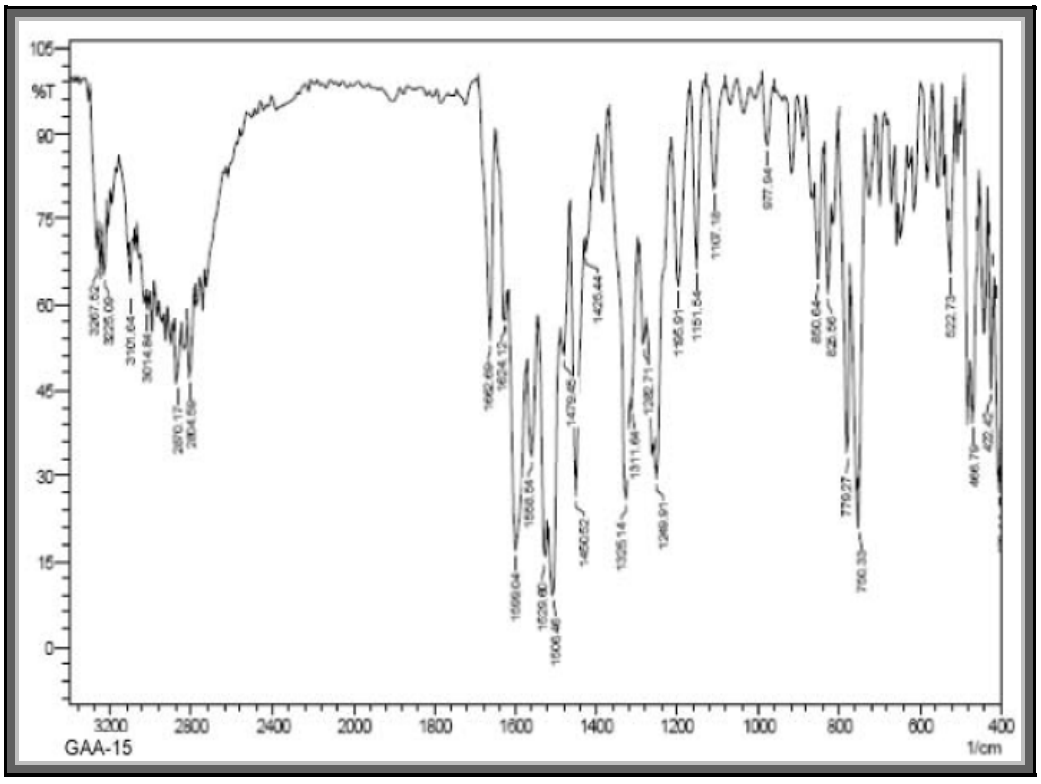
3.15.2 *MASS SPECTRUM OF GAA-01*



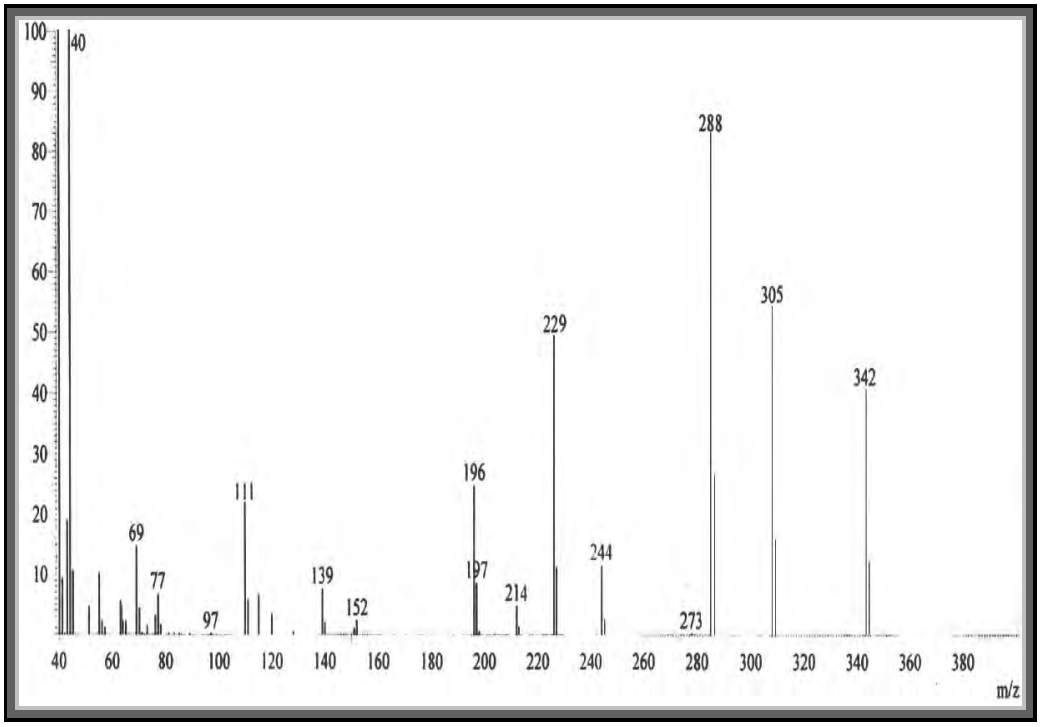
3.15.3 *^{1}H-NMR SPECTRUM OF GAA-01*



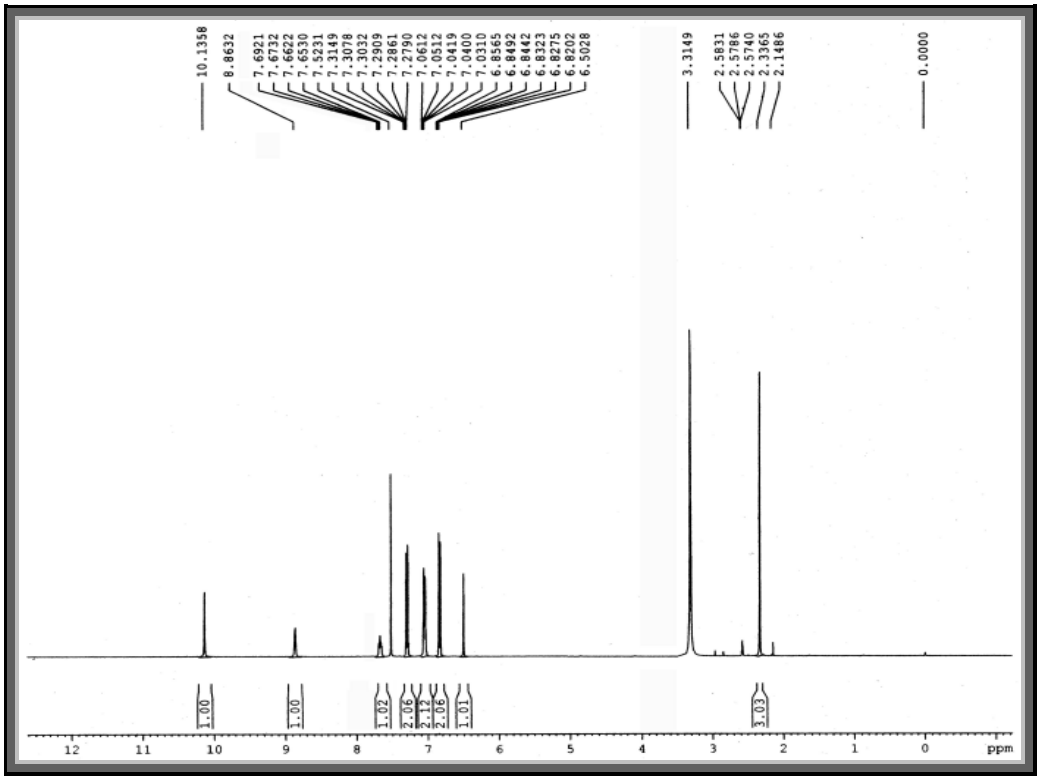
3.15.4 *IR SPECTRUM OF GAA-15*



3.15.5 *MASS SPECTRUM OF GAA-15*



3.15.6 *^{1}H-NMR SPECTRUM OF GAA-15*



3.16 RESULTS AND DISCUSSIONS

This chapter focuses on the chromenopyrimidin-4-one core structure. The literature survey revealed the fact that compounds containing benzopyran and pyrimidine-4-one motifs together embedded in the same carbon skeleton were hardly found in the literature. These compounds have been prepared using the Microwave Irradiations which constitutes to be a green chemistry approach. This methodology was found to be superior to the conventional methods as it took lesser time and also gave higher yields.

3.17 CONCLUSION

In conclusion, the compounds enlisted in this chapter are novel bio active compounds having two active motifs built into one structure. The results of the Anti-HIV activity are discussed separately in Chapter-6.

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CHAPTER – 4

Section-A

A Rapid microwave assisted synthesis of N-(2-methyl indoline-1-yl)(substituted phenyl) methanimines

4.1 INDOLE: A VERSATILE HETEROCYCLIC SYSTEM

Indole (2, 3-Benzopyrrole, ketole, 1-benzazole; C_8H_7N) is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. The participation of the nitrogen lone electron pair in the aromatic ring means that indole is not a base, and it does not behave like a simple amine.

Indole is a solid at room temperature. Indole can be produced by bacteria as a degradation product of the amino acid tryptophan. It occurs naturally in human faeces and has an intense fecal odor. At very low concentrations, however, it has a flowery smell, and is a constituent of many flower scents (such as orange blossoms) and perfumes. It also occurs in coal tar. Indoles are also used as an aromatic fragrance.

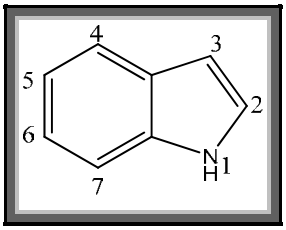


Fig. 1

Several syntheses of Indole derivatives along with its application are available in literature [1-47].

4.1.1 *PHYSICAL PROPERTIES*

Indole is a white coloured solid, melting at 52-54°C and boiling at 253-254 °C. 0.19 gm of indole is soluble in 100 ml of hot water. Indole is soluble in alcohol, ethyl acetate etc. Indole is having planar molecular shape, 1.22 g/cm^3 density and 2.11 D dipole moment in benzene. All indole derivatives show certain family resemblances to indole, but striking changes can be brought about by substitution of groups in the pyrrole ring. Thus, the fecal-like odor of skatole is the most pronounced of all the methylindoles, less pronounced for the 2-Methylindole and the 2, 3-Dimethyl indole; 1-methylindole, on the other hand, resembles methylaniline in odor. Introduction of carboxyl groups or phenolic hydroxyl groups causes elimination of the odor, and the naphthindoles are also without odor. All the common indole derivatives, like indole, form well-defined crystalline picrates, yellow to red in color.

4.1.2 *INTRODUCTION TO INDOLINE SYSTEM*

Indoline (2, 3-Dihydro-1*H*-indole; C_8H_9N) is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing ring. The compound is based on the indole structure, but the C_2-C_3 bond is saturated by oxidation / dehydrogenation it can be converted to indoles.

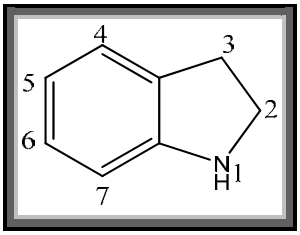


Fig. 2

4.1.2.1 *REDUCTION OF INDOLE*

Under this title, only reduction of (un)substituted-1*H*-indole system is discussed, reduction of other indole analogues is not discussed as well. Many methods have been employed in the reduction of indoles, the nature of the product(s) depending upon the method used.

The indole nucleus is not reduced by sodium-amyl alcohol [48] or by sodium-butyl alcohol, [49] but indolines are produced by electrolytic reduction of indoles in acid [50-52] and by reduction with phosphonium iodide in hydrogen iodide saturated hydriodic acid. [53]

A. Metal-Acid Reduction

The reduction of indole with zinc dust, [51] tin, [51, 54] or zinc amalgam [55] in hydrochloric acid affords indolines, but the yield of indoline produced from indole by such reductions is lowered by simultaneous polymerization of indole in the acidic media. This side reaction has been eliminated [56] by effecting this reduction with zinc dust in 85% phosphoric acid, under nitrogen to prevent aerial oxidation.

B. Birch Reduction

Early studies [62] led to the conclusion that sodium-ammonia reduction of indole affords indoline. However, the product from this reaction has been shown to be a mixture of indole and a product resulting from reduction in the benzenoid ring. [63] It was found that lithium-ammonia has very little reducing effect upon indole owing to the formation of the nonreducible indole lithium salt. [64-66]

C. Reductions using Sodium Borohydride, Lithium Aluminum Hydride and Borane

Indole is not reduced by either sodium borohydride [67] or lithium aluminum hydride, [67, 68] nor are other 1-unsubstituted indoles [69-70] reduced with lithium aluminum hydride. This nonreducibility has been illustrated in numerous cases in studies connected with the indole alkaloids where sodium borohydride [71] and lithium aluminum hydride [72] reductions of functional group(s) present in these complex molecules have been affected without reduction of the indole nucleus. Indole is reduced to indoline in 48% yield, however, by borane in tetrahydrofuran. [73] Although 1-methyl- and 1, 3-dimethylindole have been reported [68] to be reduced by lithium aluminum hydride to the corresponding indolines in 25-30% yields, later related studies [48] failed to support these observations. 1, 2, 3, 4-Tetrahydro-9-methylcarbazole remains unreduced when treated with lithium aluminum hydride, [48] and 1, 3-disubstituted oxindoles afford 1, 3-disubstituted indoles upon reduction with this reagent.

D. Catalytic Hydrogenation

Two early detailed studies on the catalytic hydrogenation of indoles were carried out. [74, 75] Whereas metal-acid reductions of indoles afford indolines as end products, catalytic hydrogenation of indoles often proceeds further than the indoline stage or occurs at positions alternative to the indolic 2, 3 double bond, and may even cause rupture of the pyrrolic ring. [76] Hydrogenation of indole with a nickel catalyst in ethanolic solution affords 1-ethyloctahydroindole by saturation of the aromatic system and reductive ethylation of the nitrogen atom. [77] Catalysts used for the hydrogenation

of indoles to indolines are platinum, nickel, nickel salts, copper, copper salts, [76] and palladium hydroxide-barium sulfate. [78] It is interesting that under vigorous conditions using a copper chromite catalyst, hydrogenation of 2, 3-dimethylindole has been reported [59] to afford only *trans*-2, 3-dimethylindoline, the *cis* isomer being the expected sole product from this reaction. It is suggested [59] that under the vigorous conditions used the hydrogenation reaches equilibrium involving appreciable dehydrogenation of the indoline and thus the indoline formed is the more stable *trans* isomer. Presumably such an equilibration-dehydrogenation, if it occurs, would have to involve the formation of some 2, 3-dimethyl-3*H*-indole in order that it could ultimately effect the formation of the *trans*-indoline. However, the establishment of the *trans* configuration of the product in this work [59] leaves much to be desired and further investigation of this product would be of interest. 1, 2, 3, 4-tetrahydrocarbazole, [48] and its 9-methyl derivative [48, 75] afford, as expected, the *cis*-indolines upon catalytic hydrogenation.

4.1.2.2 *PREPARATION OF 2-Methyl indoline*

2-methyl indoline derivatives were reported to synthesize from corresponding indole derivatives using cyano sodium borohydride [79-83] as a reducing agent and glacial acetic acid as a catalyst.

Kikugawa [80] produced 2-methyl indoline from 2-methyl indole using sodium borohydride, aluminium trichloride and pyridine as a catalyst.

Clive *et. al.* [81] reported preparation of 2-methyl indoline from 2-(phenylseleno) methyl indoline using triphenylstannane (Ph_3SnH).

Mills *et. al.* [83] reported different preparation methods for the 2-methyl indoline. (a) Treatment of ethyl acetoacetate with phenyl hydrazine and cyclization using sulfuric acid and ammonia and (b) From ethyl (2-allylphenyl) carbamate using benzene selenyl chloride and triphenylstannane.

Jackman and Scarmoutzos [84] synthesized 2-methyl indoline from 2-methyl indole using trimethylamine and borane.

Kotsuki *et. al.* [85] produced 2-methyl indoline by reducing 2-methyl indole using zinc borohydride and diethylether as a solvent.

Indoles were hydrogenated using heterogeneous catalysts in hydrocarbon solvents to achieve selective hydrogenation of the heterocyclic ring by Shaw and

Stapp.[86] Hydrogenation of indoles using Pt, Re, or in some cases, Ni catalysts (with or without sulfur compounds) occurred exclusively in the heterocyclic ring to give indolines, but conversions were affected by indole-indoline equilibrium.

The regioselective hydroamination and cyclization of aliphatic and aromatic amino olefins in the presence of $(LaHL_2)_2$ (L = η5-pentamethylcyclopentadienyl) [87] and borontrifluoride using diethylether and the divalent samarium complexes $Cp_2'Sm$ and $Cp_2'Sm(THF)_2$ (Cp' = η5-Me_5C_5) [88] and to give 2-methyl indoline was reported. Thus, $CH_2=CH(CH_2)_3NH_2$ was treated with a catalytic amount of $(LaHL_2)_2$ in a hydrocarbon solvent (toluene, cyclohexane or pentane) to give 2-methyl indoline. Kinetic and mechanistic evidence presented that the turnover-limiting step is intramolecular olefin insertion into the La-N bond followed by rapid protonolysis of the resulting La-C bond.

Lawin *et. al.* [89] reported the preparation of 2-methyl indoline from 2-methyl indole through electrolytic reduction.

Meyers and Melot [90] carried out *N*-alkylation on indoline followed by methylation at C_2 position in the presence of *t*-butyllithium and dealkylation using hydrazinehydrate resulted into corresponding indoline.

Yadav *et. al.* [91] reported that *N*-allyl anilines underwent 3-aza-Cope rearrangement in the presence of Zn^{+2} montmorillonite under microwave irradiation in the absence of solvent to afford indoline derivatives in high yields. Similarly aryl allyl thioethers were rearranged to dihydrobenzothiophenes.

Jimenez *et. al.* [92] reported mixtures of products, while 2-allyl aniline underwent reduction through beta-cyclodextrin medium.

N-methyl-2-methyl indoline was synthesized from *N*-methyl-2-methyl indole using tin and concentrated hydrochloric acid, [93] indium and ammonium chloride, [94] and from *t*-butyl 2-methyl-1*H*-indole-1-carboxylate using rhodium phosphine complex, [95] PhTRAP-ruthenium catalyst, [96] and palladium and polymethylhydrosiloxane. [97]

4.1.2.3 *PREPARATION OF N-Amino-2-methyl indoline*

The synthesis of N-Amino-2-methyl indoline follows a rather classical synthetic pathway. Nitrosation reaction is carried out in the first step followed up by the metal-acid reduction for the functional group transformation of nitroso to amino. The nitrosation is carried out using sodium nitrite and aqueous hydrochloric acid as reactants to the starting material 2-methyl indoline. The reaction was carried out between 0-5 ^{0}C to control the exothermicity and move the reaction in the forward direction. The nitroso product was then taken as the input material and was reduced using Zinc in presence of acetic acid. The product thus obtained was N-Amino-2-methyl indoline.

4.2 AIM OF THE CURRENT WORK

This laboratory is involved in the synthesis of nitrogen containing heterocycles *viz*. pyrrole, indole, 2-methyl indole, dihydropyridine, dihydropyrimidine, 4-hydroxy quinolones etc. where, pyrroles, indoles[a], dihydropyridines[b], dihydropyrimidines[c], 4-hydroxy quinolones[d] and 2-methyl indoles showed good anti-tubercular[e], anti-diabetic, anti-cancer[f] and multi drug resistance reversal[g] activity. Looking to the interesting biological profile showed by indole, 2-methyl indole and 2-methyl indoline from the literature survey and development of a simple preparation method for 2-methyl indole developed at this laboratory it was decided to prepare 2-methyl indoline-1-amine and to further explore the chemistry involving 2-methyl indoline moiety.

The current chapter aims at a novel and greener approach of synthesizing *N-benzylidene-2-methylindolin-1-amine* using the micro wave irradiation and their study for various biological activities.

[a] Crystal structure of {n- (2,6-di chlorophenyl)-2-oxoindoline-3-ylideme}. S.Thamotharan, L.Vijyalaxmi, Parthasarathi, V. & Anamik Shah. Acta Cryst E60, 212-213, **2002**

[b] Enhanced antimicrobial effect of erythromycin in the presence of 3,5-dibenzoyl 1,4-dihydropyridines. Gyongyi Gunics, Noboru Motohashi, Joseph Molnar, Sandor Karkas, Masami Kawase. Setsuo Saito, Harsukh Gevariya, Anamik Shah. Anticancer Research, 21, 269-274, **2001**.

[c] Microwave-based synthesis of novel Thienopyrimidine bioisosteres of gefitinib. Phoujdar, Manisha S.; Kathiravan, Muthu K.; Bariwal, Jitender B.; Anamik Shah.; Jain, Kishor S., Tetrahedron Letters**,** 49(7), 1269-1273, **2008**

[d] Synthesis and anti-hiv studies of some substituted pyrimidinediones, ethoxy pyrano [3, 2-c] quinolines and hydrazino pyrano [3,2-c]-quinolines. Narsinh Dodia, Anamik Shah. Ind. J. Pharma. Science, 63(3), 211-215, **2001**

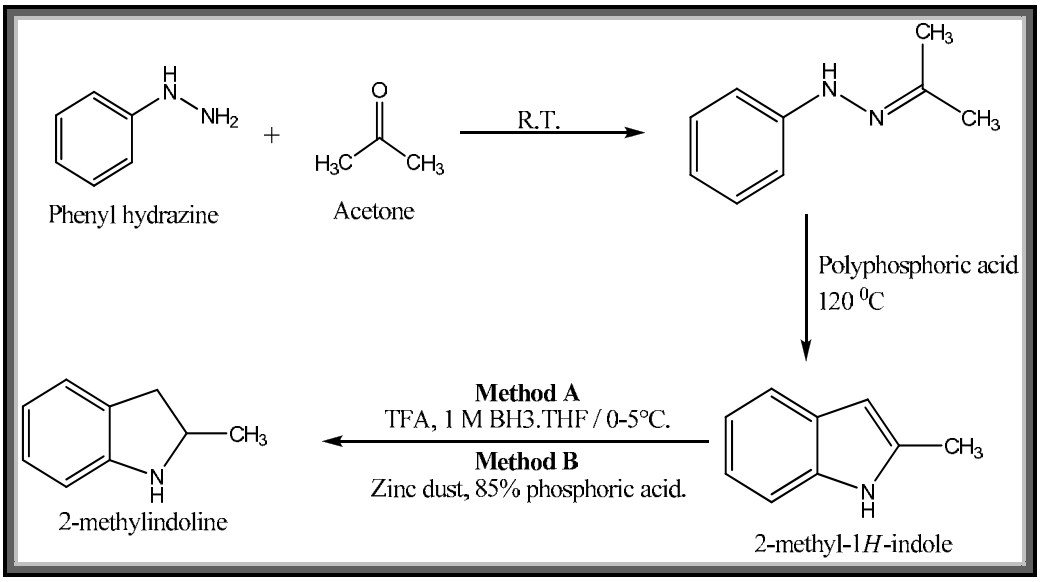
[e] Synthesis, in vitro anti-tubercular activity and 3D-QSAR study of 1,4-dihydropyridines. Atul T Manvar, M.Sc; Raghuvir R Pissurlenkar, M Pharm; Vijay R Virsodia, PhD; Kuldip D Upadhyay, PhD; Dinesh R Manvar; Arun K Mishra; Hrishikesh D Acharya; Alpesh R Parecha; Chintan D Dholakia; Anamik K Shah; Evans Clifton Coutinho, Ph.D..Molecular Diversity, **2009** [Epub ahead of print].

[f] Synthesis and Biological Activity of Stable and Potent Antitumor Agents, Aniline Nitrogen Mustards Linked to 9-Anilinoacridines via a Urea Linkage. Naval Kapuriya, Kalpana Kapuriya, Xiuguo Zhang, Ting-Chao Chou, Rajesh Kakadiya, Yu-Tse Wu, Tung-Hu Tsai, Yu-Ting Chen, Te-Chang Lee, Anamik Shah, Yogesh Naliapara, Tsann-Long Su. Bioorganic & Medicinal Chemistry, 16, 5413-5423, **2008**.

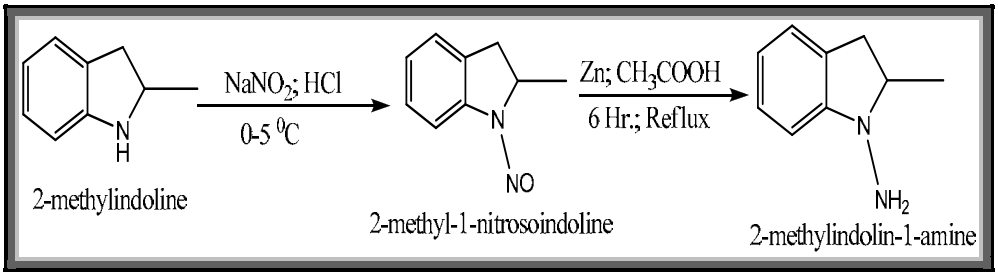
[g] Advanced Dihydropyridines as Novel Multi Drug Modifiers and Reverting Agents. Topics in Heterocyclic Chemistry,Publisher Springer Berlin/Heidelberg. Springerlink Date:.Anamik Shah, Jitender Bariwal, Joseph Molnar, Masami Kawase and Noboru Motohashi, 22/12/**2007**.

4.3 REACTION SCHEMES

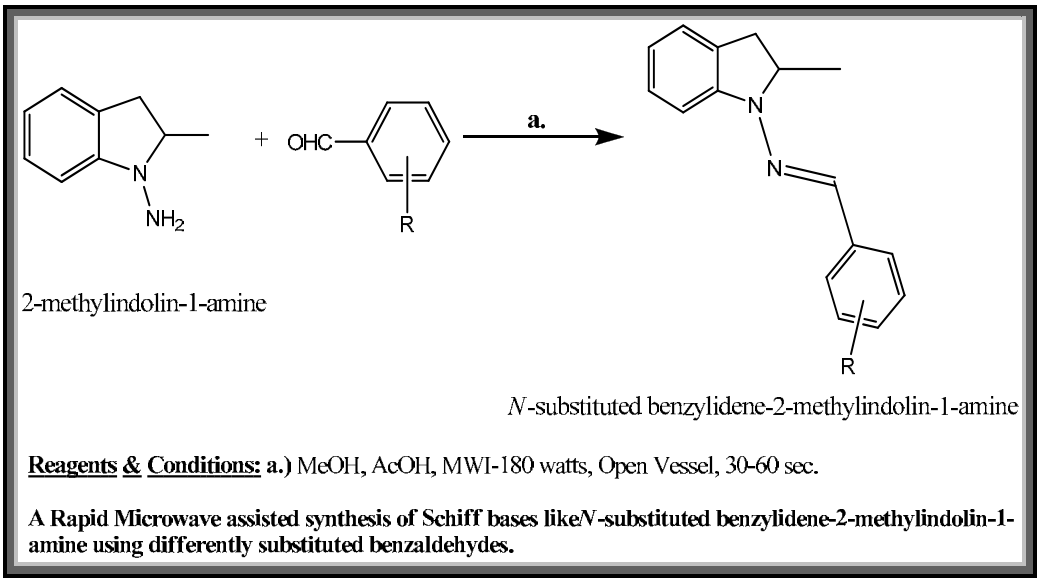
A. ***REACTION SCHEME FOR THE PREPARATION OF 2-Methyl indoline***



B. ***REACTION SCHEME FOR THE PREPARATION OF N-Amino-2-methyl indoline***



C. *REACTION SCHEME FOR THE PREPARATION OF N-substituted benzylidene-2-methyl indoline-1-amine (NAISB-01 TO NAISB-20)*



Reagents & Conditions: a.) MeOH, AcOH, MWI-180 watts, Open Vessel, 30-60 sec.

A Rapid Microwave assisted synthesis of Schiff bases like*N*-substituted benzylidene-2-methylindolin-1-amine using differently substituted benzaldehydes.

4.3.1 *PHYSICAL DATA TABLE*

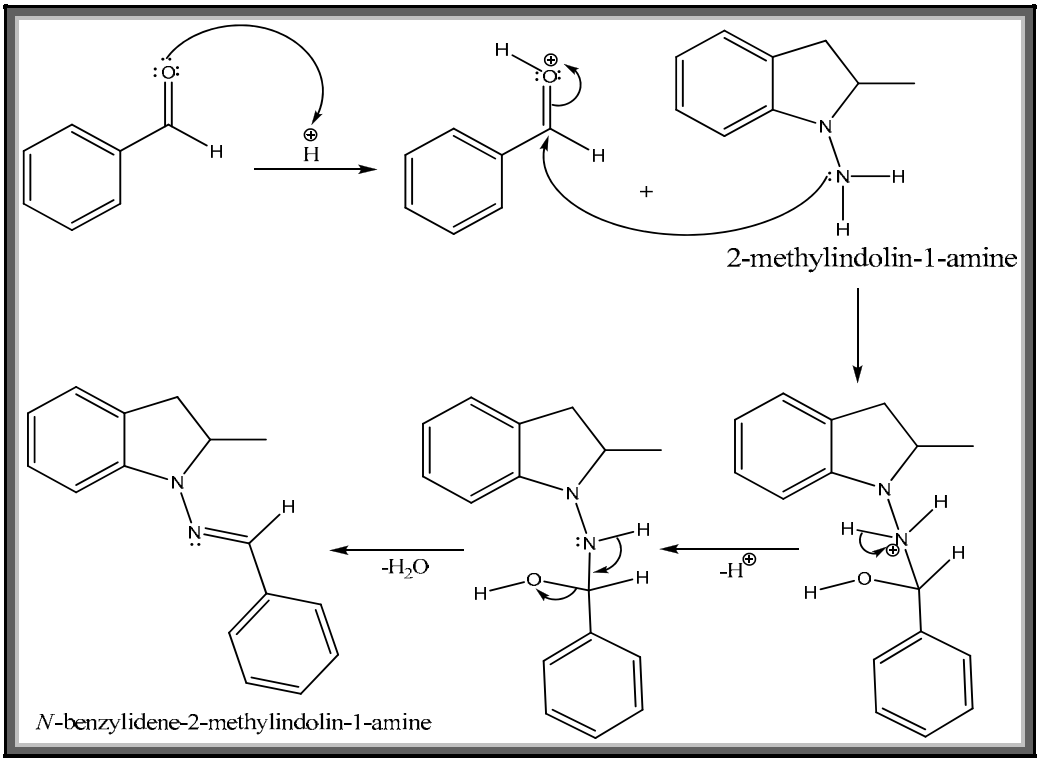
Code	R_1	M. F.	M. W.	M. P. ^0C	Time (min)	Yield %	R_{f1}
NAISB-1	H	$C_{16}H_{16}N_2$	236.31	98-100	0:30	92	0.42
NAISB-2	3-Cl	$C_{16}H_{15}ClN_2$	270.75	110-112	0:20	95	0.46
NAISB-3	3,4-OCH_3	$C_{18}H_{20}N_2O_2$	296.36	122-124	0:40	97	0.40
NAISB-4	4-OH	$C_{16}H_{16}N_2O$	252.31	102-104	0:20	98	0.42
NAISB-5	3-OCH_3, 4-OH	$C_{17}H_{18}N_2O_2$	282.33	128-130	0:30	92	0.48
NAISB-6	3-NO_2	$C_{16}H_{15}N_3O_2$	281.30	112-114	0:40	90	0.48
NAISB-7	4-Cl	$C_{16}H_{15}ClN_2$	270.75	130-132	0:40	95	0.56
NAISB-8	4-OCH_3	$C_{17}H_{18}N_2O$	266.33	132-134	0:40	98	0.50
NAISB-9	2-OH	$C_{16}H_{16}N_2O$	252.31	122-124	0:40	90	0.52
NAISB-10	4-NO_2	$C_{16}H_{15}N_3O_2$	281.30	120-122	0:40	92	0.58
NAISB-11	4-$N(CH_3)_2$	$C_{18}H_{21}N_3$	279.37	104-106	0:30	97	0.52
NAISB-12	3-OC_2H_5, 4-OH	$C_{18}H_{20}N_2O_2$	296.36	108-110	0:30	95	0.54
NAISB-13	Anthracenyl	$C_{24}H_{20}N_2$	336.42	98-100	0:50	92	0.58
NAISB-14	Pyridinyl	$C_{15}H_{15}N_3$	237.29	106-108	0:60	90	0.54
NAISB-15	Naphthyl	$C_{20}H_{18}N_2$	286.37	116-118	0:60	95	0.55
NAISB-16	Furyl	$C_{14}H_{14}N_2O$	226.27	134-136	0:40	95	0.52
NAISB-17	2-Cl	$C_{16}H_{15}ClN_2$	270.75	126-128	0:20	90	0.56
NAISB-18	3,4,5-OCH_3	$C_{19}H_{22}N_2O_3$	326.38	110-112	0:30	96	0.40
NAISB-19	3-Br	$C_{16}H_{15}BrN_2$	315.20	114-116	0:40	90	0.52
NAISB-20	2,5-OCH_3	$C_{18}H_{20}N_2O_2$	296.36	124-126	0:50	95	0.58

TLC solvent system for R_{f1} = Hexane:Ethyl acetate - 6:4.

Microwave Irradiation = 180 Watts.

4.4 PLAUSIBLE REACTION MECHANISM

4.4.1 *FORMATION OF N-benzylidene-2-methylindolin-1-amine*



The mechanism of the formation of Schiff bases is very well known. This reaction occurs in acidic media, suggesting that it would move forward in presence of protons. The lone pair of electrons on the carbonyl carbon of the aldehyde would first attack on the free proton available and hence would form a very strong electrophile. Now, the lone pair of electrons on Nitrogen of the amino group will attack on carbonyl carbon and the π electrons will then move on to oxygen atom to quench it. This will form the ammonium ion which is further quenched by the removal of the water molecule forming the desired final product.

4.5 EXPERIMENTAL

4.5.1 *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 and UV. All the reactions were carried out in Samsung MW83Y Microwave Oven which was locally modified for carrying out chemical reactions. IR spectra were recorded in 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.

4.5.2 *GENERAL PROCEDURES*

Step-1:PREPARATION OF Acetone phenyl hydrazone

25 ml of phenyl hydrazine was added drop wise to a magnetically stirred solution of 20 ml of acetone. After the completion of the addition, 5 ml of acetone was added to the reaction mixture and the reaction mixture was heated on the water bath to remove the excess of the acetone. Afterwards the reaction mixture was cooled to room temperature and it was made anhydrous by means of anhydrous sodium sulphate or anhydrous calcium chloride. The solution was filtered to give the dark yellow solution of phenyl hydrazone. Yield - 80 %, BP - 140-142°C (141-142°C)[h].

Step-2:PREPARATION OF 2-Methyl indole

30 gm of acetone phenyl hydrazone was added drop wise to a beaker containing 75 gm of polyphosphoric acid with constant stirring. The reaction mixture was heated on water bath for 2-3 hours, where the orange coloured solution became dark red-brown. After that the temperature of the reaction mixture was raised to

[h] V. Sridar, *Indian J. Chem., Sect. B*, 1996, **35**, 737.
V. Sridar, *Indian J. Chem., Sect. B*, 1997, **36**, 86.

120°C and then it was cooled to room temperature. After that 400 ml of distilled water was added to the reaction mixture to decompose the polyphosphoric acid, the whole content was steam distilled to acquire the 2-Methyl Indole as white coloured shining crystals. Yield - 79 %, MP - 58-59°C (56-57°C)[i].

Step-3: PREPARATION OF 2-Methyl indoline

Method – (A)

0.05 mole 2-methyl indole was dissolved in 110 ml of trifluoroacetic acid under nitrogen atmosphere. The solution was cooled in an ice bath and 90 ml of about 1 M BH_3.THF in tetrahydrofuran solution was added slowly over about 30 minutes. Thereafter, 50 ml of water was added, the resulting solution was stirred at room temperature for about 90 minutes. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as a mobile phase. After the reaction to be completed the mixture was then evaporated under reduced pressure to about 30 ml of semi-solid viscous oil. The oil was partitioned between methylene dichloride and aqueous sodium hydroxide solution (pH>10). The organic layer was dried over anhydrous potassium carbonate, filtered and evaporated under reduced pressure to obtain 5.65 gm of a slightly greenish transparent oily 2-methyl indoline product. Yield - 85%, BP – 224-226 °C (225-227°C)[j].

Reverse addition method

0.05 mole 2-methyl indole was dissolved in 90 ml of 1 M BH_3.THF solution in tetrahydrofuran by stirring under nitrogen atmosphere while cooling in an ice bath and the stirring was continued for about 15 minutes. Thereafter, 110 ml of trifluoroacetic acid was added drop-wise with continued stirring, cooling and maintaining a nitrogen atmosphere. 50 ml of water was added to the mixture, followed by methylene dichloride and aqueous sodium hydroxide solution (pH>10). The solution then was stirred about 30 minutes and a sample was taken for TLC, the progress and the

[i] R.J. Sundberg, The Chemistry of Indoles, Academic Press, New York, 1970, p. 78.
B. Robinson, *The Fischer Indole Synthesis*, Wiley-Interscience, New York, 1982.

[j] T. Besson, G. Guillaumet, C. Lamazzi, C. W. Rees and V. Thiéry, *J. Chem. Soc.*, *Perkin Trans. 1*, 1998, 4057.

completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as a mobile phase. The organic layer was dried over anhydrous potassium carbonate, filtered and evaporated under reduced pressure to obtain 5.70 gm of a slightly greenish transparent oily 2-methyl indoline product. Yield - 86%, BP – 224-226 °C (225-227°C)[k].

Method – (B)

To 0.36 mole of untreated zinc dust in a nitrogen atmosphere was added all at once 200 ml of 85 % phosphoric acid. The mixture was rapidly heated to 70-80°C on a steam bath and 0.12 mole of 2-methyl indole was added in portions during 30 minutes with vigorous mechanical stirring (efficient mechanical stirring was found to be extremely difficult if the mixture was kept at room temperature during the addition of indole, and the yields of indoline were only 5-15 %). After the addition to be completed stirring was continued for 3-4 hours at 80°C under nitrogen. 100 ml water was added with cooling and the mixture was basified slowly with 40% aqueous sodium hydroxide. The thick slurry was steam distilled and the extraction of the distillate with chloroform gave, after drying and concentration in *vacuuo*, greenish oil. Distillation under reduced pressure gave 65 % of 2-methyl indoline as slightly greenish transparent oil. BP – 224-226 °C[l].

B. PREPARATION OF 2-Methyl indoline-1-amine

Step-1: Preparation of 2-methyl-1-nitroso indoline

740 ml of conc. Hydrochloric acid was charged into a round bottom flask. To it was added approx. 400 gm of ice and allowed to cool for 10 min. so as to bring the temperature of the mixture below 0-5 ^{0}C. To this cooled mixture was then slowly added 200 gm of 2-Methyl indoline within the span of 1-2 hours never letting the temperature of the reaction mix cross 5 ^{0}C. This mix was then stirred for about half an hour. A solution of Sodium nitrite was prepared in the meanwhile using 110 gm of $NaNO_2$, 200 gm ice and 110 gm D.M. water. This solution was also cooled to 0-5 ^{0}C. This cooled sodium nitrite solution was then carefully added to the reaction mixture

[k k] T. Besson, G. Guillaumet, C. Lamazzi, C. W. Rees and V. Thiéry, *J. Chem. Soc.*, *Perkin Trans. 1*, 1998, 4057

[l l] T. Besson, G. Guillaumet, C. Lamazzi, C. W. Rees and V. Thiéry, *J. Chem. Soc.*, *Perkin Trans. 1*, 1998, 4057

maintaining the cooling around 0 ^{0}C. Immediately after the addition was over the reaction was checked using the Starch Iodide paper which immediately turns to blue if not more sodium nitrite solution was added in the reaction mix. The reaction mixture was then stirred for about 2 hours below 5 ^{0}C. After the completion of the reaction the mixture was allowed to come at R.T and was then filtered under vacuum and washed with chilled D.M.water which afforded the 2-Methyl-1-nitroso indoline as the main product. Yield=78 %.

Step-2 Preparation of 2-Methyl indoline-1-amine

Into a solution of 700 ml methanol and 400 ml water was dissolved 322 gm of wet cake of 2-methyl-1-nitroso indoline under cold condition. After the temperature reached 0 ^{0}C, 300 gm of zinc dust was added very slowly not letting the temperature to go beyond 5 ^{0}C. To this reaction mixture was then very carefully and very slowly added 310 ml of acetic acid. The exothermicity of this reaction was kept under control never letting the temperature to rise above 5 ^{0}C. This reaction mix was then refluxed for 6-8 hr and then allowed to cool to R.T. The work up of this reaction proceeded by extracting the organic content in toluene and then was given a charcoal treatment so as to remove any coloured impurities. Distillation of toluene under vacuum afforded us with the desired product 2-Methyl indoline-1-amine. Yield= 75 % and M.P. = 38-40 ^{0}C[m].

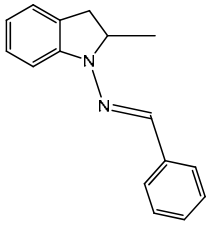
C. GENERAL PROCEDURE: N-substituted benzylidene-2-methylindolin-1-amine (NAISB-01 TO NAISB-20)

Equimolar amounts of neat reactants i.e. 2-Methyl indolin-1-amine and substituted benzaldehydes were taken in an Erlenmeyer flask, dissolved in Methanol which was taken 10 time w/v of the reactants and served as the solvent. Few drops of Acetic acid were added. The reaction mixture was then subjected to MWI for a specific time (see Physical data Table) at low power (180 W). The progress of the reaction was monitored by TLC examination at an interval of every 10 seconds. On completion of reaction, the reaction mixture was cooled at room temperature which afforded us the solid crystals of the desired product. The product thus obtained was filtered, washed with cold water, dried, and recrystallized from Rectified Spirit.

[m] B. A. Frontana-Uribe, C. Moinet and L. Toupet, *Eur. J. Org. Chem.*, 1999, 419.

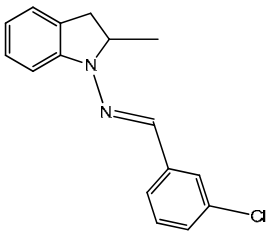
4.6 ANALYTICAL DATA

4.6.1 N-benzylidene-2-methylindolin-1-amine (NAISB-01)



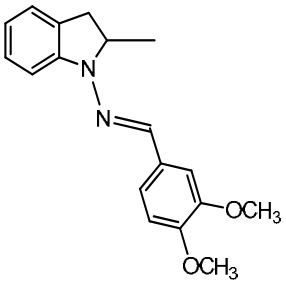
Yield: 92%; M.P.- 98-100 ºC; IR (cm^{-1}): 3045 (C-H stretching vibration of aromatic region), 958 (C-H in plane bending, aromatic region), 700 (C-C out of plane bending of mono substituted benzene ring), 748 (C-H out of plane bending of mono substituted benzene ring), 2943 (CH_3 asymmetric stretching of R-CH_3), 1477-1452 (CH_3 asymmetric bending of R-CH_3), 2980 (CH_2 asymmetric stretching of cyclopentane), 2889 (C-H symmetric stretching for cyclopentane), 922-885 (Ring stretching for cyclopentane), 1662 (C=N stretching vibration), 1400 (C-H in plane bending for alkene, =CH_2 scissoring), 1307 (C-H in plane bending for alkene, =CH rocking), 1269-1236 (N-N stretching for secondary amine); ^{1}H NMR (DMSO-d_6) δ ppm: 1.40 (s, 3H, H_1), 4.56-4.61 (m, 1H, H_2), 2.78-2.83 (d, 1H, H_3), 3.48-3.54 (q, 1H, H_3), 7.14-7.16 (d, 1H, H_4, J=8 *Hz*), 7.23-7.24 (d, 2H, H_5 & H_7, J=4 *Hz*), 7.57 (s, 1H, H_8), 7.71-7.73 (d, 2H, H_9 & H_{13}, J=8 *Hz*), 7.39-7.42 (t, 2H, H_{10} & H_{12}) 7.28-7.31 (t, 1H, H_{11}); MS: *m/z:* 236.13; Anal. Calcd. for $C_{16}H_{16}N_2$: C, 81.32; H, 6.82; N, 11.85; Found: C, 81.28; H, 6.75; N, 11.79.

4.6.2 N-(3-chlorobenzylidene)-2-methylindolin-1-amine (NAISB-02)



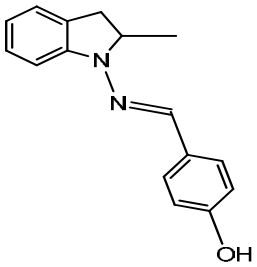
Yield: 95%; M.P.- 110-112 ºC; IR (cm^{-1}): 3040 (C-H stretching vibration of aromatic region), 952 (C-H in plane bending, aromatic region), 710 (C-C out of plane bending of mono substituted benzene ring), 742 (C-H out of plane bending of mono substituted benzene ring), 2935 (CH_3 asymmetric stretching of R-CH_3), 1470-1442 (CH_3 asymmetric bending of R-CH_3), 2970 (CH_2 asymmetric stretching of cyclopentane), 2875 (C-H symmetric stretching for cyclopentane), 922-885 (Ring stretching for cyclopentane), 1662 (C=N stretching vibration), 1400 (C-H in plane bending for alkene, =CH_2 scissoring), 1302 (C-H in plane bending for alkene, =CH rocking), 1265-1230 (N-N stretching for secondary amine) 740 (C-Cl stretching for mono chlorinated aromatic compound); MS: *m/z:* 270.09; Anal. Calcd. for $C_{16}H_{15}ClN_2$: C, 70.98; H, 5.58; Cl, 13.09; N, 10.35; Found: C, 70.93; H, 5.51; Cl, 13.02; N, 10.27.

4.6.3 N-(3,4-dimethoxybenzylidene)-2-methylindolin-1-amine (NAISB-03)



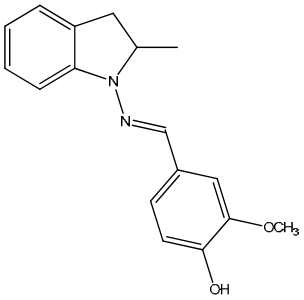
Yield: 97 %; M.P.- 122-124 ºC; IR (cm^{-1}): 3030-3015 (C-H stretching vibration of aromatic region), 1250 (C-H in plane bending, aromatic region), 700 (C-C out of plane bending of mono substituted benzene ring), 750 (C-H out of plane bending of mono substituted benzene ring), 2959 (CH_3 asymmetric stretching of R-CH_3), 1479-1454 (CH_3 asymmetric bending of R-CH_3), 2943 (CH_2 asymmetric stretching of cyclopentane), 2901-2859 (C-H symmetric stretching for cyclopentane), 940-895 (Ring stretching for cyclopentane), 1646 (C=N stretching vibration), 1415 (C-H in plane bending for alkene, =CH_2 scissoring), 1293 (C-H in plane bending for alkene, =CH rocking), 1251-1225 (N-N stretching for secondary amine) 3069 (C-H stretching for aryl ethers), 1176-1162 (C-O-C asymmetric stretching); MS: *m/z:* 296.15; Anal. Calcd. for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45; O, 10.80 Found: C, 72.88; H, 6.73; N, 9.39; O, 10.75.

4.6.4 N-(4-hydroxybenzylidene)-2-methylindolin-1-amine (NAISB-04)



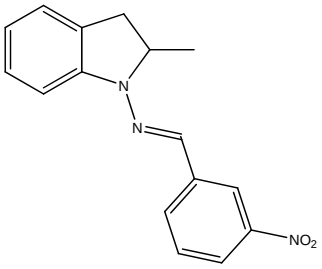
Yield: 98 %; M.P.- 102-104 ºC; IR (cm^{-1}): 3038 (C-H stretching vibration of aromatic region), 962 (C-H in plane bending, aromatic region), 710 (C-C out of plane bending of mono substituted benzene ring), 745 (C-H out of plane bending of mono substituted benzene ring), 2932 (CH_3 asymmetric stretching of R-CH_3), 1472-1468 (CH_3 asymmetric bending of R-CH_3), 2978 (CH_2 asymmetric stretching of cyclopentane), 2892 (C-H symmetric stretching for cyclopentane), 920-888 (Ring stretching for cyclopentane), 1664 (C=N stretching vibration), 1405 (C-H in plane bending for alkene, =CH_2 scissoring), 1309 (C-H in plane bending for alkene, =CH rocking), 1272-1249 (N-N stretching for secondary amine) 3615 (O-H free stretching for phenol), 1408-1358 (O-H in plane bending vibration coupled for phenol); MS: *m/z:* 252.13; Anal. Calcd. for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10; O, 6.34; Found: C, 76.11; H, 6.31; N, 11.03; O, 6.25.

4.6.5 N-(3-Methoxy-4-hydroxybenzylidene)-2-methylindolin-1-amine (NAISB-05)



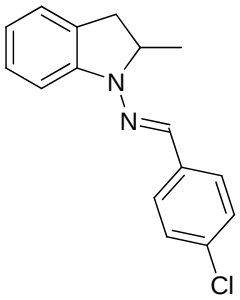
Yield: 92%; M.P.- 128-130 ºC; IR (cm^{-1}): 3032-3016 (C-H stretching vibration of aromatic region), 1246 (C-H in plane bending, aromatic region), 702 (C-C out of plane bending of mono substituted benzene ring), 749 (C-H out of plane bending of mono substituted benzene ring), 2963 (CH_3 asymmetric stretching of R-CH_3), 1481-1442 (CH_3 asymmetric bending of R-CH_3), 2931 (CH_2 asymmetric stretching of cyclopentane), 2909-2848 (C-H symmetric stretching for cyclopentane), 935-896 (Ring stretching for cyclopentane), 1653 (C=N stretching vibration), 1417 (C-H in plane bending for alkene, =CH_2 scissoring), 1295 (C-H in plane bending for alkene, =CH rocking), 1252-1219 (N-N stretching for secondary amine) 3053 (C-H stretching for aryl ethers) 1175-1159 (C-O-C asymmetric stretching), 3614 (O-H free stretching), 1401-1342 (O-H in plane bending vibration) ; MS: *m/z:* 282.14; Anal. Calcd. for $C_{17}H_{18}N_2O_2$: C, 72.32; H, 6.43; N, 9.92; O, 11.33 Found: C, 72.25; H, 6.37; N, 9.85; O, 11.26.

4.6.6 N-(3-nitrobenzylidene)-2-methyl-indolin-1-amine (NAISB-06)



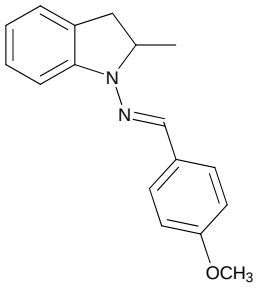
Yield: 90 %; M.P.- 112-114 ºC; IR (cm^{-1}): 3042 (C-H stretching vibration of aromatic region), 953 (C-H in plane bending, aromatic region), 701 (C-C out of plane bending of mono substituted benzene ring), 745 (C-H out of plane bending of mono substituted benzene ring), 2945 (CH_3 asymmetric stretching of R-CH_3), 1465-1451 (CH_3 asymmetric bending of R-CH_3), 2978 (CH_2 asymmetric stretching of cyclopentane), 2890 (C-H symmetric stretching for cyclopentane), 920-880 (Ring stretching for cyclopentane), 1665 (C=N stretching vibration), 1401 (C-H in plane bending for alkene, =CH_2 scissoring), 1304 (C-H in plane bending for alkene, =CH rocking), 1265-1232 (N-N stretching for secondary amine), 1545-1501 (Asymmetric stretching for nitro group), 856 (C-N stretching of Aromatic nitro group); MS: *m/z:* 281.12; Anal. Calcd. for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.37; N, 14.94; O, 11.37; Found: C, 68.22; H, 5.30; N, 14.89; O, 11.29.

4.6.7 N-(4-chlorobenzylidene)-2-methylindolin-1-amine (NAISB-07)



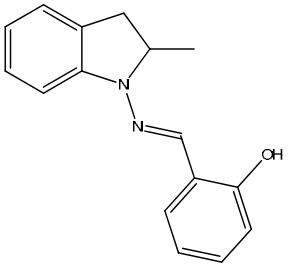
Yield: 95%; M.P.- 130-132 ºC; IR (cm^{-1}): 3039 (C-H stretching vibration of aromatic region), 952 (C-H in plane bending, aromatic region), 703 (C-C out of plane bending of mono substituted benzene ring), 745 (C-H out of plane bending of mono substituted benzene ring), 2941 (CH_3 asymmetric stretching of R-CH_3), 1469-1450 (CH_3 asymmetric bending of R-CH_3), 2977 (CH_2 asymmetric stretching of cyclopentane), 2887 (C-H symmetric stretching for cyclopentane), 924-888 (Ring stretching for cyclopentane), 1667 (C=N stretching vibration), 1402 (C-H in plane bending for alkene, =CH_2 scissoring), 1303 (C-H in plane bending for alkene, =CH rocking), 1264-1234 (N-N stretching for secondary amine), 730 (C-Cl stretching for aromatic chloro compound); MS: *m/z:* 270.09; Anal. Calcd. for $C_{16}H_{15}ClN_2$: C, 70.98; H, 5.58; Cl, 13.09; N, 10.35; Found: C, 70.91; H, 5.52; N, 10.29; Cl, 13.03.

4.6.8 N-(4-methoxybenzylidene)-2-methylindolin-1-amine (NAISB-08)



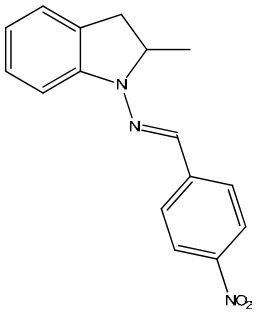
Yield: 98%; M.P.- 132-134 ºC; IR (cm^{-1}): 3030-3003 (C-H stretching vibration of aromatic region), 1249 (C-H in plane bending, aromatic region), 700 (C-C out of plane bending of mono substituted benzene ring), 750 (C-H out of plane bending of mono substituted benzene ring), 2962 (CH_3 asymmetric stretching of R-CH_3), 1483-1444 (CH_3 asymmetric bending of R-CH_3), 2933 (CH_2 asymmetric stretching of cyclopentane), 2906-2841 (C-H symmetric stretching for cyclopentane), 937-891 (Ring stretching for cyclopentane), 1651 (C=N stretching vibration), 1415 (C-H in plane bending for alkene, =CH scissoring), 1298 (C-H in plane bending for alkene, =CH rocking), 1249-1219 (N-N stretching for secondary amine), 3055 (C-H stretching for aryl ethers), 1170-1153 (C-O-C asymmetric stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 1.25-1.26 (d, 3H, H_1), 4.42-4.45 (m, 1H, H_2), 2.65-2.70 (d, 1H, H_3), 3.34-3.40 (q, 1H, H_3), 7.01-7.02 (d, 1H, H_4, *J*=7.34 *Hz*), 6.81-6.84 (d, 2H, H_5 & H_7, *J*=4.8 *Hz*), 6.69-6.73 (t, 1H, H_6), 7.46 (s, 1H, H_8), 7.05-7.09 (q, 2H, H_9 & H_{13}) 7.52-7.55 (d, 2H, H_{10}-H_{12}), 3.75 (s,3H, H_{11});MS: *m/z:* 266.14; Anal. Calcd. for $C_{17}H_{18}N_2O$: C, 76.66; H, 6.81; N, 10.52; O, 6.01, Found: C, 76.00; H, 6.75; N, 10.43; O, 5.94.

4.6.9 N-(2-hydroxybenzylidene)-2-methylindolin-1-amine (NAISB-09)



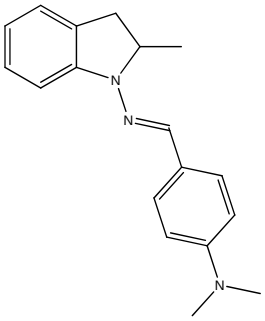
Yield: 90%; M.P.- 122-124 ºC; IR (cm^{-1}): 3037 (C-H stretching vibration of aromatic region), 967 (C-H in plane bending, aromatic region), 710 (C-C out of plane bending of mono substituted benzene ring), 745 (C-H out of plane bending of mono substituted benzene ring), 2934 (CH_3 asymmetric stretching of R-CH_3), 1472-1468 (CH_3 asymmetric bending of R-CH_3), 2978 (CH_2 asymmetric stretching of cyclopentane), 2892 (C-H symmetric stretching for cyclopentane), 934-885 (Ring stretching for cyclopentane), 1669 (C=N stretching vibration), 1408 (C-H in plane bending for alkene, =CH_2 scissoring), 1309 (C-H in plane bending for alkene, =CH rocking), 1272-1249 (N-N stretching for secondary amine) 3612 (O-H free stretching for phenol), 1403-1348 (O-H in plane bending vibration coupled for phenol); MS: *m/z:* 252.13; Anal. Calcd. for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10; O, 6.34; Found: C, 76.11; H, 6.32; N, 11.02; O, 6.25.

4.6.10 N-(4-nitrobenzylidene)-2-methyl-indolin-1-amine (NAISB-10)



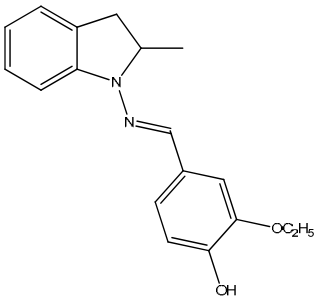
Yield: 92%; M.P.- 120-122 ºC; IR (cm^{-1}): IR (cm^{-1}): 3042 (C-H stretching vibration of aromatic region), 953 (C-H in plane bending, aromatic region), 701 (C-C out of plane bending of mono substituted benzene ring), 749 (C-H out of plane bending of mono substituted benzene ring), 2950 (CH_3 asymmetric stretching of R-CH_3), 1467-1454 (CH_3 asymmetric bending of R-CH_3), 2978 (CH_2 asymmetric stretching of cyclopentane), 2892 (C-H symmetric stretching for cyclopentane), 920-880 (Ring stretching for cyclopentane), 1669 (C=N stretching vibration), 1403 (C-H in plane bending for alkene, =CH_2 scissoring), 1304 (C-H in plane bending for alkene, =CH rocking), 1266-1230 (N-N stretching for secondary amine), 1542-1510 (Asymmetric stretching for nitro group), 853 (C-N stretching of Aromatic nitro group); MS: *m/z:* 281.12; Anal. Calcd. for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.37; N, 14.94; O, 11.37; Found: C, 68.24; H, 5.31; N, 14.88; O, 11.30.

4.6.11 N-(4-(dimethylamino)benzylidene)-2-methylindolin-1-amine (NAISB-11)



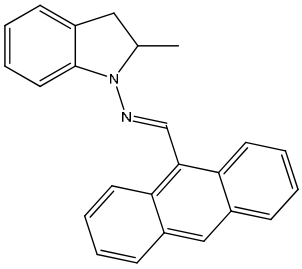
Yield: 97%; M.P.- 104-106 ºC; IR (cm^{-1}): 3040 (C-H stretching vibration of aromatic region), 956 (C-H in plane bending, aromatic region), 704 (C-C out of plane bending of mono substituted benzene ring), 742 (C-H out of plane bending of mono substituted benzene ring), 2948 (CH_3 asymmetric stretching of R-CH_3), 1475-1450 (CH_3 asymmetric bending of R-CH_3), 2983 (CH_2 asymmetric stretching of cyclopentane), 2888 (C-H symmetric stretching for cyclopentane), 926-882 (Ring stretching for cyclopentane), 1665 (C=N stretching vibration), 1403 (C-H in plane bending for alkene, =CH_2 scissoring), 1309 (C-H in plane bending for alkene, =CH rocking), 1272-1234 (N-N stretching for secondary amine), 1343 (C-N stretching for aryl tertiary amine); MS: *m/z:* 279.17; Anal. Calcd. for $C_{18}H_{21}N_3$: C, 77.38; H, 7.58; N, 15.04; Found: C, 77.29; H, 7.50; N, 14.95.

4.6.12 N-(3-Ethoxy-4-hydroxybenzylidene)-2-methylindolin-1-amine (NAISB-12)



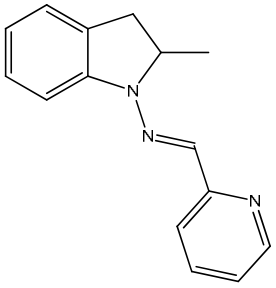
Yield: 95%; M.P.- 108-110 ºC; IR (cm^{-1}): 3032-3016 (C-H stretching vibration of aromatic region), 1246 (C-H in plane bending, aromatic region), 702 (C-C out of plane bending of mono substituted benzene ring), 749 (C-H out of plane bending of mono substituted benzene ring), 2963 (CH_3 asymmetric stretching of R-CH_3), 1481-1442 (CH_3 asymmetric bending of R-CH_3), 2931 (CH_2 asymmetric stretching of cyclopentane), 2909-2848 (C-H symmetric stretching for cyclopentane), 935-896 (Ring stretching for cyclopentane), 1653 (C=N stretching vibration), 1417 (C-H in plane bending for alkene, =CH_2 scissoring), 1295 (C-H in plane bending for alkene, =CH rocking), 1252-1219 (N-N stretching for secondary amine) 3055 (C-H stretching for aryl ethers) 1170-1160 (C-O-C asymmetric stretching), 3612 (O-H free stretching), 1404-1347 (O-H in plane bending vibration); MS: *m/z:* 296.15; Anal. Calcd. for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45; O, 10.80 Found: C, 72.87; H, 6.72; N, 9.37; O, 10.73.

4.6.13 N-(anthracen-9-ylmethylene)-2-methylindolin-1-amine (NAISB-13)



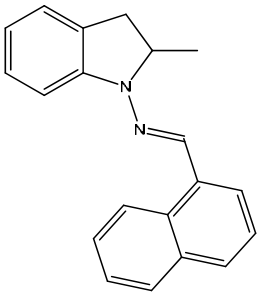
Yield: 92%; M.P.- 98-100 ºC; IR (cm^{-1}): 3040 (C-H stretching vibration of aromatic region), 950 (C-H in plane bending, aromatic region), 700 (C-C out of plane bending of mono substituted benzene ring), 740 (C-H out of plane bending of mono substituted benzene ring), 2940 (CH_3 asymmetric stretching of R-CH_3), 1470-1450 (CH_3 asymmetric bending of R-CH_3), 2980 (CH_2 asymmetric stretching of cyclopentane), 2880 (C-H symmetric stretching for cyclopentane), 920-885 (Ring stretching for cyclopentane), 1660 (C=N stretching vibration), 1400 (C-H in plane bending for alkene, =CH_2 scissoring), 1307 (C-H in plane bending for alkene, =CH rocking), 1269-1236 (N-N stretching for secondary amine), 1635 (a medium intensity band confirming anthracene), 866 (a strong band again confirming anthracene); MS: *m/z:* 336.16; Anal. Calcd. for $C_{24}H_{20}N_2$: C, 85.68; H, 5.99; N, 8.33; Found: C, 85.60; H, 5.90; N, 8.25;.

4.6.14 2-Methyl-N-(pyridin-2-ylmethylene)indolin-1-amine (NAISB-14)



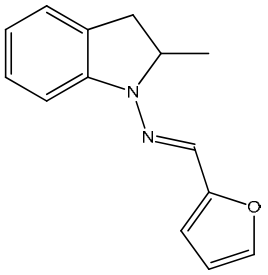
Yield: 90%; M.P.- 106-108 ºC; IR (cm^{-1}): 3046 (C-H stretching vibration of aromatic region), 957 (C-H in plane bending, aromatic region), 707 (C-C out of plane bending of mono substituted benzene ring), 745 (C-H out of plane bending of mono substituted benzene ring), 2946 (CH_3 asymmetric stretching of R-CH_3), 1472-1452 (CH_3 asymmetric bending of R-CH_3), 2986 (CH_2 asymmetric stretching of cyclopentane), 2889 (C-H symmetric stretching for cyclopentane), 921-882 (Ring stretching for cyclopentane), 1659 (C=N stretching vibration), 1402 (C-H in plane bending for alkene, =CH_2 scissoring), 1306 (C-H in plane bending for alkene, =CH rocking), 1264-1231 (N-N stretching for secondary amine); MS: *m/z:* 237.13; Anal. Calcd. for $C_{15}H_{15}N_3$: C, 75.92; H, 6.37; N, 17.71; Found: C, 75.86; H, 6.30; N, 17.66.

4.6.15 2-Methyl-N-(naphthalen-1-ylmethylene)indolin-1-amine (NAISB-15)



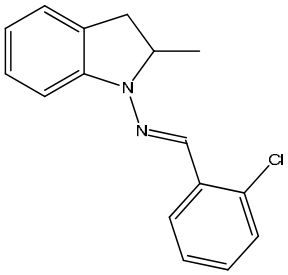
Yield: 95%; M.P.- 116-118 ºC; IR (cm^{-1}): 3048 (C-H stretching vibration of aromatic region), 958 (C-H in plane bending, aromatic region), 710 (C-C out of plane bending of mono substituted benzene ring), 758 (C-H out of plane bending of mono substituted benzene ring), 2953 (CH_3 asymmetric stretching of R-CH_3), 1467-1442 (CH_3 asymmetric bending of R-CH_3), 2990 (CH_2 asymmetric stretching of cyclopentane), 2899 (C-H symmetric stretching for cyclopentane), 912-875 (Ring stretching for cyclopentane), 1672 (C=N stretching vibration), 1403 (C-H in plane bending for alkene, =CH_2 scissoring), 1310 (C-H in plane bending for alkene, =CH rocking), 1269-1236 (N-N stretching for secondary amine), 760 (C-H out of plane bending in naphthalene); MS: *m/z:* 286.15; Anal. Calcd. for $C_{20}H_{18}N_2$: C, 83.88; H, 6.34; N, 9.78; Found: C, 83.80; H, 6.28; N, 9.70.

4.6.16 N-(furan-2-ylmethylene)-2-methylindolin-1-amine (NAISB-16)



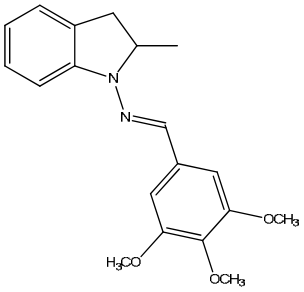
Yield: 95%; M.P.- 134-136 ºC; IR (cm^{-1}): 3049 (C-H stretching vibration of aromatic region), 953 (C-H in plane bending, aromatic region), 703 (C-C out of plane bending of mono substituted benzene ring), 748 (C-H out of plane bending of mono substituted benzene ring), 2943 (CH_3 asymmetric stretching of R-CH_3), 1477-1452 (CH_3 asymmetric bending of R-CH_3), 2984 (CH_2 asymmetric stretching of cyclopentane), 2879 (C-H symmetric stretching for cyclopentane), 920-885 (Ring stretching for cyclopentane), 1665 (C=N stretching vibration), 1412 (C-H in plane bending for alkene, =CH_2 scissoring), 1307 (C-H in plane bending for alkene, =CH rocking), 1275-1235 (N-N stretching for secondary amine), 3130 (C-H stretching in furan), 1569 (ring stretching of furan); MS: *m/z:* 226.11; Anal. Calcd. for $C_{14}H_{14}N_2O$: C, 74.31; H, 6.24; N, 12.38; O, 7.07; Found: C, 74.27; H, 6.18; N, 12.31; O, 7.02.

4.6.17 N-(2-chlorobenzylidene)-2-methylindolin-1-amine (NAISB-17)



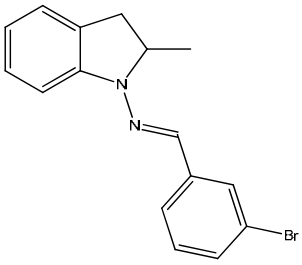
Yield: 90%; M.P.- 126-128 ºC; IR (cm^{-1}): 3045 (C-H stretching vibration of aromatic region), 953 (C-H in plane bending, aromatic region), 709 (C-C out of plane bending of mono substituted benzene ring), 765 (C-H out of plane bending of mono substituted benzene ring), 2941 (CH_3 asymmetric stretching of R-CH_3), 1469-1450 (CH_3 asymmetric bending of R-CH_3), 2977 (CH_2 asymmetric stretching of cyclopentane), 2887 (C-H symmetric stretching for cyclopentane), 924-888 (Ring stretching for cyclopentane), 1667 (C=N stretching vibration), 1402 (C-H in plane bending for alkene, =CH_2 scissoring), 1303 (C-H in plane bending for alkene, =CH rocking), 1262-1235 (N-N stretching for secondary amine), 735 (C-Cl stretching for aromatic chloro compound); MS: *m/z:* 270.09; Anal. Calcd. for $C_{16}H_{15}ClN_2$: C, 70.98; H, 5.58; Cl, 13.09; N, 10.35; Found: C, 70.93; H, 5.51; Cl, 13.02; N, 10.29.

4.6.18 2-Methyl-N-(3,4,5-trimethoxybenzylidene)indolin-1-amine (NAISB-18)



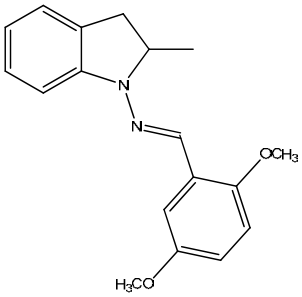
Yield: 96%; M.P.- 110-112 ºC; IR (cm^{-1}): 3033-3003 (C-H stretching vibration of aromatic region), 1253 (C-H in plane bending, aromatic region), 704 (C-C out of plane bending of mono substituted benzene ring), 757 (C-H out of plane bending of mono substituted benzene ring), 2962 (CH_3 asymmetric stretching of R-CH_3), 1483-1444 (CH_3 asymmetric bending of R-CH_3), 2933 (CH_2 asymmetric stretching of cyclopentane), 2906-2841 (C-H symmetric stretching for cyclopentane), 937-891 (Ring stretching for cyclopentane), 1651 (C=N stretching vibration), 1415 (C-H in plane bending for alkene, =CH scissoring), 1293 (C-H in plane bending for alkene, =CH rocking), 1253-1220 (N-N stretching for secondary amine), 3058 (C-H stretching for aryl ethers), 1173-1156 (C-O-C asymmetric stretching); MS: *m/z:* 326.16; Anal. Calcd. for $C_{19}H_{22}N_2O_3$: C, 69.92; H, 6.79; N, 8.58; O, 14.71; Found: C, 69.86; H, 6.73; N, 8.52; O, 14.66.

4.6.19 N-(3-bromobenzylidene)-2-methylindolin-1-amine (NAISB-19)



Yield: 90%; M.P.- 114-116 ºC; IR (cm^{-1}): 3045 (C-H stretching vibration of aromatic region), 958 (C-H in plane bending, aromatic region), 700 (C-C out of plane bending of mono substituted benzene ring), 748 (C-H out of plane bending of mono substituted benzene ring), 2943 (CH_3 asymmetric stretching of R-CH_3), 1477-1452 (CH_3 asymmetric bending of R-CH_3), 2980 (CH_2 asymmetric stretching of cyclopentane), 2889 (C-H symmetric stretching for cyclopentane), 922-885 (Ring stretching for cyclopentane), 1662 (C=N stretching vibration), 1400 (C-H in plane bending for alkene, =CH_2 scissoring), 1307 (C-H in plane bending for alkene, =CH rocking), 1269-1236 (N-N stretching for secondary amine), 587 (C-Br stretching for aromatic bromo compounds); MS: *m/z:* 314.04; Anal. Calcd. for $C_{16}H_{15}BrN_2$: C, 60.97; H, 4.80; Br, 25.35; N, 8.89; Found: C, 60.93; H, 4.75; Br, 25.30; N, 8.83.

4.6.20 2-Methyl-N-(2,5-dimethoxybenzylidene)indolin-1-amine (NAISB-20)



Yield: 95%; M.P.- 124-126 ºC; IR (cm^{-1}): 3036-3010 (C-H stretching vibration of aromatic region), 1257 (C-H in plane bending, aromatic region), 707 (C-C out of plane bending of mono substituted benzene ring), 752 (C-H out of plane bending of mono substituted benzene ring), 2975 (CH_3 asymmetric stretching of R-CH_3), 1487-1445 (CH_3 asymmetric bending of R-CH_3), 2934 (CH_2 asymmetric stretching of cyclopentane), 2903-2845 (C-H symmetric stretching for cyclopentane), 937-890 (Ring stretching for cyclopentane), 1656 (C=N stretching vibration), 1413 (C-H in plane bending for alkene, =CH scissoring), 1290 (C-H in plane bending for alkene, =CH rocking), 1255-1227 (N-N stretching for secondary amine), 3057 (C-H stretching for aryl ethers), 1175-1150 (C-O-C asymmetric stretching); MS: *m/z:* 296.15; Anal. Calcd. for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45; O, 10.80; Found: C, 72.88; H, 6.73; N, 9.39; O, 10.74.

4.7 SPECTRAL DISCUSSION

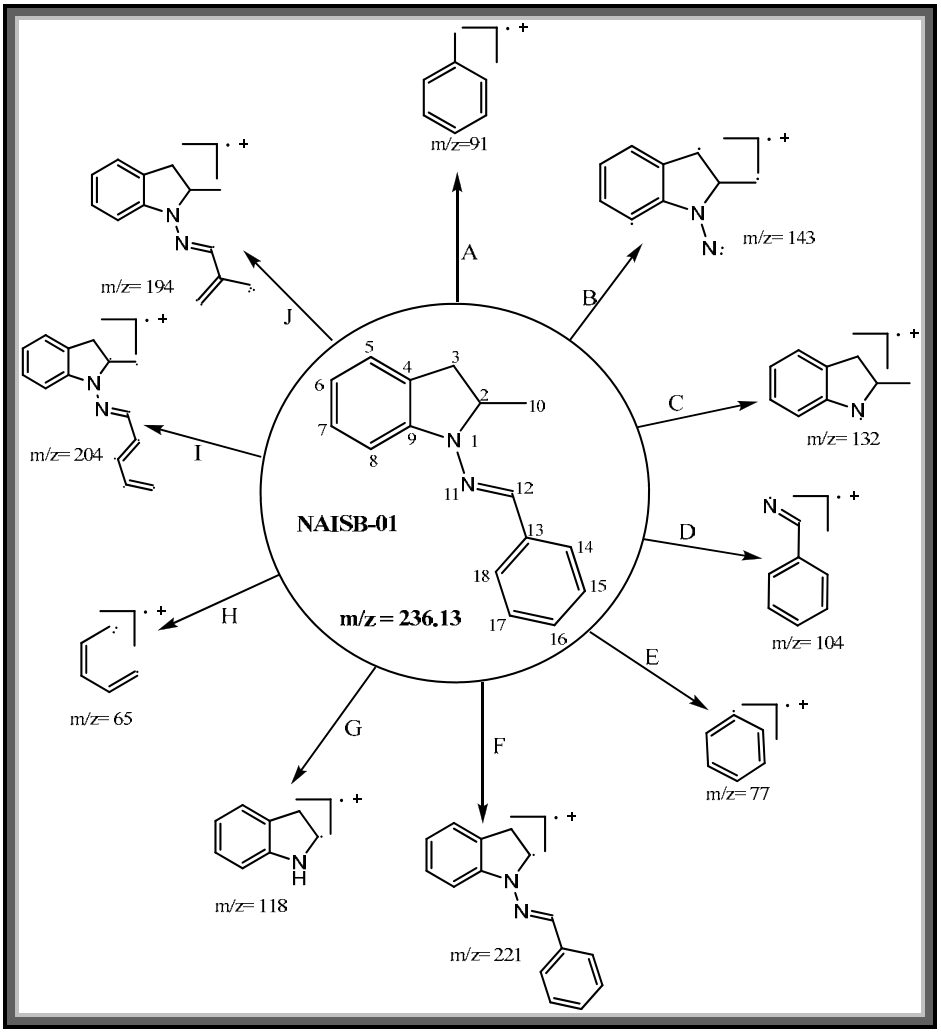
4.7.1 *IR SPECTRAL STUDY*

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. The characteristic bands of Hydroxyl groups were obtained for stretching at 3400-3650 cm^{-1}, and those for bending were obtained at 1050-1250 cm^{-1}. The characteristic bands for aromatic region were obtained for C-H stretching between 3095-3015 cm^{-1}, the in plane bending vibrations of a phenyl ring were observed between 1248-950 cm^{-1}. The general aromatic C-C stretching bands were observed at 1460-1408 cm^{-1} while the out of plane bending frequency of C-H was seen between 952-696 cm^{-1}. The characteristic bands for halogen groups like chlorine and bromine were found at 740-700 cm^{-1} & 600-500 cm^{-1}. Also characteristic stretching frequencies of 1,3-Disubstituted and 1,4-Disubstituted phenyl ring were found at 671 cm^{-1} and 823 cm^{-1} respectively, the C-H symmetric stretching for a cyclo pentane ring was observed between 2970-2860 cm^{-1}, the ring stretching for the cyclopentane ring was observed between 935-885 cm^{-1}, while the C=N stretching vibrations were obtained between 1600-1680 cm^{-1} suggesting the correct formation of the desired products (NAISB-01 to NAISB-20).

4.7.2 *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. The probable Mass fragmentation pattern for the representative compound of each series is discussed hereinafter.

4.7.2.1 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF NAISB-01*

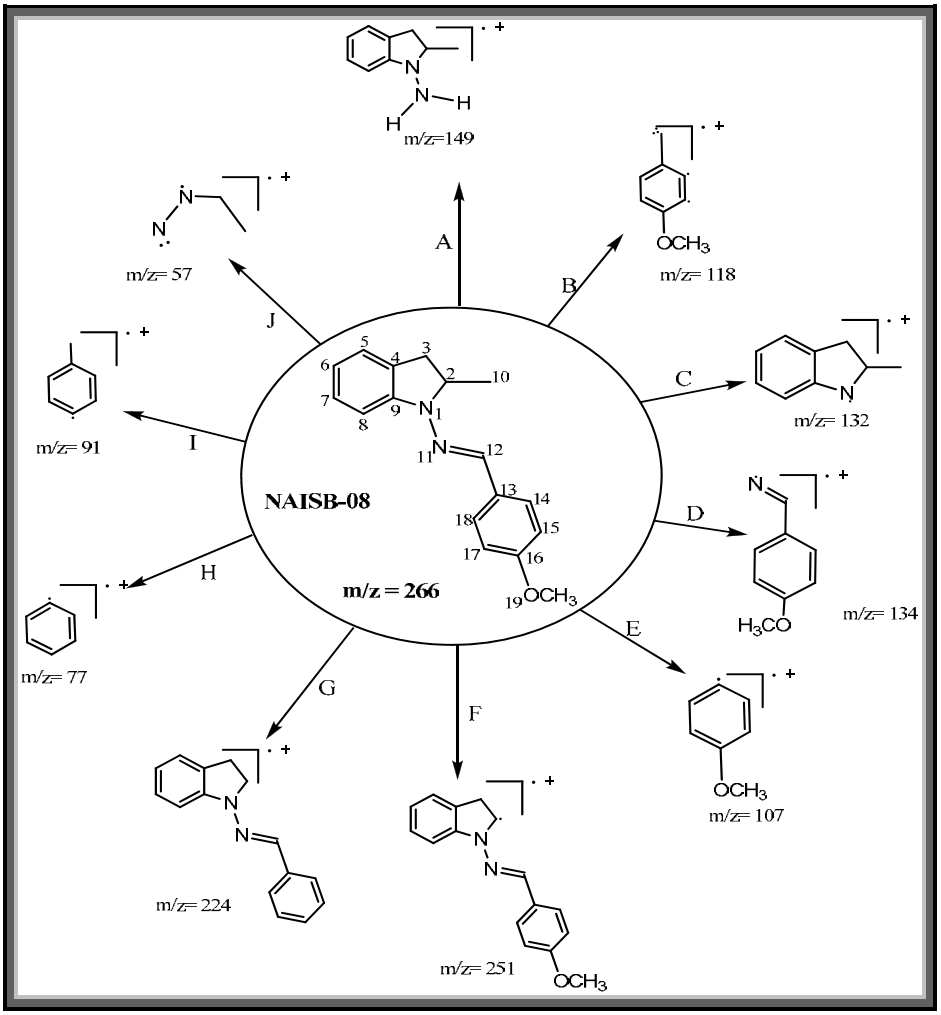


N-benzylidene-2-methylindolin-1-amine (NAISB-01)

1. The target compound showed the characteristic molecular ion peak 236 *m/z*.
2. The bond cleavage between N_{11}-C_{12} generated a molecular ion which corresponds to a characteristic peak at 91 *m/z* **(A).**
3. A bond cleavage between N_{11}-C_{12} generated another molecular ion which corresponds to a characteristic peak at 143 *m/z* **(B)**.
4. Bond cleavages between N_1-N_{11} generated a molecular ion which corresponds to a characteristic peak at 132 *m/z* **(C)**.
5. Bond cleavages between N_1-N_{11} generated another molecular ion which corresponds to a characteristic peak at 104 *m/z* **(D)**.

6. Bond cleavages between C_{12}-C_{13} generated a molecular ion which corresponds to a characteristic peak at 77 *m/z* **(E)**.
7. Bond cleavages between C_2-C_{10} generated a molecular ion which corresponds to a characteristic peak at 221 *m/z* **(F)**.
8. Bond cleavages between C_2-C_{10}, and N_1-N_{11} generated a molecular ion which corresponds to a characteristic peak at 118 *m/z* **(G)**.
9. Bond cleavages between C_4-C_9, C_4-C_5 and N_1-C_9 generated a molecular ion which corresponds to a characteristic peak at 65 *m/z* **(H)**.
10. Bond cleavages between C_{13}-C_{14}, C_{15}-C_{16} generated a molecular ion which corresponds to a characteristic peak at 204 *m/z* **(I)**.
11. Bond cleavages between C_{14}-C_{15}, C_{17}-C_{18} generated a molecular ion which corresponds to a characteristic peak at 194 *m/z* **(J)**.

4.7.2.2 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF NAISB-08*



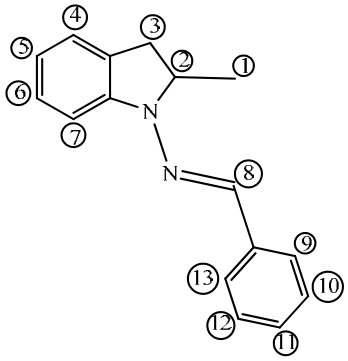
N-(4-methoxybenzylidene)-2-methylindolin-1-amine (NAISB-08)

1. The target compound shows the desired characteristic molecular ion peak of 266 *m/z*.
2. The bond cleavage between N_{11}-C_{12} generated a molecular ion which corresponds to a characteristic peak at 149 *m/z* **(A).**
3. A bond cleavage between N_{11}-C_{12} generated another molecular ion which corresponds to a characteristic peak at 118 *m/z* **(B)**.
4. Bond cleavages between N_1-N_{11} generated a molecular ion which corresponds to a characteristic peak at 132 *m/z* **(C)**.
5. Bond cleavages between N_1-N_{11} generated another molecular ion which corresponds to a characteristic peak at 134 *m/z* **(D)**.
6. Bond cleavages between C_{12}-C_{13} generated a molecular ion which corresponds to a characteristic peak at 107 *m/z* **(E)**.
7. Bond cleavages between C_2-C_{10} generated a molecular ion which corresponds to a characteristic peak at 251 *m/z* **(F)**.
8. Bond cleavages between C_2-C_{10}, and C_{16}-O_{19} generated a molecular ion which corresponds to a characteristic peak at 224 *m/z* **(G)**.
9. Bond cleavages between C_{12}-C_{13}, and C_{16}-O_{19} generated a molecular ion which corresponds to a characteristic peak at 77 *m/z* **(H)**.
10. Bond cleavages between N_{11}-C_{12}, C_{16}-O_{19} generated a molecular ion which corresponds to a characteristic peak at 91 *m/z* **(I)**.
11. Bond cleavages between C_2-C_3, N_1-C_9 and N_{11}-C_{12} generated a molecular ion which corresponds to a characteristic peak at 57 *m/z* **(J)**.

4.7.3 *^{1}H-NMR SPECTRAL STUDY*

^{1}H-NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in DMSO solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from H-NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. The spectral interpretation can be discussed as under.

4.7.3.1 N-benzylidene-2-methylindolin-1-amine (NAISB-01)



1. The proton no. 1 i.e. the methyl protons are observed as a strong singlet in the NMR spectrum at 1.40 δ ppm. The singlet is a strong broad signal between 1.38 δ ppm to 1.40 δ ppm but the splitting in the signal is actually not observed. This may happen due to one hydrogen that is present on the next carbon.
2. The proton no. 2 i.e. the methine proton is surrounded by 5 protons which are bonded to the carbon next to it. A broad multiplet is observed for a single proton between 4.56 δ ppm to 4.61 δ ppm. This broad multiplet is assigned to this proton as it would definitely give a broad multiplet due the presence of 5 protons in its vicinity.
3. Now the methylene protons i.e. proton no. 3 has 2 protons attached to the same carbon atom which means they are geminal protons but their chemical environment would be different as compared to each other. It is very clearly observed in the NMR spectrum as we observe 2 different signals for one proton each. A Doublet is observed for one and a multiplet for the other as it seems. When the expanded spectra is studied for the same, the doublet observed between 2.78 δ ppm and 2.83 δ ppm is further more split into a doublet, this can be explained by the fact that the doublet is observed due to the methine proton which is on the carbon next to it and further doublet is due to the geminal coupling. The same happens to the other proton but it goes a bit downfield as it is nearer to the phenyl nucleus and hence the signal is not as sharp as its geminal counterpart. The signal for other proton is observed between 3.48 δ ppm and 3.54 δ ppm. Thus, 2 signals for the 2 geminal protons are observed in the NMR spectrums which are clearly seen in the expanded spectra.

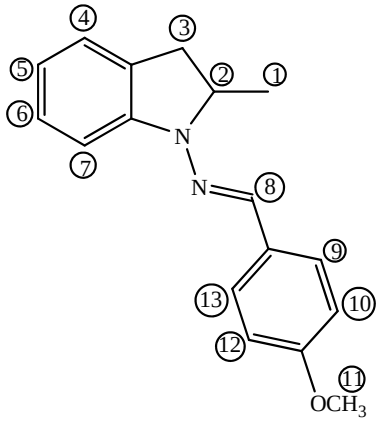
4. Now the proton no. 4 on the phenyl ring is very clearly observed as a doublet between 7.16 δ ppm and 7.14 δ ppm. The calculation of the J value comes out to be exactly 8Hz which furthermore justifies the structural elucidation pointing it out that this proton is ortho coupled to another proton in its vicinity. This is clearly seen in the structure where proton 4 to ortho to proton no. 5.
5. Studying the expanded spectra for the aromatic region it is observed that a strong doublet is observed between 7.24 δ ppm and 7.23 δ ppm for 2 protons. This doublet is assigned to proton nos. 5 and 7. The J value was calculated to be 4 Hz which is in accordance with the theoretical value as both these protons are meta coupled to each other. The J value is on a bit higher side than for normal meta coupled proton because both these protons, proton no. 5 and proton no. 7 are ortho coupled to their adjacent protons as well.
6. Again, on observing the same expanded spectra of this compound a multiplet is observed for a single proton between 6.84 δ ppm to 6.88 δ ppm, where the signal has split into 7 lines. Without any doubt this signal assigned to proton no. 6 which is ortho coupled with 2 protons; proton no. 5 and 7 and is also meta coupled to proton no.4.
7. The proton no. 8 is not coupled to any proton hence we should observe a strong singlet. This strong singlet is observed at 7.57 δ ppm in the NMR spectrum and is clearly seen in the expanded spectra. There are two reasons for this signal to be at such a downfield region. One, its carbon is directly bonded by a double bond to an N-N-C system i.e. is directly bonded to an electronegative nitrogen atom. The other reason is that this same carbon is also bonded to the phenyl ring which acts as an electron sink. Due to these two reasons the proton no. 8 becomes highly deshielded and is observed at such a down field region as 7.57 δ ppm.
8. Proton nos. 9 and 13 has identical chemical environments. On observing the expanded spectra we observe a clear doublet between 7.73 δ ppm and 7.71 δ ppm. The J value was calculated to be exactly 8 Hz which suggest that it is ortho coupled to other proton which is evident. Thus, this doublet at 7.7 δ ppm is assigned for proton nos. 9 and 13.
9. Same is the case with the proton no. 10 and 12, they have identical chemical environment. The only difference is that they have two protons in their

vicinity. Hence a triplet for two protons should be observed. This triplet for two protons is very clearly observed between 7.39 δ ppm to 7.42 δ ppm in the expanded spectra which is assigned to proton no. 10 and 12.

10. The Proton no. 11 has two protons in its vicinity hence a triplet for a single proton should be found which is again very clearly seen in the expanded spectra between 7.28 δ ppm to 7.31 δ ppm. Thus, this triplet for single proton is assigned to proton no. 11.

Thus, by observing and assigning the peaks in the ^{1}H-NMR spectrum and by the calculation of the J values for each of the above proton it can clearly be suggested that the proposed structure for compound no. NAISB-01 has been confirmed.

4.7.3.2 N-(4-methoxybenzylidene)-2-methylindolin-1-amine (NAISB-08)



1. The proton no. 1 i.e. the methyl protons gives a characteristic strong doublet in the NMR spectrum between 1.25 δ ppm. to 1.26 δ ppm. The strong doublet is clearly seen in the expanded spectra of this compound. The occurrence of the doublet is due to the methine proton in its proximity.
2. Just as in the case of NAISB-01 the proton no. 2 i.e. the methine proton is surrounded by 5 protons which are bonded to the carbons next to it. A broad multiplet for a single proton is observed between 4.42 δ ppm to 4.45 δ ppm. This broad multiplet is assigned to this methine proton as it would definitely give a broad multiplet due the presence of 5 protons in its vicinity.
3. Now the methylene protons i.e. proton no. 3 has two protons attached to the same carbon atom which means they are geminal protons but their chemical environment would be different as compared to each other. It is very clearly

observed in the NMR spectrum that there are two different signals for one proton each; a Doublet for one and a multiplet for the other. When the expanded spectra is studied for the same there is a doublet observed between 2.65 δ ppm and 2.70 δ ppm is further more split into a doublet, this can be explained by the fact that the doublet is observed due to the methine proton which is on the carbon next to it and further doublet is due to the geminal coupling. The same happens to the other proton but it goes a bit downfield as it is nearer to the phenyl nucleus and hence the signal is not as sharp as its geminal counterpart. The signal for other proton is observed between 3.34 δ ppm and 3.40 δ ppm. Thus, two signals for two geminal protons are clearly seen in the expanded spectra.

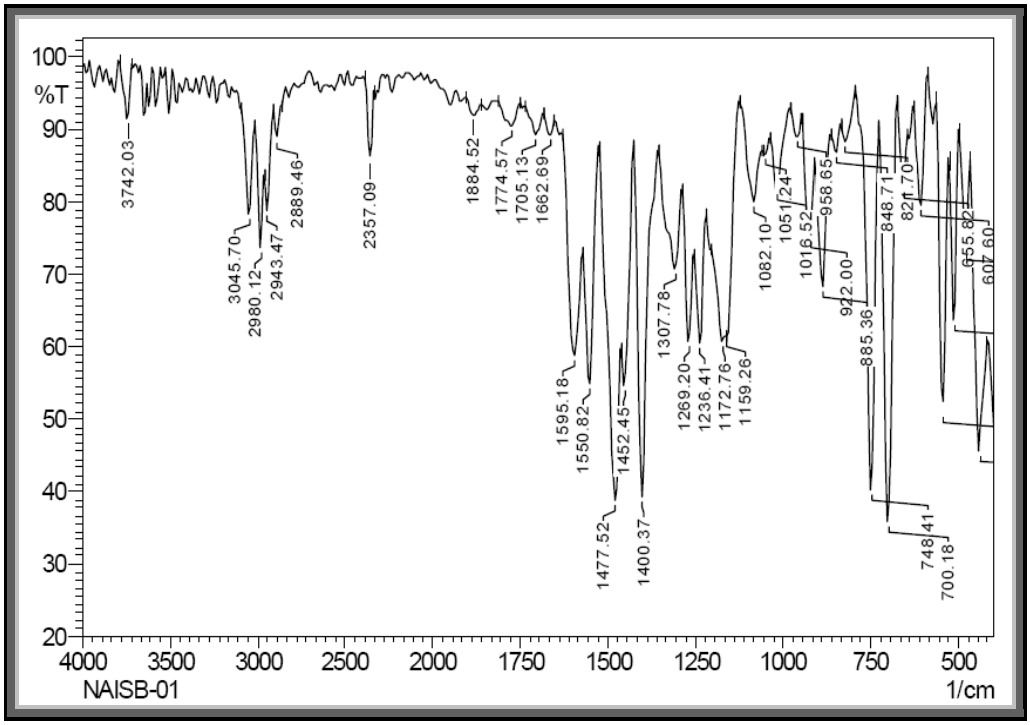
4. Studying the expanded spectra of this compound a formation of a doublet and a triplet are assigned to 3 protons in the spectrum. The doublet is clearly distinguished from the other signal and this signal is found between 7.01 and 7.02 δ ppm. The J value was calculated to be 7.34 Hz which further more supports our structure elucidation. Thus this doublet is assigned to proton no. 4.
5. Studying the expanded spectra for the aromatic region, a strong doublet is observed between 6.81 δ ppm and 6.84 δ ppm for 2 protons. This doublet is assigned to proton nos. 5 and 7. This doublet is almost splitting on its peaks, which can be explained by the fact that both proton 5 and 7 have protons next to them. Proton 5 is surrounded by protons 4 and 6 and proton 7 is having proton 6 in its vicinity. Thus both are ortho coupled to protons 4 and 6 respectively. This fact is further supported by calculating the J value for the over all spectrum range which is calculated to be 14.3 Hz. The J value for each of the signal is 4.8 Hz. This suggests that they are meta coupled as well this is only true for protons 5 and 7. Thus, by this overall strong argument and the study of J values we assign the 2 protons between 6.81 δ ppm and 6.84 δ ppm as protons 5 and 7 respectively.
6. Again, on observing the same expanded spectra of this compound a triplet is observed for a single proton between 6.69 δ ppm to 6.73 δ ppm, where the signal has split on its peak. Without any doubt this signal is assigned to proton no. 6 which is ortho coupled with 2 protons, proton no. 5 and 7 and also meta coupled to proton no.4.

7. The proton no. 8 is not coupled to any proton hence is observed as a strong singlet. This strong singlet is observed at 7.46 δ ppm in the NMR spectrum and is clearly seen in the expanded spectra. There are two reasons for this signal to be at such a downfield region. One, its carbon is directly bonded by a double bond to an N-N-C system i.e. is directly bonded to an electronegative nitrogen atom. The other reason is that this same carbon is also bonded to the phenyl ring which acts as an electron sink. Due to these two reasons the proton no. 8 becomes highly deshielded and is observed at such a down field region as 7.46 δ ppm.
8. Proton nos. 9 and 13 has identical chemical environments. The signal for these two protons is observed in the region between 7.05 δ ppm and 7.09 δ ppm. This signal is along with the doublet of proton no. 4. Together they are seen as 3 protons in the NMR spectrum. The signal should normally be a doublet but it is found as a quartet is due to some coupling with the neighboring protons. Thus, this multiplet found between 7.01 δ ppm and 7.09 δ ppm for 3 protons is assigned to proton nos. 4 and proton nos. 9 and 13 respectively.
9. Same is the case with the proton no. 10 and 12, they have identical chemical environment. The only difference is that there are two protons in their vicinity and also the methoxy group will push their signal a bit down field. Hence a doublet is observed for two protons. This doublet for two protons is very clearly observed between 7.52 δ ppm to 7.55 δ ppm in the expanded spectra which is assigned to proton no. 10 and 12.
10. The Proton no. 11 is the methoxy proton which is very clearly seen as a characteristic singlet in the NMR spectrum at 3.75 δ ppm. As it's carbon is bonded directly to the oxygen atom the protons are deshielded and thus observed at such downfield region.

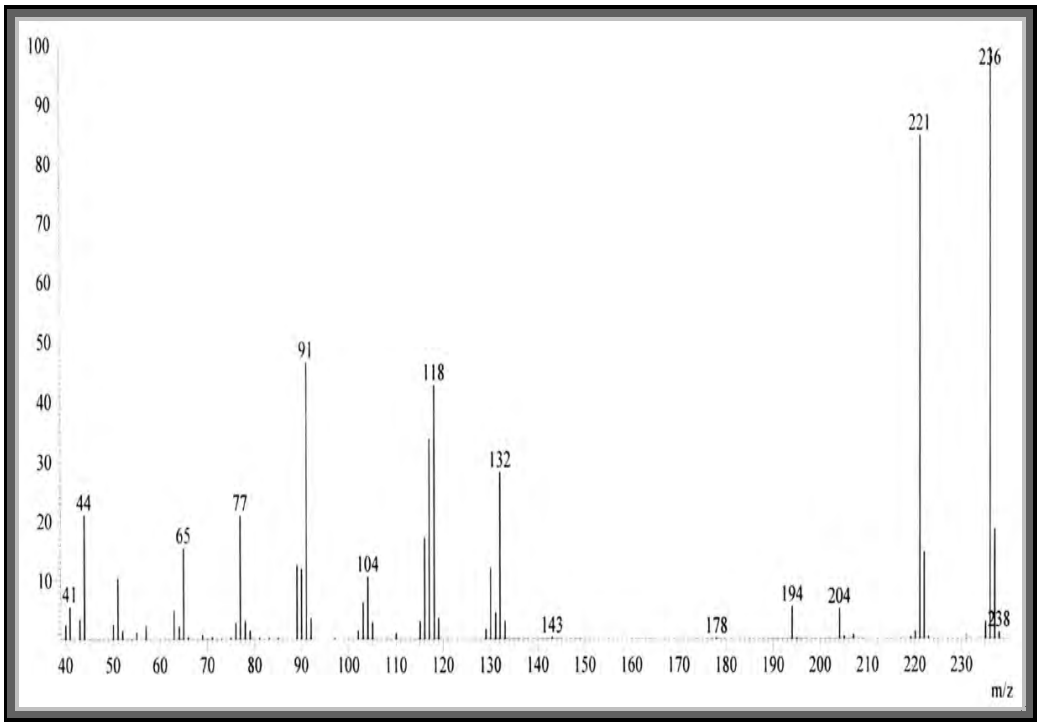
Thus, by observing and assigning the peaks in the ^{1}H-NMR spectrum and by the calculation of the J values for each of the above proton it can clearly be suggested that the proposed structure for compound no. NAISB-08 has been confirmed.

4.8 SPECTRAL REPRESENTATIONS OF THE COMPOUNDS

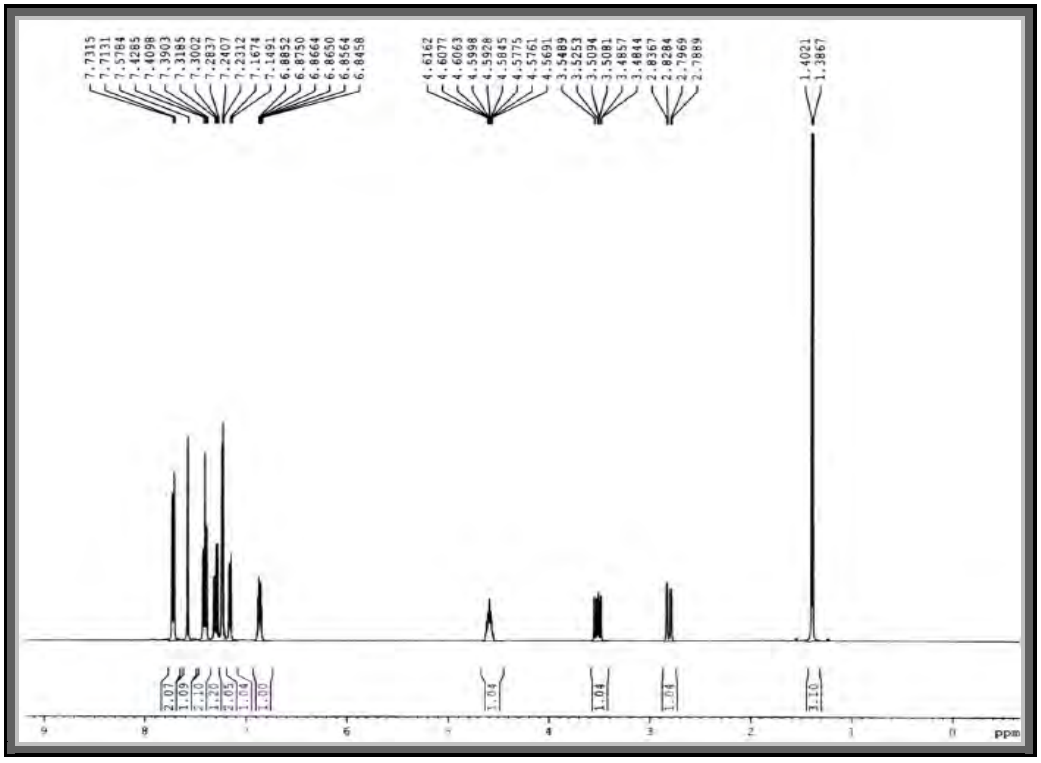
4.8.1 *IR Spectrum of NAISB-01*



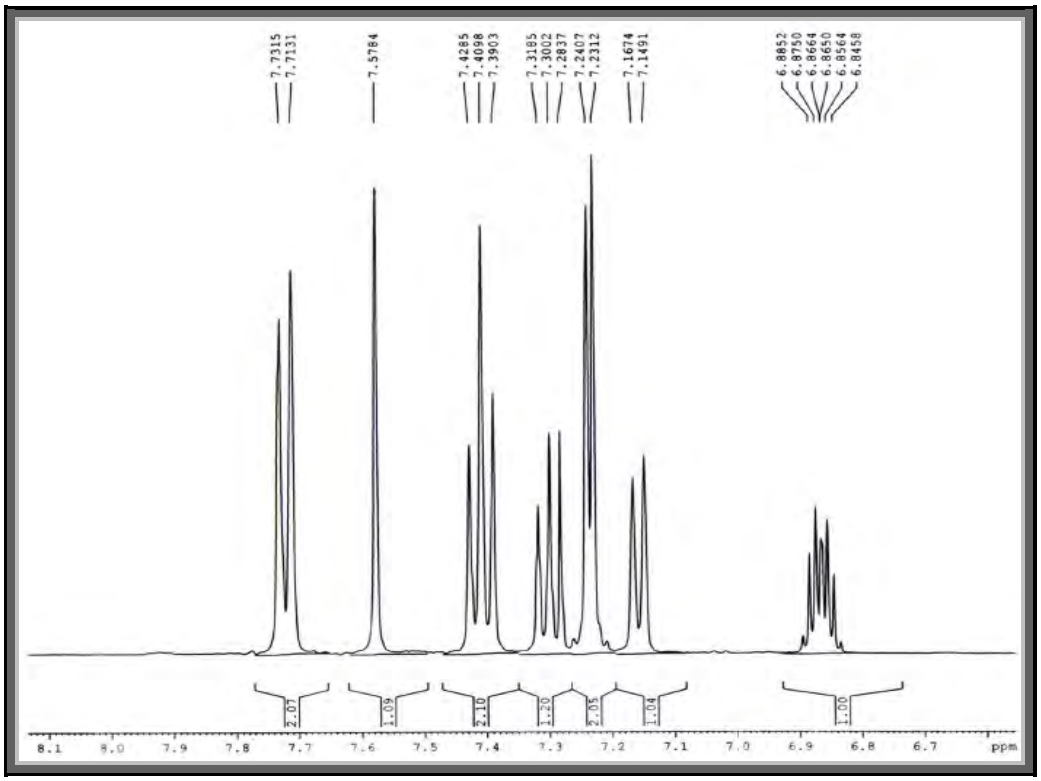
4.8.2 *Mass Spectrum of NAISB-01*



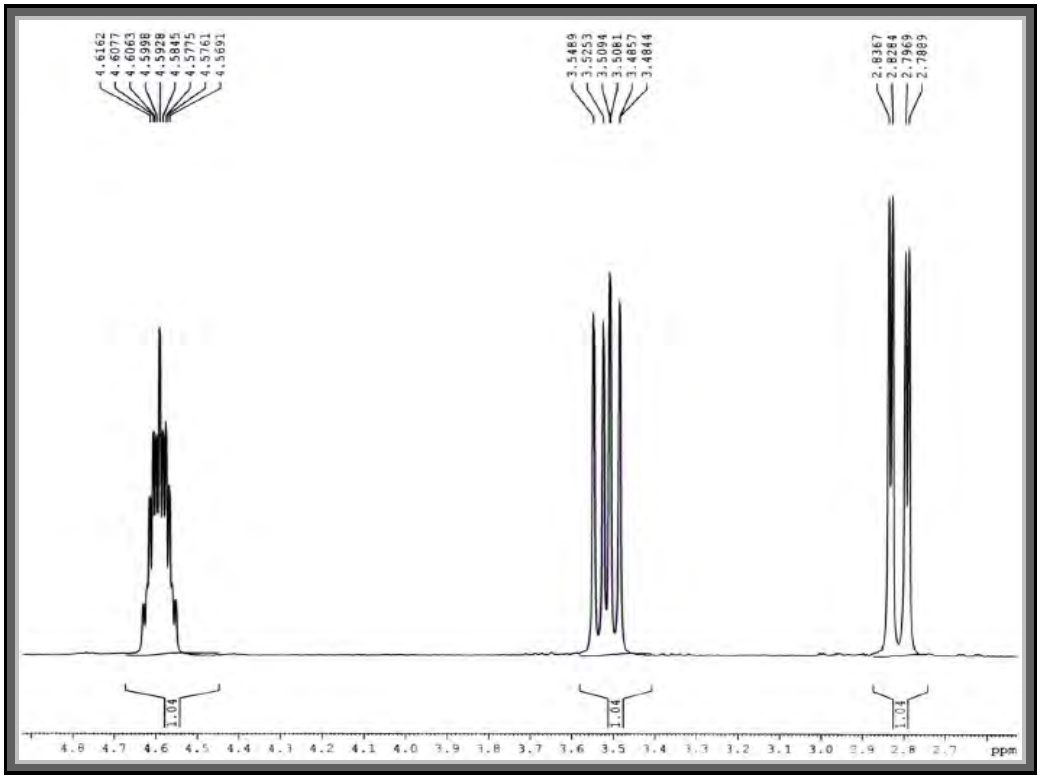
4.8.3 *^{1}H-NMR Spectrum of NAISB-01*



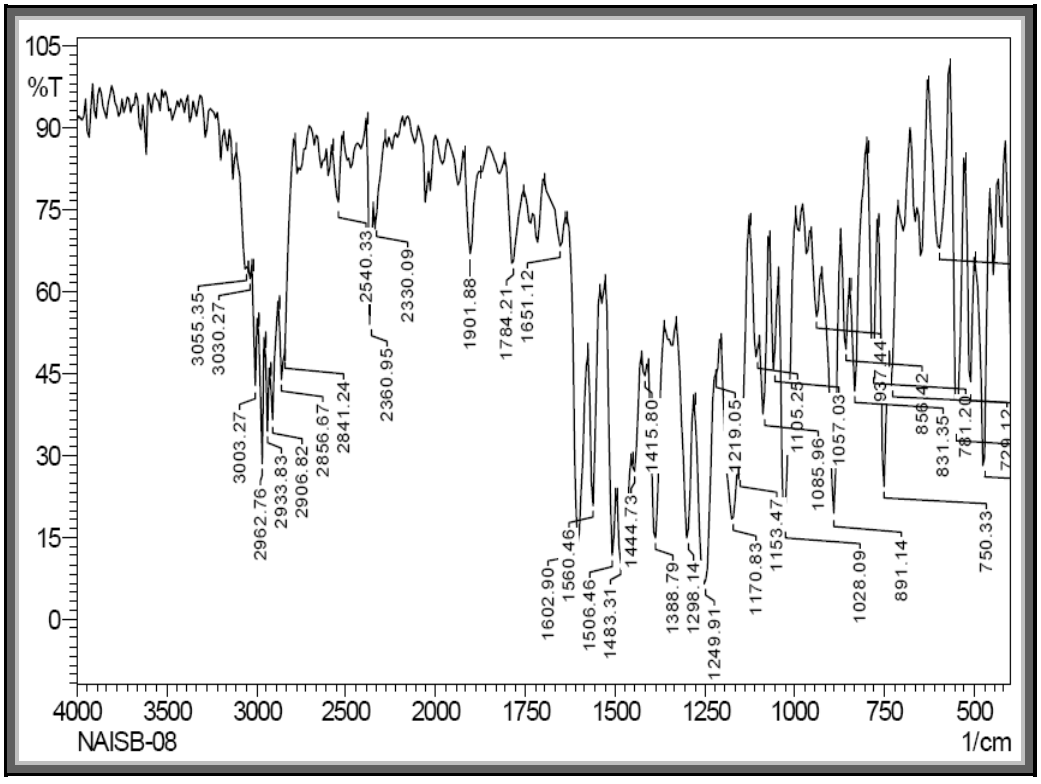
4.8.3.1 *Expanded ^{1}H-NMR spectrum of NAISB-01*



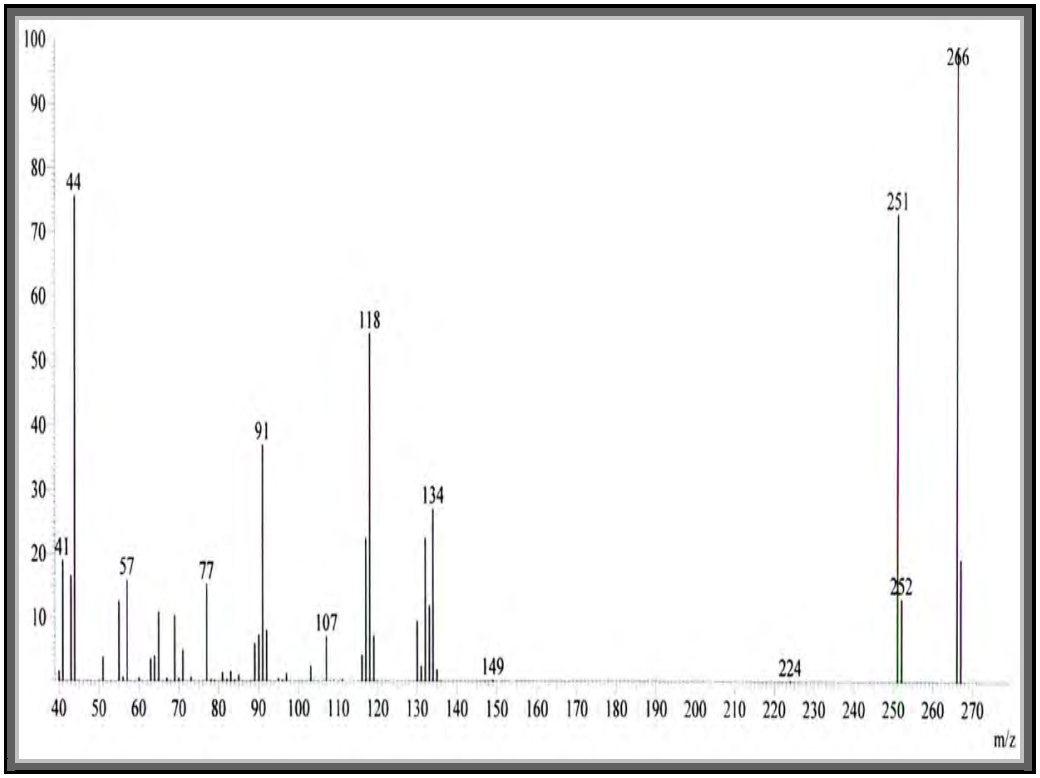
4.8.3.2 *Expanded ^{1}H-NMR spectrum of NAISB-01*



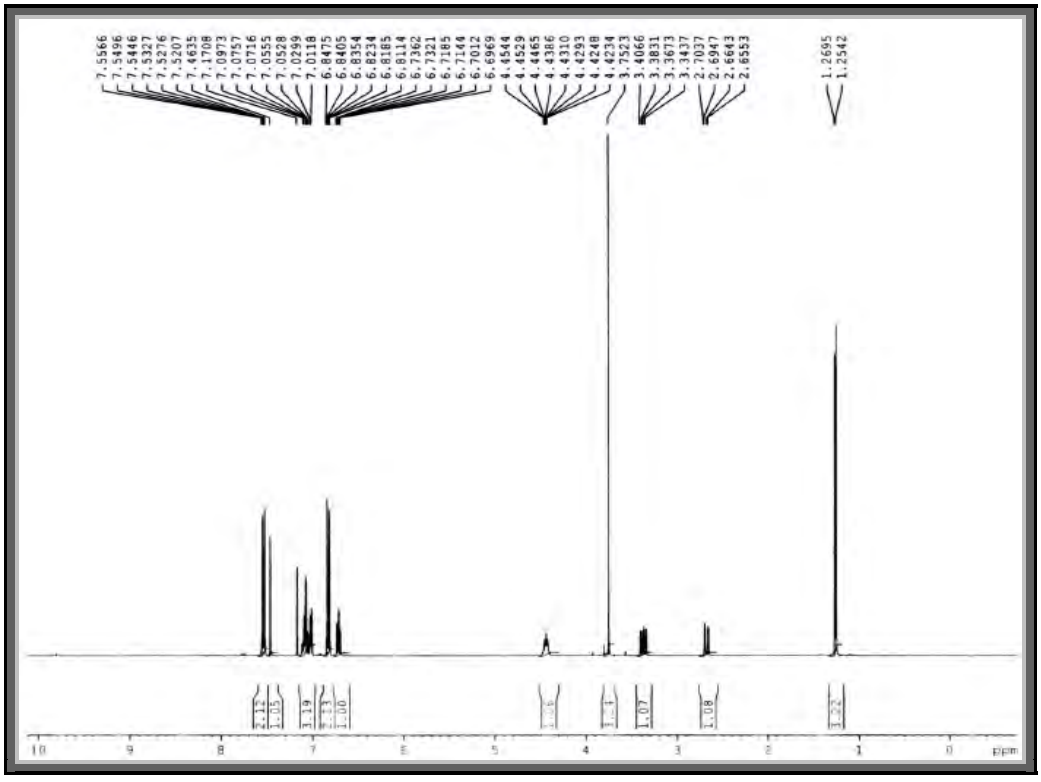
4.8.4 *IR Spectrum of NAISB-08*



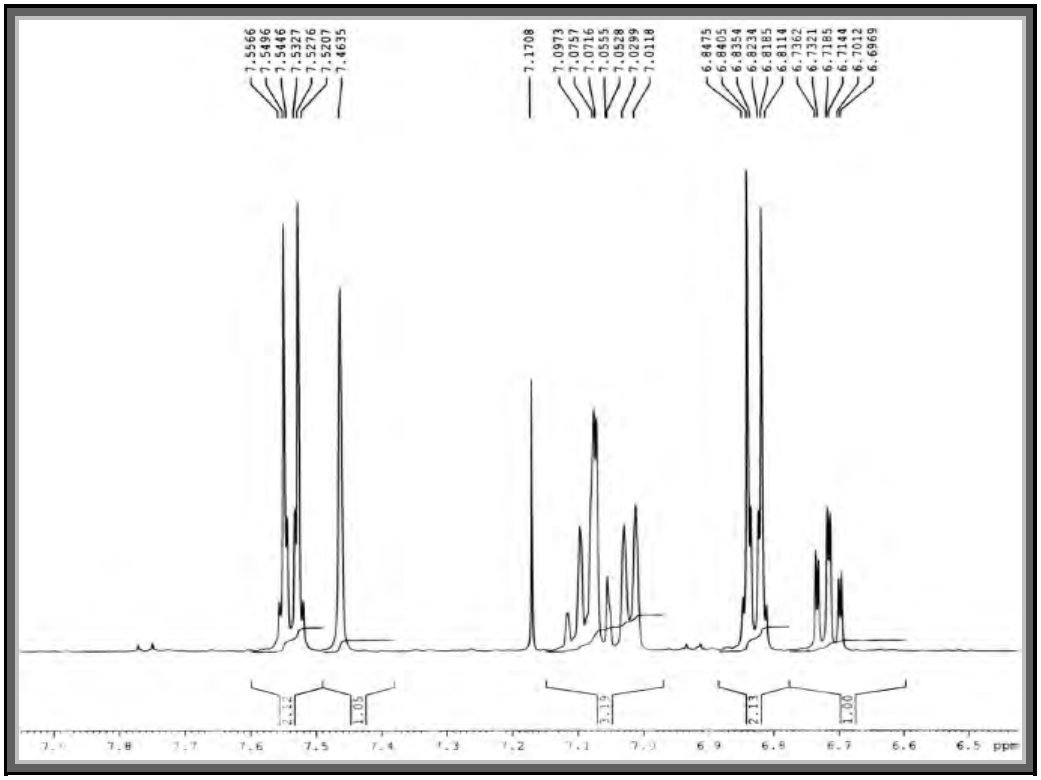
4.8.5 *Mass Spectrum of NAISB-08*



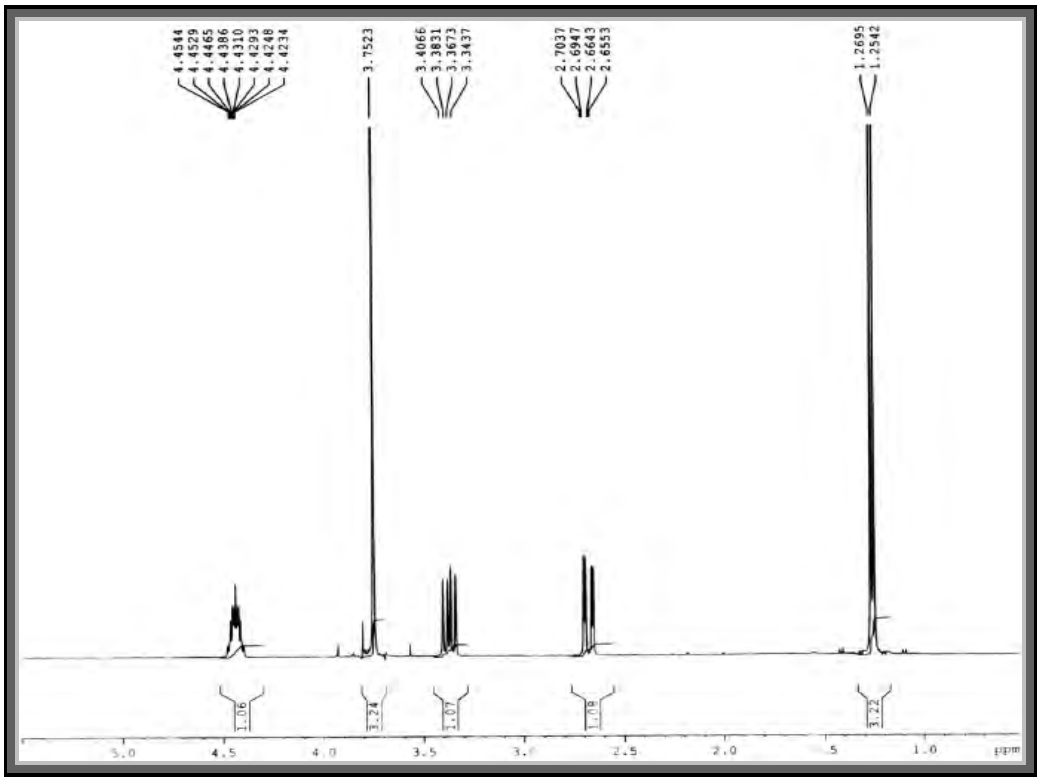
4.8.6 *^{1}H-NMR Spectrum of NAISB-08*



4.8.6.1 *Expanded ^{1}H-NMR Spectrum of NAISB-08*



4.8.6.2 *Expanded ^{1}H-NMR Spectrum of NAISB-08*



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CHAPTER – 4

Section-B

A Rapid microwave assisted synthesis of N-(2-methylindoline-1-yl)(substituted-1,3-diphenyl-1H-pyrazole-4-yl)methanimines

4.9 PYRAZOLES AS A BIOACTIVE CORE STRUCTURE

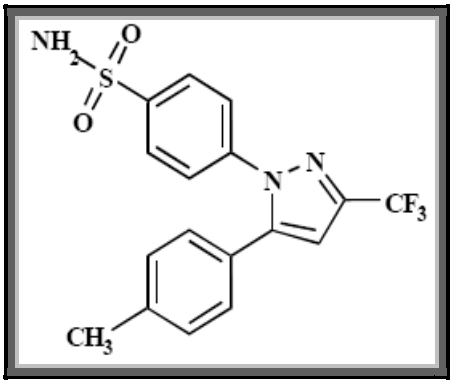
Pyrazole refers both to the class of simple aromatic ring organic compounds of the heterocyclic series characterized by a five-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions and to the unsubstituted parent compound. Being so composed and having pharmacological effects on humans, they are classified as alkaloids, although they are rare in nature.

The synthesis of pyrazoles remains of great interest owing to the wide applications in pharmaceutical and agrochemical industry due to their herbicidal, fungicidal, insecticidal, analgesic, antipyretic and anti-inflammatory properties. [1, 2] Some methods have been developed in recent years, though the most important method is the reaction between hydrazines and β-dicarbonyl compounds. [3] This reaction involves the double condensation of 1, 3-diketones or α, β-unsaturated ketones with hydrazine or its derivatives. [4, 5] However, the appealing generality of this method is somewhat vitiated by the severe reaction conditions or the multistep sequences usually required to access the starting materials. [6] Thus, continuous efforts have been devoted to the development of more general and versatile synthetic methodologies for this class of compounds. [7]

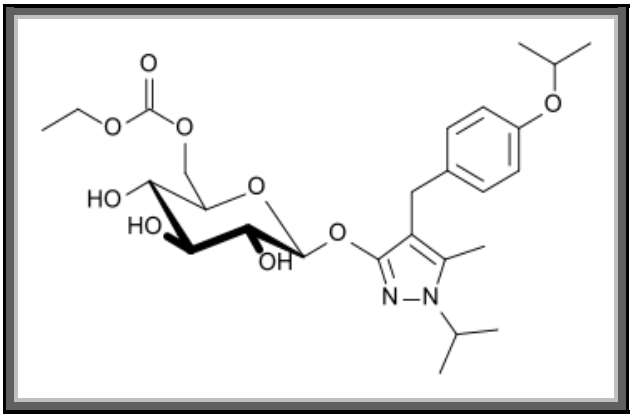
The application of Vilsmeier–Haack (VH) reagent ($POCl_3$ / DMF) for formylation of a variety of both aromatic and heteroaromatic substrates is well documented. [8] Besides this, the reagent has also been extensively used for effecting various chemical transformations from other classes of compounds. Many of these reactions have led to novel and convenient routes for the synthesis of various heterocyclic compounds. [9] A notable example that finds significant application in heterocyclic chemistry is the synthesis of 4-formylpyrazoles from the double formylation of hydrazones with Vilsmeier-Haack (VH) reagent. [10, 11] These observations, coupled with the recent developments on the simple synthesis of pyrazole derivatives, [1, 2] especially 4-functionalized 1, 3-diphenylpyrazoles as antibacterial, [12] anti-inflammatory, [13, 14] antiparasitic, [15] and antidiabetic [16] drugs, prompted chemistry research to undertake the synthesis of pyrazole-4-carboxldehyde derivatives using Vilsmeier-Haack (VH) [17, 18, 19]. The study is particularly aimed at developing a one-pot synthesis of pyrazole-4-carboxaldehyde oximes starting from

acetophenone phenylhydrazones. Given below are some examples of drugs comprising of pyrazole nucleus and their structures.

Celebrex (celecoxib) is chemically designated as 4-[5-(4-methylphenyl)- 3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide and is a diaryl-substituted pyrazole.



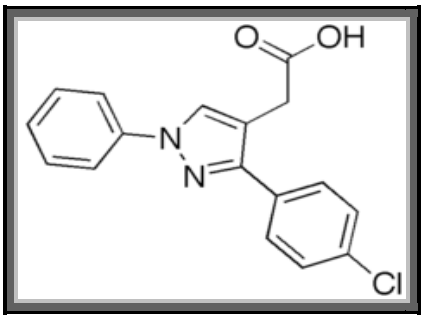
Remogliflozin etabonate (INN/USAN)[a] is a proposed drug for the treatment of type 2 diabetes being investigated by GlaxoSmithKline.[b]



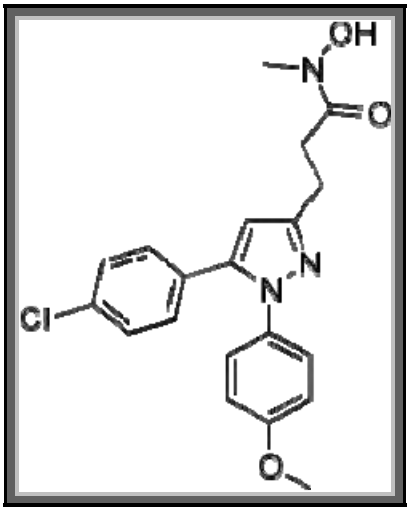
[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl]acetic acid also better known as *Lonazolac* is a non-steroidal anti-inflammatory drug the structure of which is given below.

[a] Statement on a nonproprietory name adopted by the USAN council

[b] Fujimori Y, Katsuno K, Nakashima I, Ishikawa-Takemura Y, Fujikura H, Isaji M (June 2008). "Remogliflozin etabonate, in a Novel Category of Selective Low-Affinity / High-Capacity Sodium Glucose Cotransporter (SGLT2) Inhibitors, Exhibits Antidiabetic Efficacy in Rodent Models". *J. Pharmacol. Exp. Ther.*.doi:10.1124/jpet.108.140210



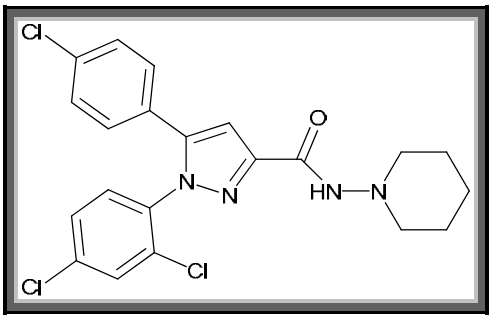
Tepoxalin is a nonsteroidal anti-inflammatory drug approved for veterinary use in the United States and the European Union. It is primarily used to reduce inflammation and relief of pain caused by musculoskeletal disorders such as hip dysplasia and Arthritis, particularly in dogs. It is generally marketed under the brand name Zubrin.



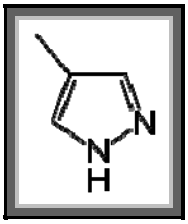
Rimonabant, also known by the systematic name [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride)], is a 1,5-diarylpyrazole CB_1 receptor antagonist (Figure 2).[c] Rimonabant is not only a potent and highly selective ligand of the CB_1 receptor, but it is also orally active and antagonizes most of the effects of cannabinoid agonists, such as THC, both *in vitro* and *in vivo*. Rimonabant has shown clear clinical efficacy for the treatment of obesity.[d] The structure for this compound is given below.

[c] Rinaldi – Carmona, M.; Barth, F.; Héaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J. *et al*. (1994), "SR141716A, a potent and selective antagonist of the brain cannabinoid receptor", *FEBS Letters* **350**: 240 – 244, doi:10.1016/0014-5793(94)00773

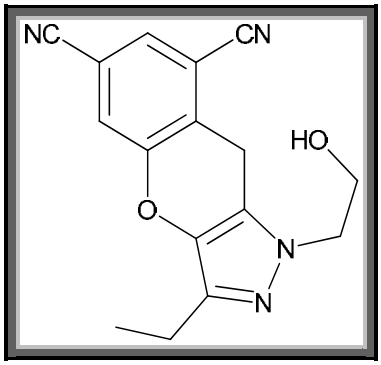
[d] Muccioli, G.G.; Lambert, D.M. (2005), "Current Knowledge on the Antagonists and Inverse Agonists of Cannabinoid Receptors", *Current Medicinal Chemistry* **12** (12): 1361–1394,doi:10.2174/0929867054020891



Fomepizole or 4-methylpyrazole is indicated for use as an antidote in confirmed or suspected methanol[e] or ethylene glycol[f] poisoning. It may be used alone or in combination with hemodialysis. Apart from medical uses, the role of 4-methylpyrazole in coordination chemistryhas been studied.[g]

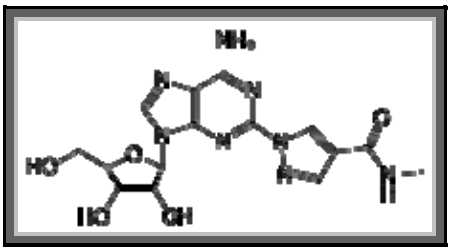


Lersivirine belongs to the pyrazole family and is another next generation NNRTI in clinical trials developed by the pharmaceutical company Pfizer. The resistance profile is similar to that of other next generation NNRTI's. In the end of 2009 lersivirine was in phase IIb.



[e] International Programme on Chemical Safety (IPCS): Methanol
[f] Velez LI, Shepherd G, Lee YC, Keyes DC (September 2007). "Ethylene glycol ingestion treated only with fomepizole". *J Med Toxicol* **3** (3): 125–8.PMID
[g] Vos, Johannes G. (1979). "Pyrazolato and related anions. Part V. Transition metal salts of 4-methylpyrazole". *Transition Metal Chemistry* **4**: 137

Regadenoson is an A_{2A} adenosine receptor agonist that is a coronary vasodilator. It produces maximal hyperemia quickly and maintains it for an optimal duration that is practical for radionuclide myocardial perfusion imaging.[h] It was approved by the United States Food and Drug Administration on April 10, 2008 and it will be marketed under the tradename Lexiscan. It has not yet gained approval in the European Union. Regadenoson has a 2-3 minute biological half-life, as compared with adenosine's 30 second half life. Regadenoson stress tests are not affected by the presence of beta blockers, as regadenoson vasodilates but do not stimulate beta adrenergic receptors.



There are quite a few other drug like molecules which fall in the class of Pyrazoles but have never made it to the market due to failure in the clinical trials. The above cited examples are proof enough to show that the pyrazole nucleus is a very interesting bioactive motif.

Several researchers have explored chemistry as well as biology of the indoline system as well as pyrazole nucleus in the recent years and fairly good reviews and publications are cited in the literature [20-48].

[h] Cerqueira MD (July 2004). "The future of pharmacologic stress: selective A2A adenosine receptor agonists". *Am. J. Cardiol.* **94** (2A): 33D–40D; discussion 40D–42D. doi:10.1016/j.amjcard.2004.04.017

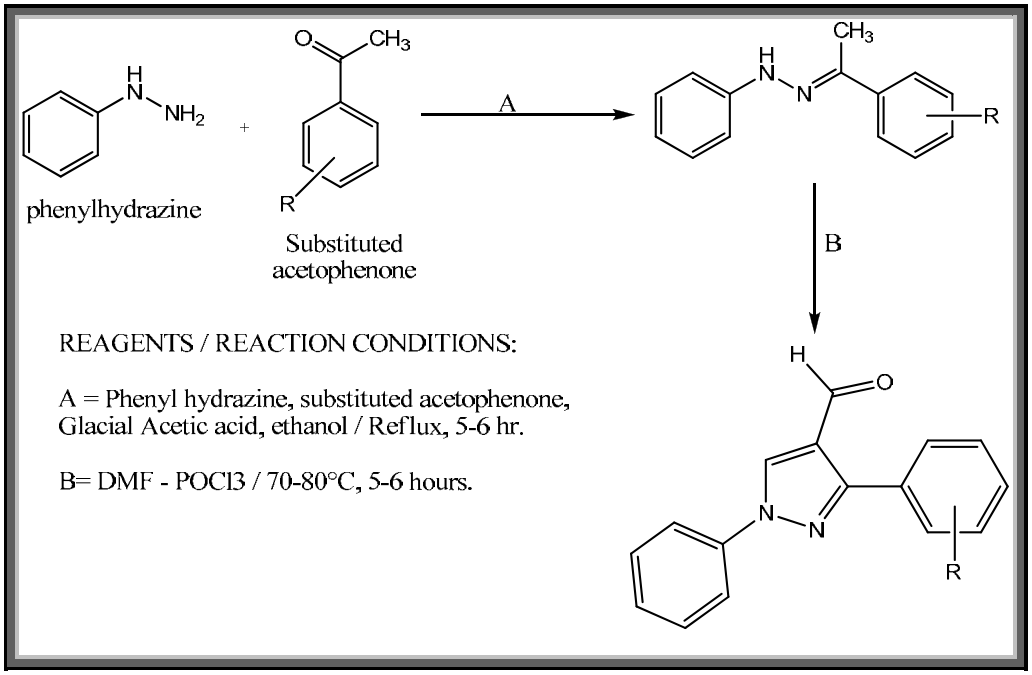
4.10 THE AIM OF CURRENT WORK

The synthesis of pyrazoles remain of great interest owing to the wide applications in pharmaceutical and agrochemical industry due to their herbicidal, fungicidal, insecticidal, analgesic, antipyretic, anti inflammatory, anti bacterial, anti parasitic and anti diabetic properties. Earlier, from this laboratory, some indolinone derivatives were prepared and tested for anti cancer activity on colon cancer cell line (SW 620), which showed good results[a]. In continuation of previous work, we aimed at synthesizing some novel compounds comprising of both these interesting building blocks viz. pyrazolaldehydes as well as 2-Methyl indoline.

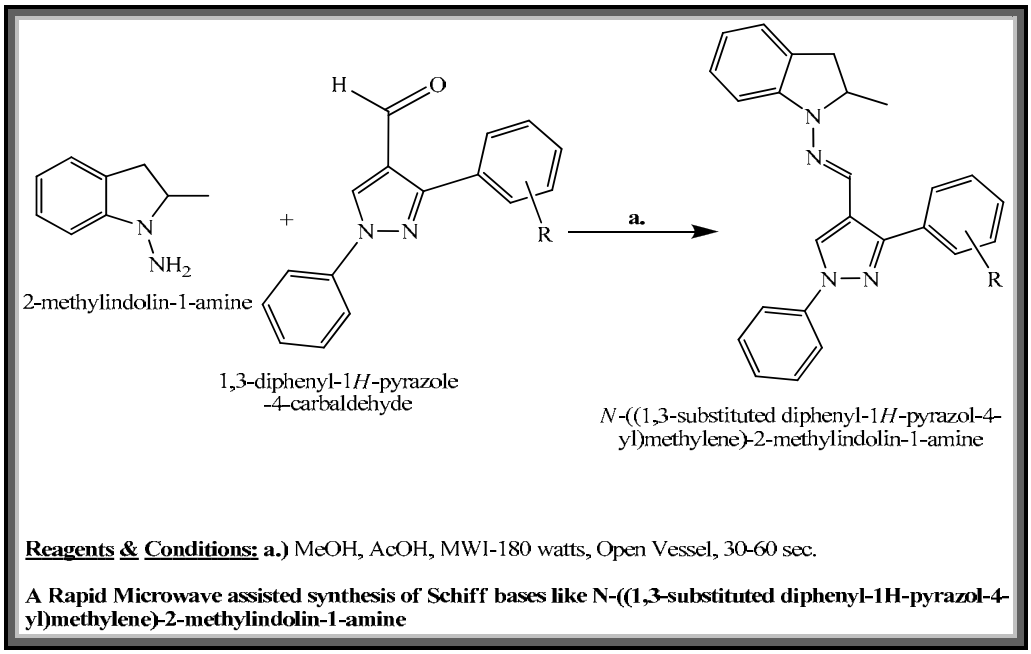
[a] V. Virsodia, A. Manvar, K. Upadhyay, R. Loriya, D. Karia, M. Jaggi, A. Singh, R. Mukherjee, M. S. Shaikh, E. C. Coutinho and A. Shah; *Eur. J. Med. Chem.*, **2008**, 1-8.

4.11 REACTION SCHEMES

A. *PREPARATION OF PYRAZOLE ALDEHYDES*



B. *PREPARATION OF N-((1,3-substituted diphenyl-1H-pyrazole-4-yl) methylene)-2-methylindoline-1-amine (NAIPAL-01 TO NAIPAL-09)*



4.11.1 *PHYSICAL DATA TABLE*

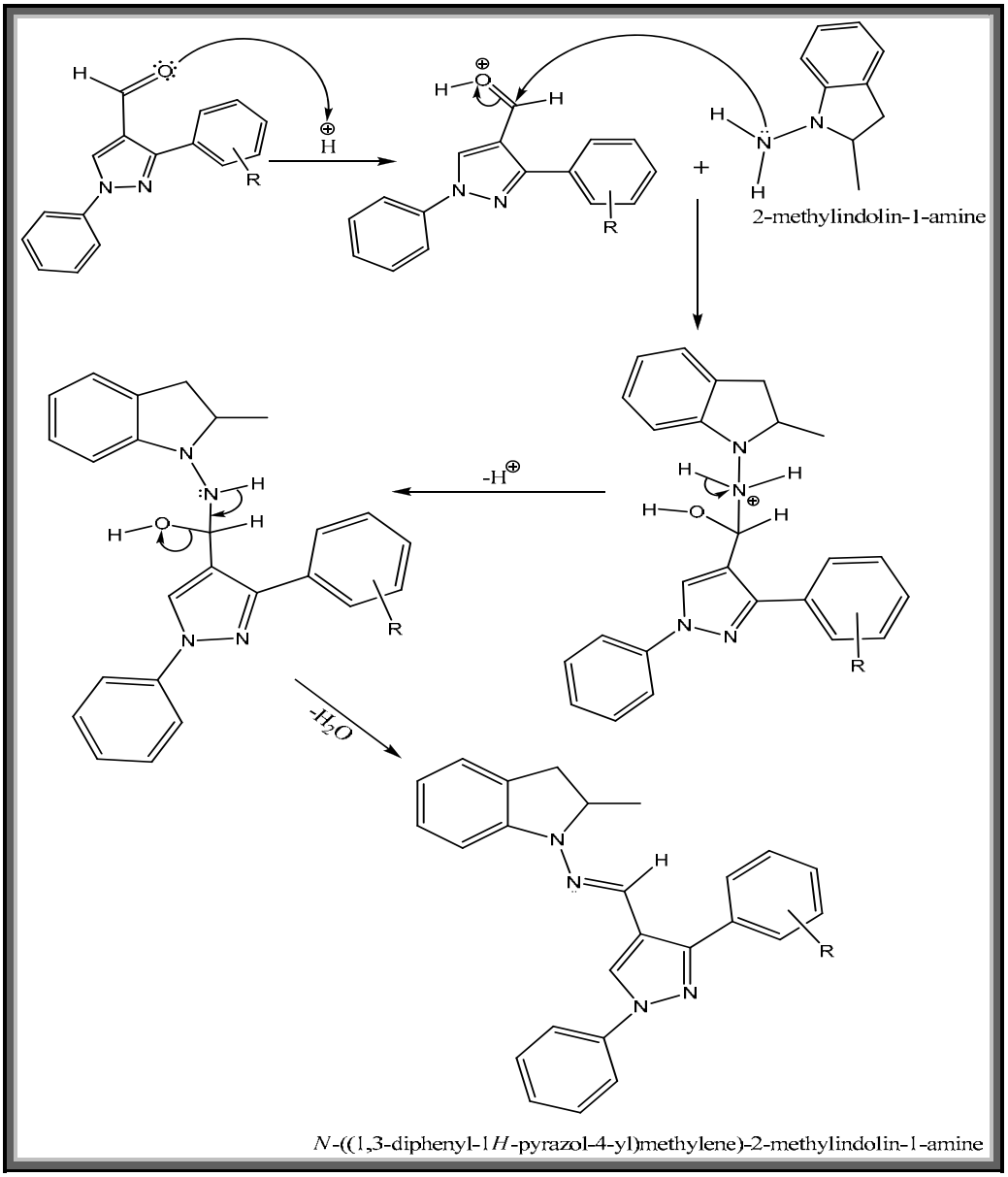
Code	R	M. F.	M. W.	M. P. ^{0}C	Time (min)	Yield %	R_f
NAIPAL-1	H	$C_{25}H_{22}N_4$	378.46	140-142	0:40	95	0.45
NAIPAL -2	4-Cl	$C_{25}H_{21}ClN_4$	412.91	148-150	0:20	96	0.48
NAIPAL -3	4-NO_2	$C_{25}H_{21}N_5O_2$	423.46	188-190	0:30	98	0.44
NAIPAL -4	4-OH	$C_{25}H_{22}N_4O$	394.46	166-168	0:20	97	0.45
NAIPAL -5	4-OCH_3	$C_{26}H_{24}N_4O$	408.49	172-174	0:30	93	0.48
NAIPAL -6	2-NO_2	$C_{25}H_{21}N_5O_2$	423.46	182-184	0:40	92	0.50
NAIPAL- 7	4-Br	$C_{25}H_{21}BrN_4$	457.36	142-144	0:30	95	0.58
NAIPAL -8	Thiophenyl	$C_{23}H_{20}N_4S$	384.49	146-148	0:30	96	0.54
NAIPAL -9	4-F	$C_{25}H_{21}FN_4$	396.45	158-160	0:30	90	0.52

TLC solvent system for R_f = Hexane:Ethyl acetate - 6:4.

Microwave Irradiation= 180 Watts.

4.12 PLAUSIBLE REACTION MECHANISM

4.12.1 *N-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-2-methylindolin-1-amine*



As mentioned in Section A, the mechanism proceeds via the protonation of the carbonyl oxygen which gets a positive charge. This positive charge migrates to the nitrogen of the 2-methyl indoline-1-amine after a charge transfer to form an ammonium ion. A subsequent loss of water molecule will result in the formation of the desired final product i.e. N-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-2-methylindolin-1-amine.

4.13 EXPERIMENTAL

4.13.1 *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 and UV. All the reactions were carried out in Samsung MW83Y Microwave Oven which was locally modified for carrying out chemical reactions. IR spectra were recorded in 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.

4.13.2 *GENERAL PROCEDURES*

A. *General procedure for the synthesis of Pyrazole Aldehydes*

Step-1: ***Synthesis of 1-Phenyl-2-(1-substituted phenylethylidene) hydrazine*** [i]

0.1 mole of appropriately substituted acetophenone was dissolved in 50 ml of ethanol into 250 ml round bottom flask. 0.1 mole of Phenyl hydrazine was added to above flask along with 3-4 drops of glacial acetic acid. The reaction mixture was refluxed for 5-6 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene: ethyl acetate (7: 3) as a mobile phase. After the reaction to be completed, the reaction mixture was cooled to room temperature and the crystalline product was separated by filtration. The product was washed with ethanol and dried to give substituted acetone phenyl hydrazine in good yield which was pure enough to use as such for the next step.

[i] Joshipura D. N. Shah, A. K. Thesis in Chemistry, Department of Chemistry, Saurashtra University

Step-2: ***Synthesis of Pyrazole aldehydes from Acetone phenyl hydrazines***[j]

25 ml of dry dimethylformamide was transferred into 250 ml flat bottom flask. 3 ml of phosphorous oxychloride was added drop wise to above flask under stirring at 0-5°C. After completion of the addition, the mixture was stirred at this temperature for 10-15 min. 0.03 mole of freshly prepared acetophenone phenyl hydrazone was added to above mixture and the content was heated on water bath for 5-6 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene: ethyl acetate (7: 3) as a mobile phase. After the reaction to be completed, the reaction mixture was cooled to room temperature and the content of the flask was poured on crushed ice to isolate the product. The separated product was filtered off and it was washed with cold water to remove acidity. It was dried at 65°C and recrystallized from the mixture of DMF-Methanol to give crystalline pyrazole aldehyde in good yield.

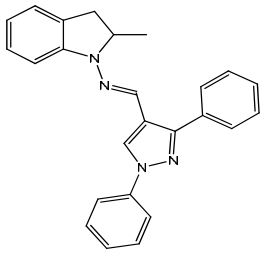
B. ***Synthesis of N-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-2-methylindolin-1-amines (NAIPAL-01 to NAIPAL-09)***

Equimolar amounts of neat reactants i.e. 2-Methyl indolin-1-amine and substituted pyrazolaldehydes were taken in an Erlenmeyer flask, dissolved in Methanol which was taken 10 time w/v of the reactants and served as the solvent. Very few drops of Acetic acid were added in the reaction mixture to catalyze the reaction in the forward direction. The reaction mixture was then subjected to MWI for a specific time (see Physical data Table) at low power (180 W). The progress of the reaction was monitored by TLC examination at an interval of every 10 seconds. On completion of reaction, the reaction mixture was cooled at R.T. which afforded us the solid crystals of the desired product. The product thus obtained was filtered, washed with cold water, dried, and recrystallized from Rectified Spirit.

[j] Joshipura D. N. Shah, A. K. Thesis in Chemistry, Department of Chemistry, Saurashtra University

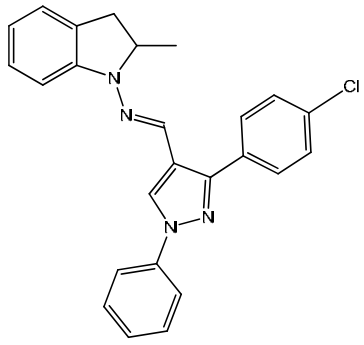
4.14 ANALYTICAL DATA

4.14.1 N-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-2-methylindolin-1-amine (NAIPAL-01)



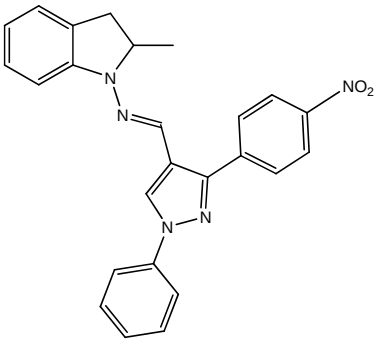
Yield: 95%; M.P.- 140-142 ºC; IR (cm^{-1}): 3492 (N-H stretching in Indole), 1491 (ring stretching indole), 2850 (CH_3 symmetric stretching vibration of Ar-CH_3), 2926 (CH_3 asymmetric stretching in Ar-CH_3), 1472 (CH_3 asymmetric bending), 2973 (symmetric C-H stretching in Cyclopentane), 887 (Ring stretching in cyclopentane), 1590-1533 (C=N stretching frequency), 1405 (C=N in plane bending, scissoring), 3048 (Aromatic C-H stretching vibration), 1250 (C-H in plane bending in phenyl ring), 750 (C-H out of plane bending in phenyl ring); MS: *m/z:* 378.18; Anal. Calcd. for $C_{25}H_{22}N_4$: C, 79.34; H, 5.86; N, 14.80; Found: C, 79.30; H, 5.81; N, 14.75.

4.14.2 N-((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-2 - methylindolin-1-amine (NAIPAL-02)



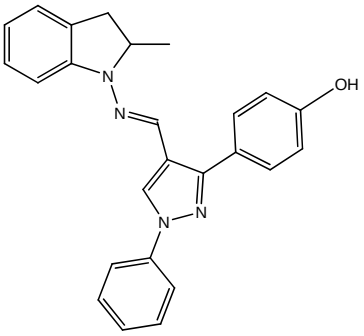
Yield: 96%; M.P.- 148-150 ºC; IR (cm^{-1}): 3495 (N-H stretching in Indole), 1498 (ring stretching indole), 2856 (CH_3 symmetric stretching vibration of Ar-CH_3), 2931 (CH_3 asymmetric stretching in Ar-CH_3), 1475 (CH_3 asymmetric bending), 2970 (symmetric C-H stretching in cyclopentane), 889 (ring stretching in cyclopentane), 1599-1529 (C=N stretching frequency), 1408 (C=N in plane bending, scissoring), 3051 (Aromatic C-H stretching vibration), 1251 (C-H in plane bending in phenyl ring), 750 (C-H out of plane bending in phenyl ring), 750-686 (C-Cl stretching for mono chlorinated aromatic compound); ^{1}H NMR (DMSO-d_6) δ ppm: 1.35-1.37 (d, 3H, H_1), 4.46-4.51 (m, 1H, H_2), 2.77-2.81 (d, 1H, H_3), 3.46-3.52 (q, 1H, H_3), 7.09-7.15 (q, 2H, H_4 & H_7), 6.82-6.86 (t, 1H, H_5), 7.33-7.37 (t, 1H, H_6), 7.60 (s, 1H, H_8), 8.40 (s, 1H, H_9), 7.47-7.53 (m, 4H, $H_{10,}$ H_{11}, H_{13}, H_{14}), 7.33-7.37 (t, 1H, H_{12}), 7.71-7.75 (d, 2H, H_{15}, H_{18}), 7.81-7.85 (d, 2H, H_{16}, H_{17}); MS: *m/z:* 412.15; Anal. Calcd. for $C_{25}H_{21}ClN_4$: C, 72.72; H, 5.13; Cl, 8.59; N, 13.57; Found: C, 72.65; H, 5.09; Cl, 8.55; N, 13.52.

4.14.3 N-((3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-2-methyl-indolin-1-amine (NAIPAL-03)



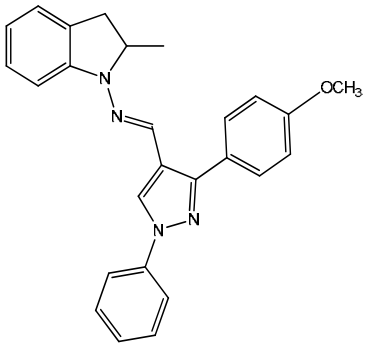
Yield: 98 %; M.P.- 188-190 ºC; IR (cm^{-1}): 3497 (N-H stretching in Indole), 1493 (ring stretching indole), 2855 (CH_3 symmetric stretching vibration of Ar-CH_3), 2930 (CH_3 asymmetric stretching in Ar-CH_3), 1473 (CH_3 asymmetric bending), 2970 (symmetric C-H stretching in cyclopentane), 885 (ring stretching in cyclopentane), 1590-1530 (C=N stretching frequency), 1405 (C=N in plane bending, scissoring), 3057 (Aromatic C-H stretching vibration), 1257 (C-H in plane bending in phenyl ring), 750 (C-H out of plane bending in phenyl ring), 1515 (NO_2 asymmetric stretching frequency); MS: *m/z:* 423.17; Anal. Calcd. for $C_{25}H_{21}N_5O_2$: C, 70.91; H, 5.00; N, 16.54; O, 7.56 Found: C, 70.86; H, 4.96; N, 16.50; O, 7.51.

4.14.4 N-((3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-2-methyl-indolin-1-amine (NAIPAL-04)



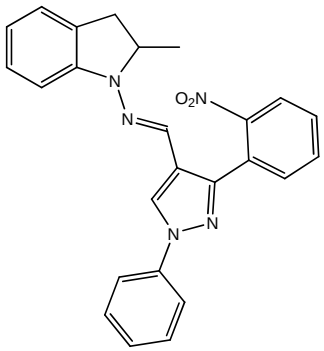
Yield: 97 %; M.P.- 166-168 ºC; IR (cm^{-1}): 3492 (N-H stretching in Indole), 1489 (ring stretching indole), 2851 (CH_3 symmetric stretching vibration of Ar-CH_3), 2931 (CH_3 asymmetric stretching in Ar-CH_3), 1475 (CH_3 asymmetric bending), 2975 (symmetric C-H stretching in cyclopentane), 889 (ring stretching in cyclopentane), 1587-1533 (C=N stretching frequency), 1408 (C=N in plane bending, scissoring), 3049 (Aromatic C-H stretching vibration), 1250 (C-H in plane bending in phenyl ring), 750 (C-H out of plane bending in phenyl ring), 3625 (O-H stretching for free alcohol), 678 (O-H out of plane bending);MS: *m/z:* 394.18; Anal. Calcd. for $C_{25}H_{22}N_4O$: C, 76.12; H, 5.62; N, 14.20; O, 4.06; Found: C, 76.07; H, 5.58; N, 14.14; O, 4.00.

4.14.5 N-((3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-2-methylindolin-1-amine (NAIPAL-05)



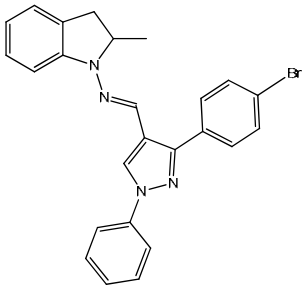
Yield: 93 %; M.P.- 172-174 ºC; IR (cm^{-1}): 3497 (N-H stretching in Indole), 1493 (ring stretching indole), 2852 (CH_3 symmetric stretching vibration of Ar-CH_3), 2939 (CH_3 asymmetric stretching in Ar-CH_3), 1474 (CH_3 asymmetric bending), 2970 (symmetric C-H stretching in cyclopentane), 883 (ring stretching in cyclopentane), 1589-1530 (C=N stretching frequency), 1402 (C=N in plane bending, scissoring), 3055 (Aromatic C-H stretching vibration), 1256 (C-H in plane bending in phenyl ring), 750 (C-H out of plane bending in phenyl ring), 3147-3054 (C-H stretching in aryl ethers), 1278 (C-O-C asymmetric stretching in ethers); MS: *m/z:* 408.20; Anal. Calcd. for $C_{26}H_{24}N_4O$: C, 76.45; H, 5.92; N, 13.72; O, 3.92; Found: C, 76.41; H, 5.88; N, 13.69; O, 3.87.

4.14.6 2-Methyl-N-((3-(2-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene) indolin-1-amine (NAIPAL-06)



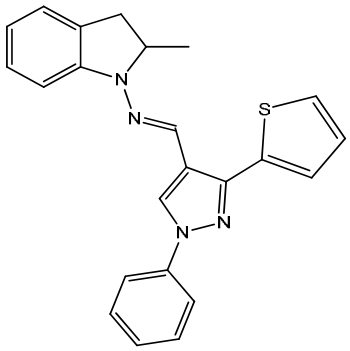
Yield: 92 %; M.P.- 182-184 ºC; IR (cm^{-1}): 3498 (N-H stretching in Indole), 1496 (ring stretching indole), 2857 (CH_3 symmetric stretching vibration of Ar-CH_3), 2932 (CH_3 asymmetric stretching in Ar-CH_3), 1475 (CH_3 asymmetric bending), 2974 (symmetric C-H stretching in cyclopentane), 886 (ring stretching in cyclopentane), 1590-1534 (C=N stretching frequency), 1407 (C=N in plane bending, scissoring), 3052 (Aromatic C-H stretching vibration), 1250 (C-H in plane bending in phenyl ring), 750 (C-H out of plane bending in phenyl ring), 1510 (NO_2 asymmetric stretching frequency); MS: *m/z:* 423.17; Anal. Calcd. for $C_{25}H_{21}N_5O_2$: C, 70.91; H, 5.00; N, 16.54; O, 7.56; Found: C, 70.85; H, 4.97; N, 16.50; O, 7.53.

4.14.7 N-((3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-2-methylindolin-1-amine (NAIPAL-07)



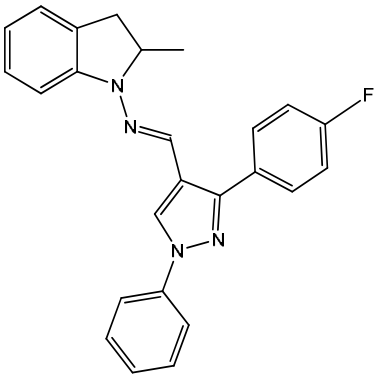
Yield: 95 %; M.P.- 142-144 ºC; IR (cm^{-1}): 3490 (N-H stretching in Indole), 1495 (ring stretching indole), 2858 (CH_3 symmetric stretching vibration of Ar-CH_3), 2935 (CH_3 asymmetric stretching in Ar-CH_3), 1470 (CH_3 asymmetric bending), 2976 (symmetric C-H stretching in cyclopentane), 890 (ring stretching in cyclopentane), 1600-1529 (C=N stretching frequency), 1405 (C=N in plane bending, scissoring), 3055 (Aromatic C-H stretching vibration), 1250 (C-H in plane bending in phenyl ring), 750 (C-H out of plane bending in phenyl ring), 587 (C-Br stretching for mono brominated aromatic compound); MS: *m/z:* 456.09; Anal. Calcd. for $C_{25}H_{21}BrN_4$: C, 65.65; H, 4.63; Br, 17.47; N, 12.25; Found: C, 65.61; H, 4.60; Br, 17.44; N, 12.19.

4.14.8 2-Methyl-N-((1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-methylene)-indolin-1-amine (NAIPAL-08)



Yield: 96 %; M.P.- 146-148 ºC; IR (cm^{-1}): 3485-3423 (N-H stretching indole), 1597-1564 (ring stretching indole 2 characteristic bands), 1485 (CH_3 asymmetric bending), 1683-1647 (C=N stretching frequency), 1402 (C=N in plane bending, scissoring), 1298 (C=N rocking vibration), 3275 (C-H stretching for aromatic region), 1267 (C-H in plane bending for phenyl ring), 704 (C-C out of plane bending for mono substituted benzene ring), 750 (C-H out of plane bending for mono substituted benzene ring), 3389-3304 (C-H stretching frequency for thiophene), 1219, 1367, 1383, 1531 (several bands due to ring stretching in thiophene), 715-663 (strongest of the thiophene bands for 2-substituted thiophene); ^{1}H NMR (DMSO-d_6) δ ppm: 1.27-1.29 (d, 3H, H_1), 4.38-4.43 (m, 1H, H_2), 2.64-2.69 (d, 1H, H_3), 3.34-3.40 (q, 1H, H_3), 7.00-7.11 (m, 4H, H_4, H_6, H_7, H_{13}), 6.70-6.74 (t, 1H, H_5), 7.64 (s, 1H, H_8), 8.23 (s, 1H, H_9), 7.68-7.70 (d, 2H, H_{10}, H_{14}, *J* Value = *10.4 Hz*), 7.27-7.28 (d, 1H, H_{11}, *J Value = 4.8 Hz*), 7.18-7.22 (t, 1H, H_{12}), 7.34-7.40 (m, 3H, Thiophenyl); MS: *m/z:* 384.14; Anal. Calcd. for $C_{23}H_{20}N_4S$: C, 71.85; H, 5.24; N, 14.57; S, 8.34, Found: C, 71.80; H, 5.19; N, 14.52; S, 8.30.

4.14.9 N-((3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-2-methylindolin-1-amine (NAIPAL-09)



Yield: 90 %; M.P.- 158-160 ºC; IR (cm^{-1}): 3495 (N-H stretching in Indole), 1490 (ring stretching indole), 2854 (CH_3 symmetric stretching vibration of Ar-CH_3), 2930 (CH_3 asymmetric stretching in Ar-CH_3), 1475 (CH_3 asymmetric bending), 2973 (symmetric C-H stretching in cyclopentane), 887 (ring stretching in cyclopentane), 1599-1534 (C=N stretching frequency), 1409 (C=N in plane bending, scissoring), 3047 (Aromatic C-H stretching vibration), 1257 (C-H in plane bending in phenyl ring), 750 (C-H out of plane bending in phenyl ring), 1074 (C-F stretching for mono fluorinated aromatic compound); MS: *m/z:* 396.18; Anal. Calcd. for $C_{25}H_{21}FN_4$: C, 75.74; H, 5.34; F, 4.79; N, 14.13; Found: C, 75.70; H, 5.29; F, 4.73; N, 14.09.

4.15 SPECTRAL DISCUSSION

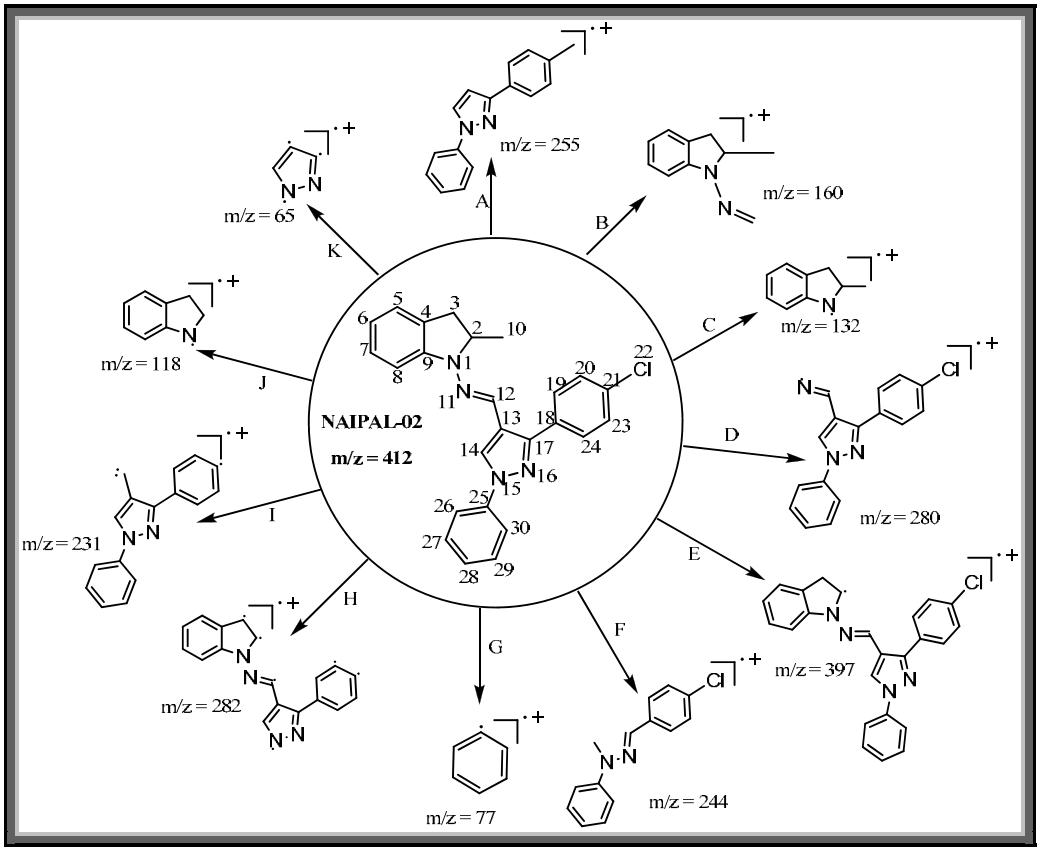
4.15.1 *IR SPECTRAL STUDY*

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. The N-H stretching frequency of Indole was found between 3485-3450 cm^{-1}, the Ring stretching frequency in indole was observed at around 1490 cm^{-1}, the CH_3 symmetric stretching vibration of Ar-CH_3 were observed between 2870-2845 cm^{-1}, CH_3 asymmetric stretching in Ar-CH_3 was observed around 2930 cm^{-1}, the CH_3 asymmetric bending vibrations were observed at around 1475 cm^{-1}, the symmetric C-H stretching in Cyclopentane was found between 2930-2950 cm^{-1}, the Ring stretching in cyclopentane was found at 885 cm^{-1}, C=N stretching frequency were observed between 1599-1534 cm^{-1}, C=N in plane bending for scissoring type vibrations were seen at 1410 cm^{-1}, Aromatic C-H stretching vibration were observed around 3040-3010 cm^{-1} , the C-H in plane bending in phenyl ring was observed around 1250 cm^{-1}, the C-H out of plane bending in phenyl ring was also seen around 750 cm^{-1}, the halogenated derivatives showed the characteristic C-Halogen stretching frequency between 1100-550 cm^{-1}; respectively suggesting the correct formation of the desired products (NAIPAL-01 to NAIPAL-09).

4.15.2 *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. The probable mass fragmentation pattern for the representative compound of each series is discussed below.

4.15.2.1 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF NAIPAL-02*

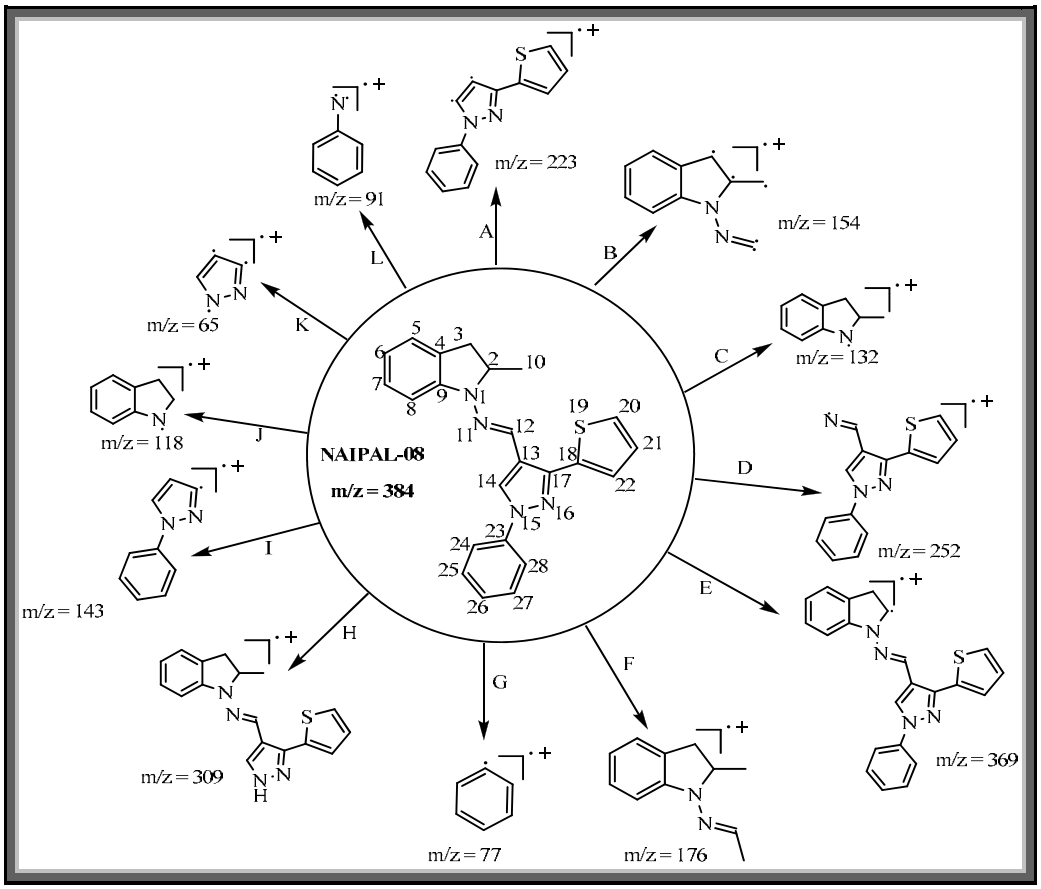


N-((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-2 -methylindolin-1-amine (NAIPAL-02)

1. The target compound showed the characteristic molecular ion peak 412 *m/z*.
2. The bond cleavage between C_{12}-C_{13} generated a molecular ion which corresponds to a characteristic peak at 255 *m/z* **(A).**
3. A bond cleavage between C_{12}-C_{13} generated another molecular ion which corresponds to a characteristic peak at 160 *m/z* **(B)**.
4. Bond cleavages between N_1-N_{11} generated a molecular ion which corresponds to a characteristic peak at 132 *m/z* **(C)**.
5. Bond cleavages between N_1-N_{11} generated another molecular ion which corresponds to a characteristic peak at 280 *m/z* **(D)**.
6. Bond cleavages between C_2-C_{10} generated a molecular ion which corresponds to a characteristic peak at 397 *m/z* **(E)**.

7. Bond cleavages between C_{13}-C_{17}, and C_{13}-C_{14} generated a molecular ion which corresponds to a characteristic peak at 244 *m/z* **(F)**.
8. Bond cleavages between N_{15}-C_{25} generated a molecular ion which corresponds to a characteristic peak at 77 *m/z* **(G)**.
9. Bond cleavages between C_2-C_{10}, N_{15}-C_{25} and C_{21}-Cl_{22} generated a molecular ion which corresponds to a characteristic peak at 282 *m/z* **(H)**.
10. Bond cleavages between N_{11}-C_{12}, C_{21}-Cl_{22} generated a molecular ion which corresponds to a characteristic peak at 231 *m/z* **(I)**.
11. Bond cleavages between N_1-N_{11}, C_2-C_{10}, generated a molecular ion which corresponds to a characteristic peak at 118 *m/z* **(J)**.
12. Bond cleavages between C_{12}-C_{13}, C_{17}-C_{18}, and N_{15}-C_{25} generated a molecular ion which corresponds to a characteristic peak at 65 *m/z* **(K)**.

4.15.2.2 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF NAIPAL-08*



2-Methyl-N-((1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-methylene)-indolin-1-amine (NAIPAL-08)

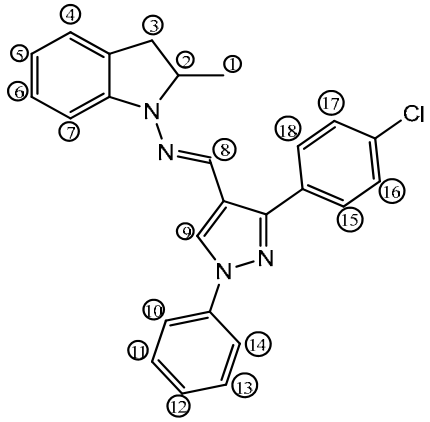
1. The target compound showed the characteristic molecular ion peak 384 *m/z*.
2. The bond cleavage between C_{12}-C_{13} generated a molecular ion which corresponds to a characteristic peak at 223 *m/z* **(A).**
3. A bond cleavage between C_{12}-C_{13} generated another molecular ion which corresponds to a characteristic peak at 154 *m/z* **(B)**.
4. Bond cleavages between N_1-N_{11} generated a molecular ion which corresponds to a characteristic peak at 132 *m/z* **(C)**.
5. Bond cleavages between N_1-N_{11} generated another molecular ion which corresponds to a characteristic peak at 252 *m/z* **(D)**.
6. Bond cleavages between C_2-C_{10} generated a molecular ion which corresponds to a characteristic peak at 369 *m/z* **(E)**.
7. Bond cleavages between C_{13}-C_{17}, and C_{13}-C_{14} generated a molecular ion which corresponds to a characteristic peak at 176 *m/z* **(F)**.
8. Bond cleavages between N_{15}-C_{23} generated a molecular ion which corresponds to a characteristic peak at 77 *m/z* **(G)**.
9. Bond cleavages between N_{15}-C_{23} generated another molecular ion which corresponds to a characteristic peak at 309 *m/z* **(H)**.
10. Bond cleavages between C_{12}-C_{13}, C_{17}-C_{18} generated a molecular ion which corresponds to a characteristic peak at 143 *m/z* **(I)**.
11. Bond cleavages between N_1-N_{11}, C_2-C_{10}, generated a molecular ion which corresponds to a characteristic peak at 118 *m/z* **(J)**.
12. Bond cleavages between C_{12}-C_{13}, C_{17}-C_{18}, and N_{15}-C_{23} generated a molecular ion which corresponds to a characteristic peak at 65 *m/z* **(K)**.
13. Bond cleavages between C_{14}-N_{15}, N_{15}-N_{16} generated a molecular ion which corresponds to a characteristic peak at 91 *m/z* **(L)**.

Similarly, other newly synthesized compounds can also be explained of their formation by the similar mass fragmentation as above.

4.15.3 *^{1}H-NMR SPECTRAL STUDY*

^{1}H-NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in DMSO solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from H-NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. The spectral interpretation can be discussed as under.

4.15.3.1 N-((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-2 -methylindolin-1-amine (NAIPAL-02)



1. The proton no. 1 i.e. the methyl protons has one proton on the adjacent carbon hence a doublet should be observed in the NMR spectrum at an upfield region This Characteristic strong doublet is seen between 1.37 δ ppm to 1.35 δ ppm in the NMR spectrum. Without doubt it is assigned to the methyl protons no.1.
2. By observing the structure it could be seen that the methine proton i.e. proton no.2 is surrounded by 5 protons from both the adjacent carbon atoms. On theoretical basis there should be a multiplet signal for that methine proton which is very clearly seen in the NMR spectrum between 4.51 δ ppm to 4.46 δ ppm. This multiplet accounts only for a single proton hence this multiplet signal of the spectrum is assigned to proton no.2.
3. As discussed in the section A of this chapter the two geminal protons or the methylene protons i.e. proton no. 3 would have different chemical environments and hence two different signals for both theseprotons would be

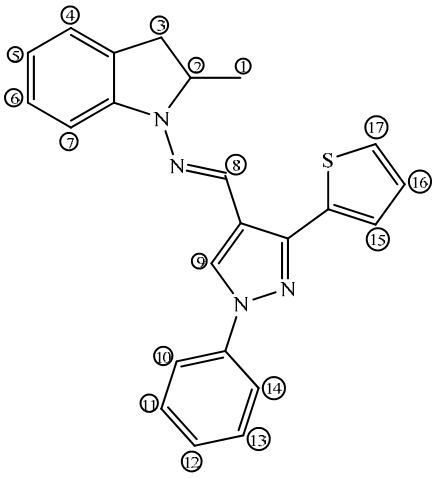
found. On studying the expanded spectra of this compound same pattern as in compounds of Section A is observed. One doublet and one quartet accounting for one proton each is observed. The doublet is observed between 2.77 δ ppm and 2.81 δ ppm and their peaks are again narrowly split due to the geminal coupling hence this is definitely one of the methylene protons. Where as the quartet is seen between 3.46 δ ppm and 3.52 δ ppm this splitting is due to two reasons one is the proton on the adjacent carbon which splits it into a doublet and the other is the geminal coupling which further splits it to give a quartet and as it accounts only for a single proton it can be said that this is the second methylene proton.

4. On further studying the expanded NMR spectrum in the aromatic region it is observed that there is a quartet signal accounting for 2 protons between 7.09 δ ppm and 7.15 δ ppm. Next to this quartet there are two triplets accounting for one proton each. Now proton no. 4 has only one adjacent proton hence it should give a doublet and same is the case with the proton no. 7. The signal that looks like a quartet is not actually a quartet but they are two doublets accounting for two protons and this has to proton no. 4 as well as proton no. 7. Both these doublets are so near that it looks like a quartet but they are independent doublets of proton no. 4 and proton no. 7 respectively.
5. By studying the expanded NMR spectrum further, the first triplet signal in the aromatic region is observed between 6.82 δ ppm and 6.86 δ ppm. This triplet signal is assigned to proton no. 5 in the aromatic region. The reason behind this triplet is the coupling of proton no. 5 with the two protons on the two adjacent carbons respectively.
6. Similar to proton 5, another triplet signal is observed in the NMR spectrum between 7.33 δ ppm and 7.37 δ ppm which is assigned to proton no. 6 as it is also surrounded by a number of protons around it like proton no. 5 and no. 7.
7. The proton no. 8 is a single proton not surrounded by any other proton in its vicinity hence a singlet should be observed for it; moreover as its carbon is directly attached to the electronegative nitrogen moiety or more particularly the C-N-N system hence it should be extremely deshielded and thus found in the downfield region. This is precisely the case; on observing the expanded spectra a strong singlet at 7.60 δ ppm is observed which is assigned to the proton no. 8 owing to the above arguments.

8. The proton no. 9 is also not surrounded by any other proton in its vicinity and hence one more singlet should be observed. Also, as it is inside a pyrazole ring with two nitrogen atoms the proton would be extremely deshielded and observed at even more downfield region. A strong singlet at 8.4 δ ppm is observed which is assigned to the proton no. 9
9. On studying the aromatic region a multiplet between 7.47 δ ppm and 7.53 δ ppm which accounts for 4 protons is observed. This multiplet is assigned to four protons i.e. proton no. 10, 11, 13 and 14. Moreover, proton no.12 gives a characteristic signal of a triplet between 7.33 δ ppm and 7.37 δ ppm. A triplet is observed due to the proton no. 11 and 13 in its vicinity which will split the signal into a triplet.
10. On further investigation two doublets are observed which are on the verge of further splitting, accounting for 2 protons each. proton no. 15 and 18 have similar chemical environment and hence will give the same characteristic signal and so is the case with proton nos. 16 and 17, they also have similar chemical environments and will thus have the same characteristic signal. The only difference is that protons 16 and 17, due to its proximity with the electronegative chlorine atom will be more deshielded and hence would be observed in the downfield region as compared to proton no. 15 and 18. This is very clearly observed in the spectra. The characteristic doublet between 7.71 δ ppm and 7.75 δ ppm can be successfully assigned to protons 15 and 18 where as the characteristic doublet between 7.81 δ ppm to 7.85 δ ppm can be successfully assigned to proton no. 16 and 17 owing to the above arguments.

Thus, by observing and assigning the peaks and signals in the NMR spectrum and by the calculation of the J values for each of the above proton it can be clearly suggested that the proposed structure for compound no. NAIPAL-02 has been confirmed.

4.15.3.2 2-Methyl-N-((1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-methylene)-indolin-1-amine (NAIPAL-08)



1. The proton no. 1 i.e. the methyl protons has one proton on the adjacent carbon hence a doubletshould be observed in the NMR spectrum at an upfield region This Characteristic strong doublet is seen between 1.27 δ ppm to 1.29 δ ppm in the NMR spectrum. Without doubt it is assigned to the methyl protons no.1.
2. By observing the structure its evidently seen that the methine proton i.e. proton no.2 is surrounded by 5 protons from both the adjacent carbon atoms. On theoretical basis a multiplet signal should be found for that methine proton which is very clearly seen in the NMR spectrum between 4.38 δ ppm to 4.43 δ ppm. This multiplet accounts only for a single proton hence this multiplet signal of the spectrum is assigned to proton no.2.
3. As discussed in the section A of this chapter the two geminal protons or the methylene protons i.e. proton no. 3 would have different chemical environments and hence two different signals for both theseprotons would be observed. On studying the expanded spectra of this compound it is clear that the same pattern as in compounds of Section A is observed. One doublet and one quartet accounting for one proton each is observed. The doublet is observed between 2.64 δ ppm and 2.69 δ ppm and their peaks are again narrowly split due to the geminal coupling hence this is definitely one of the methylene protons. Whereas the quartet is seen between 3.34 δ ppm and 3.40 δ ppm this splitting is due to two reasons one is the proton on the adjacent carbon which splits it into a doublet and the other is the geminal coupling

which further splits it to give a quartet and as it accounts only for a single proton it can be said that this is the second methylene proton.

4. On further studying the expanded NMR spectrum in the aromatic region it is observed that there is a multiplet signal accounting for 4 protons between 7.00 δ ppm and 7.11 δ ppm. The other signal near the multiplet is a triplet for one proton; hence it is suggested that out of the 4 protons of multiplet 3 protons are proton no. 4, 6 and 7. Where as the triplet next to the multiplet found in the NMR spectrum between 6.70 δ ppm to 6.74 δ ppm is nothing but the signal for proton no. 5.
5. The proton no. 8 is a single proton not surrounded by any other proton in its vicinity hence a singlet should be observed for it; moreover as its carbon is directly attached to the electronegative nitrogen moiety or more particularly the C-N-N system hence it should be extremely deshielded and thus found in the downfield region. This is precisely the case; on observing the expanded spectra a strong singlet at 7.64 δ ppm is observed which is assigned to the proton no. 8 owing to the above arguments.
6. The proton no. 9 is also not surrounded by any other proton in its vicinity and hence one more singlet should be observed. Also, as it is inside a pyrazole ring with two nitrogen atoms the proton would be extremely deshielded and observed at even more downfield region. A strong singlet at 8.23 δ ppm is observed which is assigned to the proton no. 9.
7. Again studying the aromatic region in the spectrum a doublet between 7.68 δ ppm to 7.70 δ ppm is observed accounting for two protons. This signal is assigned to protons 10 and 14 as they have similar chemical environment and as there is one proton in its vicinity they will show a doublet. Calculating the J value for the above doublet it comes out to be 10.4 Hz which suggests that they are ortho coupled to another protons hence without doubt this signal is for proton no. 10 and 14.
8. Now, there is one doublet between 7.27 δ ppm and 7.28 δ ppm accounting for one proton. The J value was calculated to be 4.8 Hz which suggests that it is meta coupled to another proton. Hence this signal is assigned to proton no.11 as it is meta coupled to proton no.13. The signal for proton no.13 can be found in the NMR spectrum as a multiplet which accounted for 4 protons. Out of the multiplet we assigned 3 protons as proton no. 4, 6 and 7 while the fourth

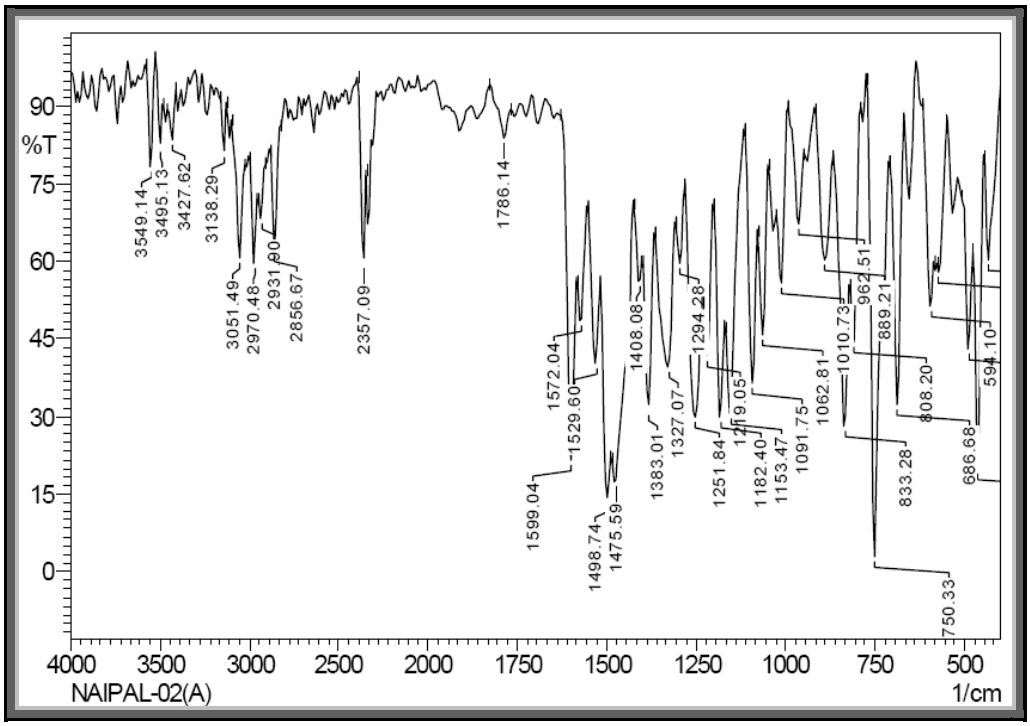
proton is the proton no. 13 in that multiplet. The proton no.12 is shown in the NMR spectrum as a triplet between 7.18 δ ppm and 7.22 δ ppm. This triplet is due to the coupling of proton 12 with proton no. 11 and 13.

9. As for the thiophenyl ring we observe all its three protons as a multiplet in the NMR spectrum between 7.34 δ ppm to 7.40 δ ppm.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton it can clearly be suggested that the proposed structure for compound no. NAIPAL-08 has been confirmed.

4.16 SPECTRAL REPRESENTATIONS OF THE COMPOUNDS

4.16.1 *IR SPECTRUM OF NAIPAL-02*



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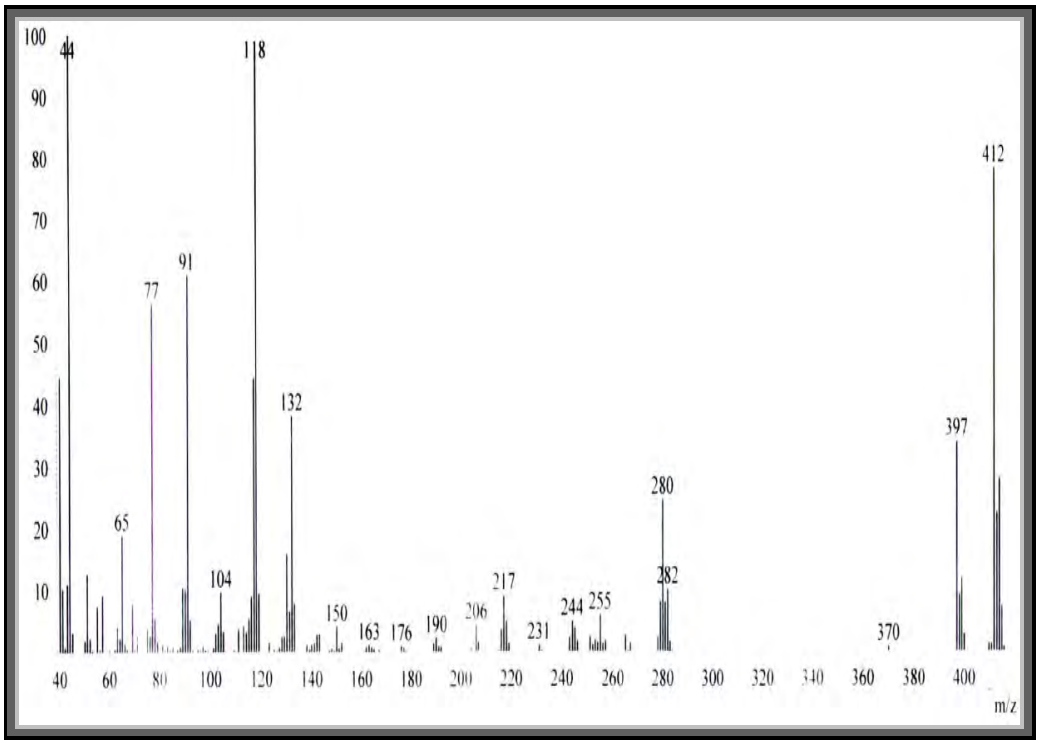

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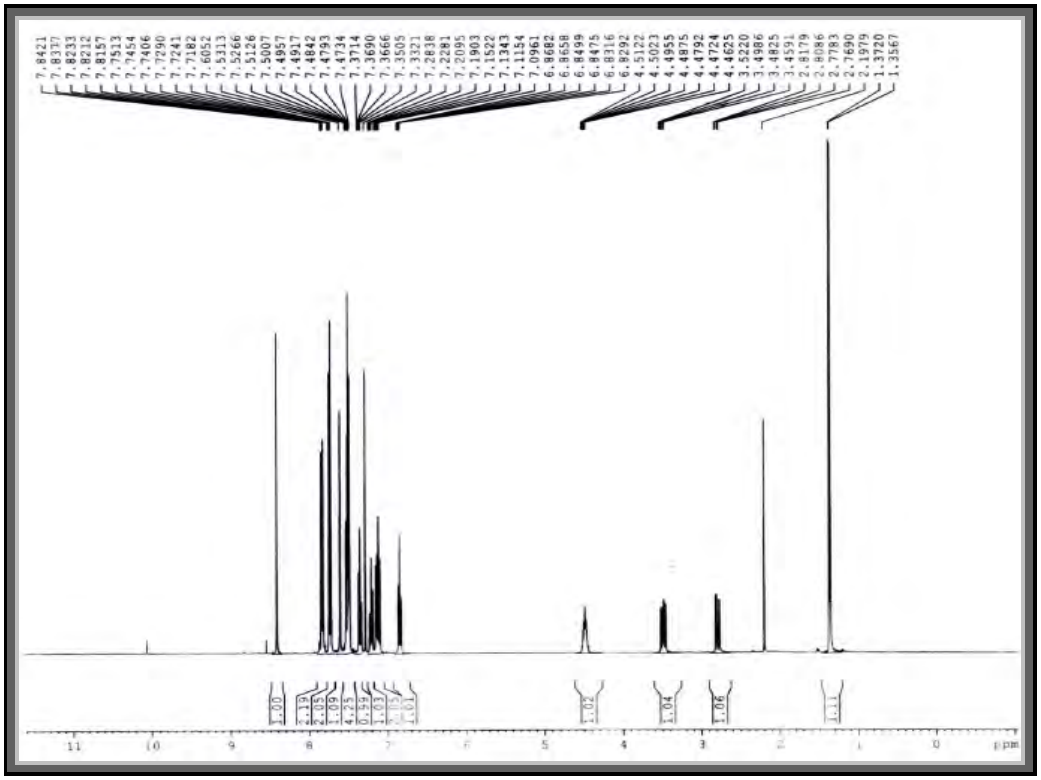
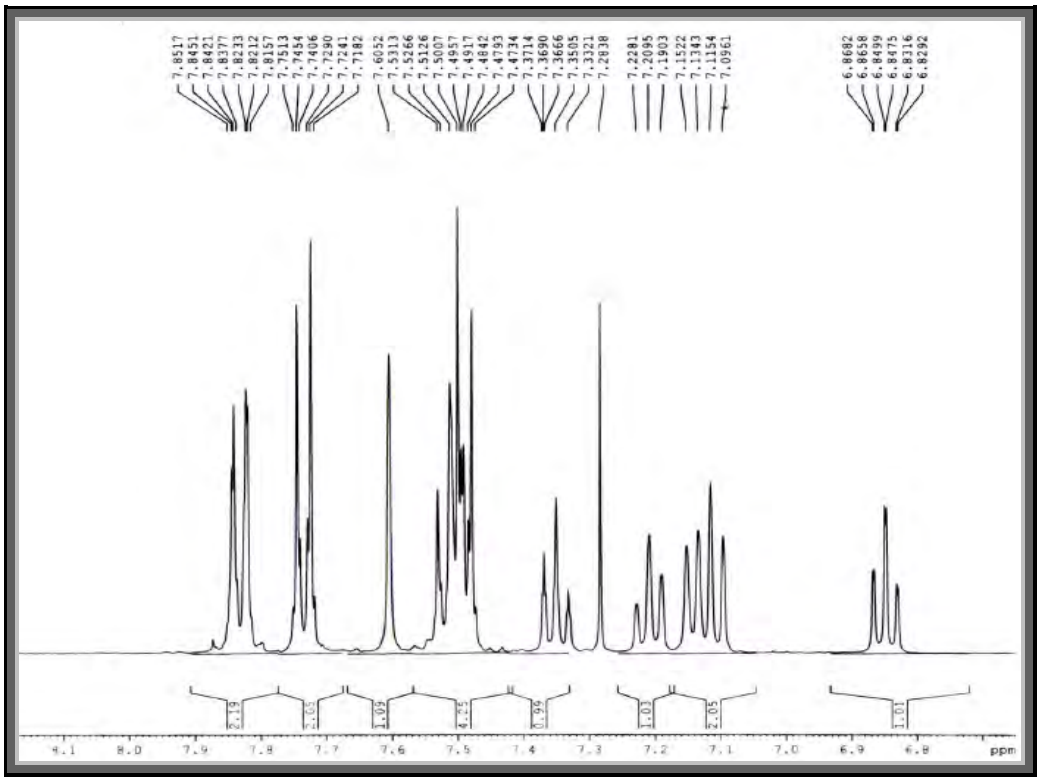


4.16.3.1 *EXPANDED ^{1}H-NMR SPECTRUM OF NAIPAL-02*



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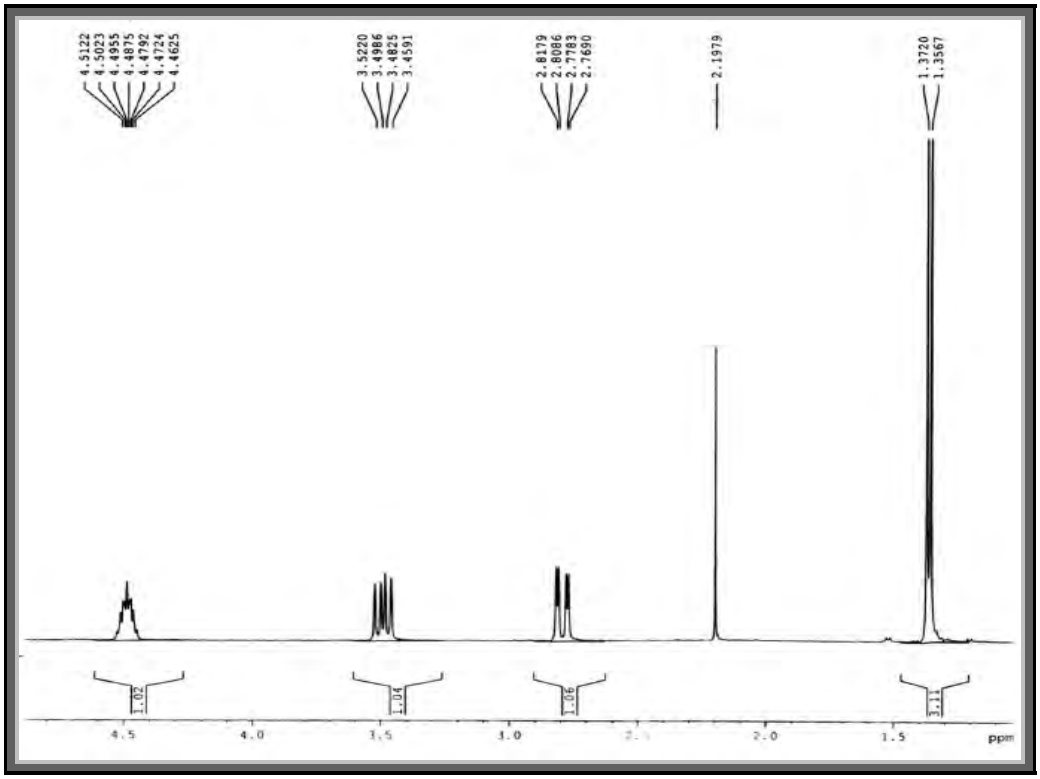
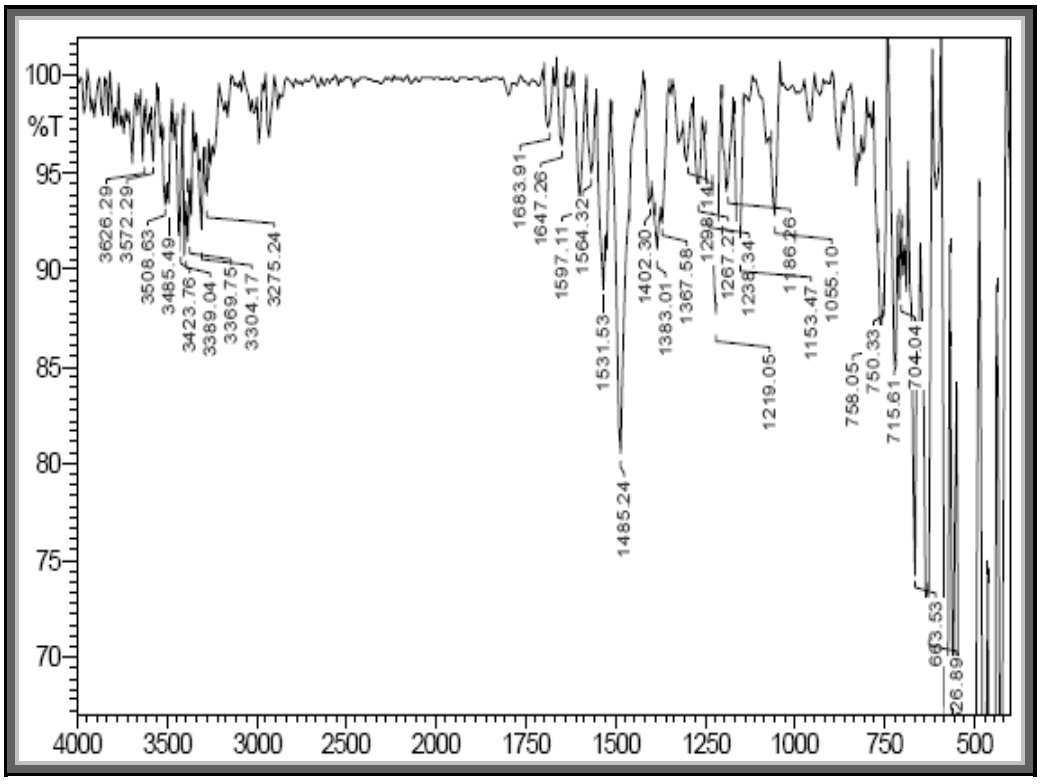
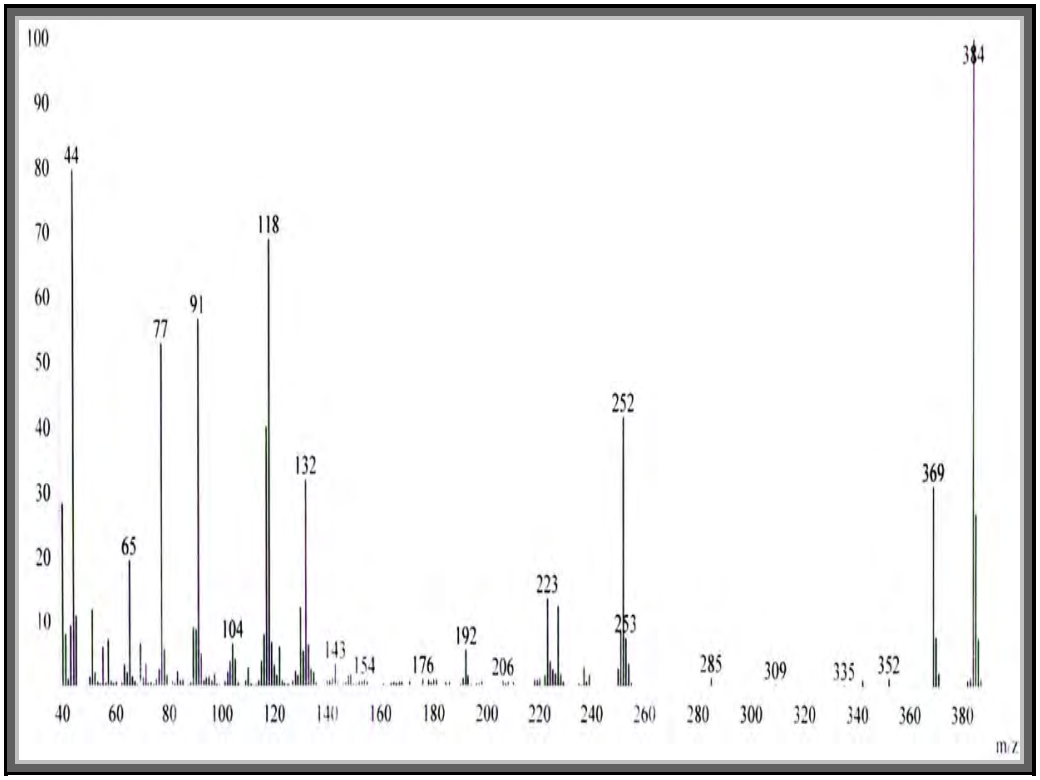


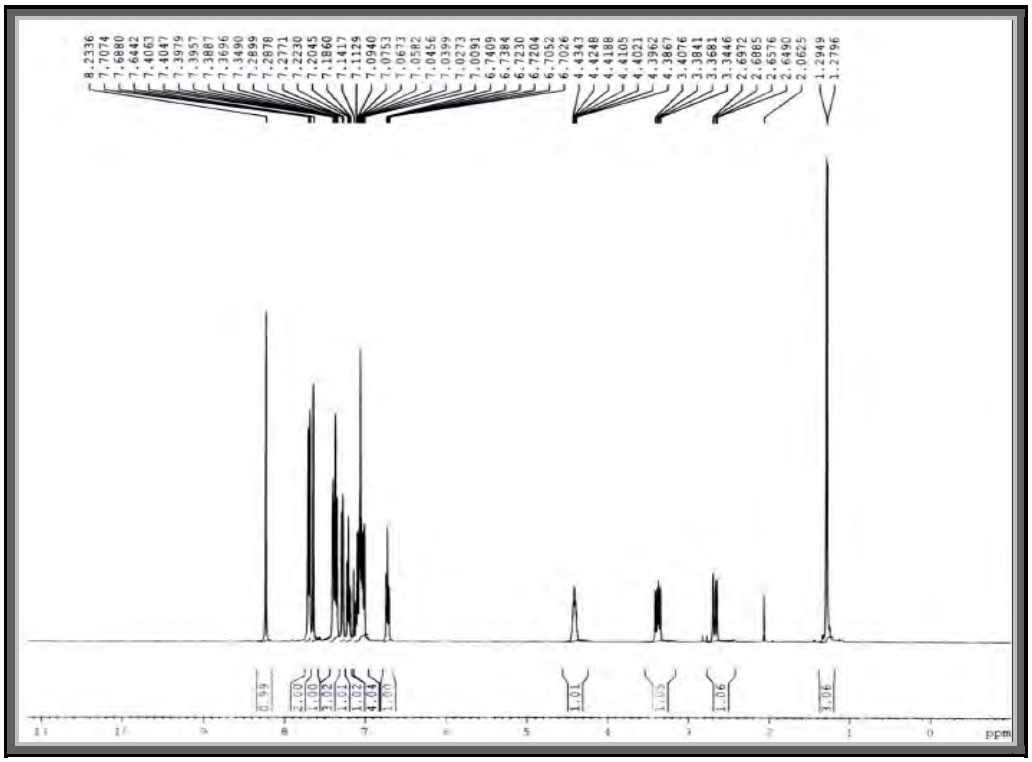
4.16.4 *IR SPECTRUM OF NAIPAL -08*



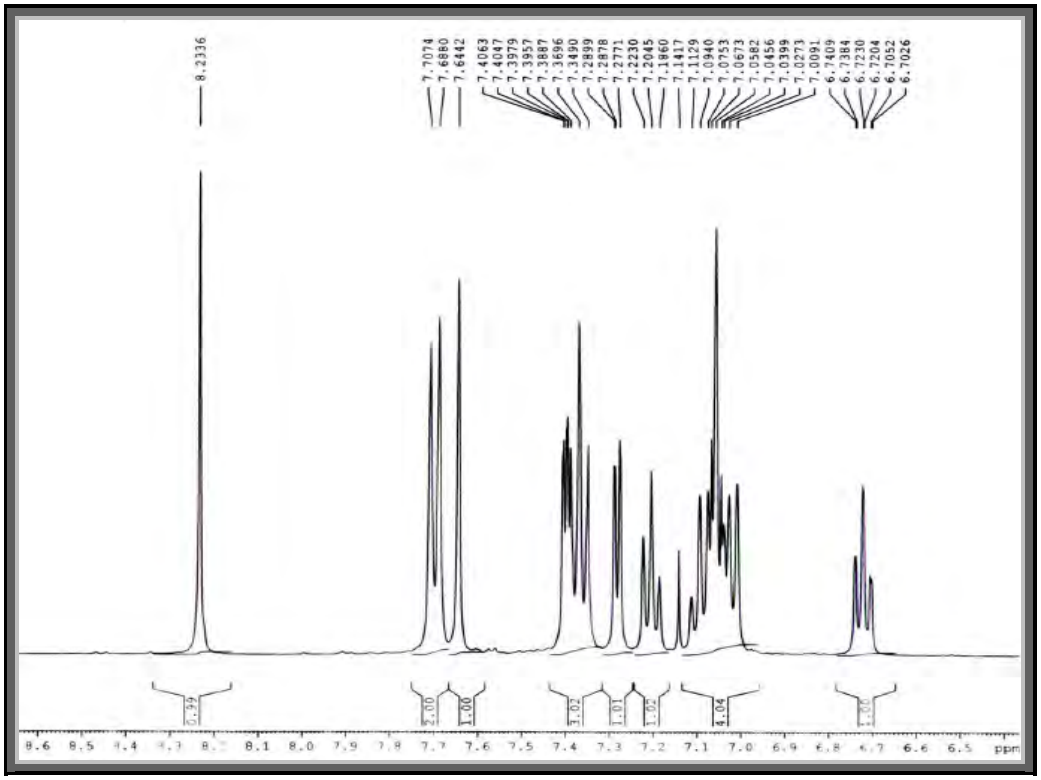
4.16.5 *MASS SPECTRUM OF NAIPAL-08*



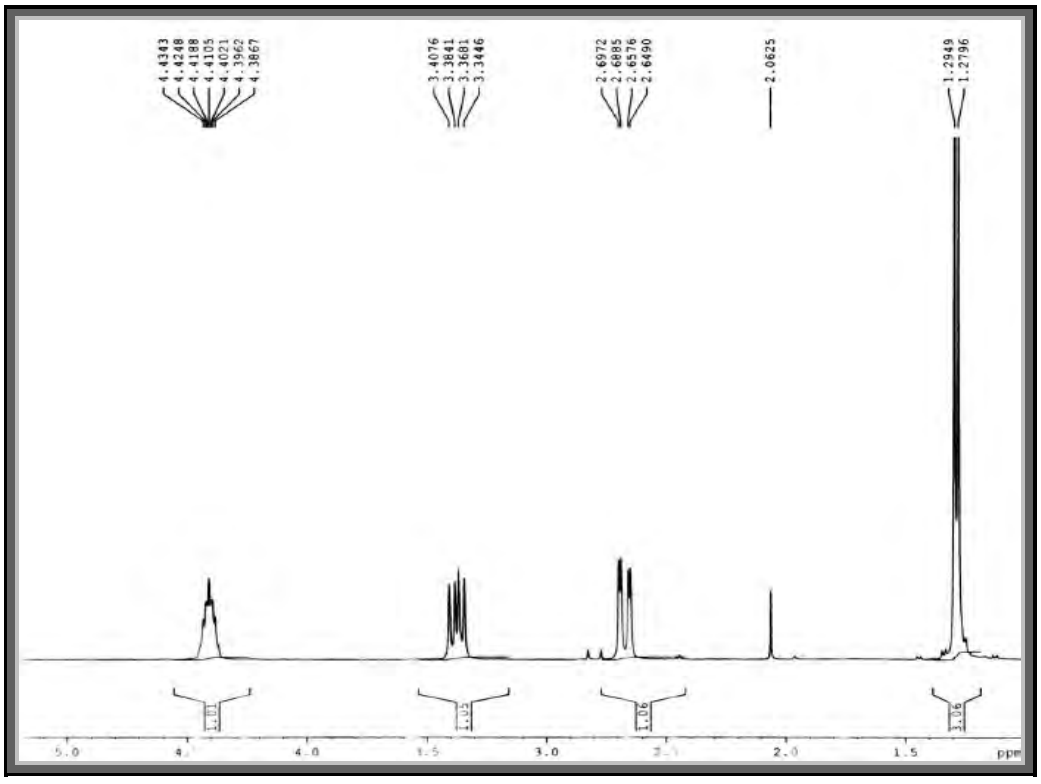
4.16.6 *^{1}H-NMR SPECTRUM OF NAIPAL-08*



4.16.6.1 *EXPANDED ^{1}H-NMR SPECTRUM OF NAIPAL-08*



4.16.6.2 *EXPANDED ^{1}H-NMR SPECTRUM OF NAIPAL-08*



4.17 RESULTS AND DISCUSSIONS

This chapter is based on the investigations on the 2-Methylindoline-1-amine core structure. No work was reported on their Schiff bases and no references were found pertaining to the biological activities of these compounds. Two kinds of aldehydes have been employed to synthesize these Schiff bases, the differently substituted benzaldehydes as well as differently substituted pyrazole aldehydes. More so ever, reactions have been carried out under Microwave irradiation which is a cleaner and a greener method of synthesis. Under normal conditions the Schiff bases are prepared by refluxing the aldehydes as well as amines in different solvents for 3 to 6 hours. The Microwave assisted synthesis was found to be extremely fast and the yields obtained after the reaction were also quite impressive.

Thus the chemical entities enlisted above in both the sections of this chapter, especially the Schiff bases involving the pyrazolaldehydes, are novel and have been prepared by a new synthetic methodology.

4.18 CONCLUSION

The indole nucleuses as well as pyrazole nucleus both are privilege structures. The compounds containing both these functions embedded in their carbon skeleton and their biological activity study was the main rational behind our synthesizing these new chemical entities. Our group has achieved some success in finding some nitrogen containing heterocycles such as 2-methyl indoles, dihydropyridines, dihydropyrimidines etc. which yielded good to excellent biological activity. Hence, knowing that both these nucleus have a very wide applications in pharmaceutical as well as agrochemical industry owing to their herbicidal, insecticidal, antifungal, antipyretic, anti-inflammatory, analgesic, anticancer as well as MDR activities we decided to go further in synthesizing these moieties and study their biological activities.

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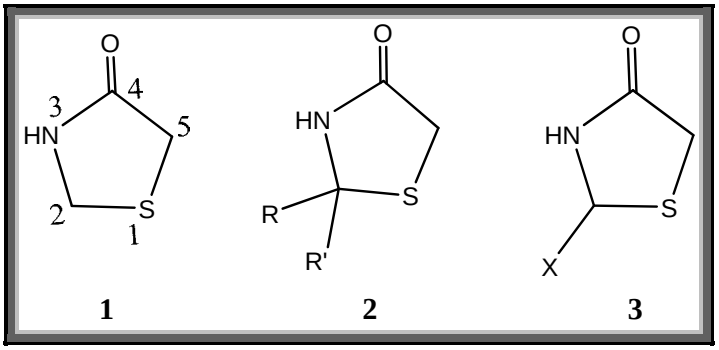
CHAPTER – 5

A Microwave Assisted Synthesis of Thiazolidinones like 3-(2-Methylindoline-1-yl)-2-substituted phenylthiazolidin-4-ones using thioglycolic acid

5.1 SYNTHETIC STRATEGIES FOR 4-THIAZOLIDINONE

Thiazolidinone, a saturated form of thiazoles with carbonyl group on fourth carbon, has been considered as a moiety of choice as it possesses a broad spectrum of pharmacological activities against several targets. This array of biological response profile has attracted the attention of scientists' the world over to further investigate the potential of this organic motif. The referencing on this topic revealed that a lot of work has been done in this particular field in past.

4-Thiazolidinones are derivatives of thiazolidine with a carbonyl group at the 4-position (1). Substituents in the 2-, 3-, and 5-positions may be varied, but the greatest difference in structure and properties is exerted by the group attached to the carbon atom in the 2-position (R and R' in 2 or X in 3). Variations in the substituents attached to the nitrogen atom and the methylene carbon atom are possible for the structures represented by 2 and 3.



1 **2** **3**

Several protocols for the synthesis of 4-thiazolidinones are available in the literature [1-9]. Essentially they are three component reactions involving an amine, a carbonyl compound, and a mercapto-acid. The process can be either a one-pot three-component condensation or a two-step process [10-12].

An improved protocol has been reported wherein N,N-dicyclohexyl carbodiimide (DCC) or 2-(1H-benzotriazo-1-yl)-1,1,3,3-tetramethyl uranium hexafluorophospate (HBTU) is used as a dehydrating agent to accelerate the intramolecular cyclization resulting in faster reaction and improved yields [13-14]. The DCC/HBTU-mediated protocol has the advantage of mild reaction conditions, a very

short reaction time, and product formation in almost quantitative yields. More importantly, yields of the 4-thiazolidinones are independent of the nature of the reactants. This modification is compatible with a solid-phase combinatorial approach to generate a library of compounds.

Cesur et al. and Vicini et al. have reported another method of synthesis of 4-thiazolidinones by the use of thiocyanate, alkylisothiocynate and ammonium thiocyanate with hydrazide/ acetamide, followed by the treatment with ethyl bromoacetate and sodium acetate [15-16].

Ottana et al. also reported synthesis of 4-thiazolidinones using the starting material N-propyl-N0-phenylthiourea, obtained by the reaction of propylamine and phenylisothiocyanate in chloroform at room temperature for 4 h followed by workup under acidic conditions [17].

Fraga-Dubreuil et al. pioneered the use of task-specific ionic liquid as synthetic equivalent of ionic liquid-phase matrice for the preparation of a small library of 4-thiazolidinones [18]. The starting ionic liquid-phase (ethyleneglycol) is functionalized in good yields with 4-(formylphenoxy)butyric acid by using usual esterification reaction conditions (DCC/ DMAP as catalyst). The synthesis of the ionic liquid-phase bound 4-thiazolidinones was performed by a one-pot threecomponent condensation under microwave dielectric heating.

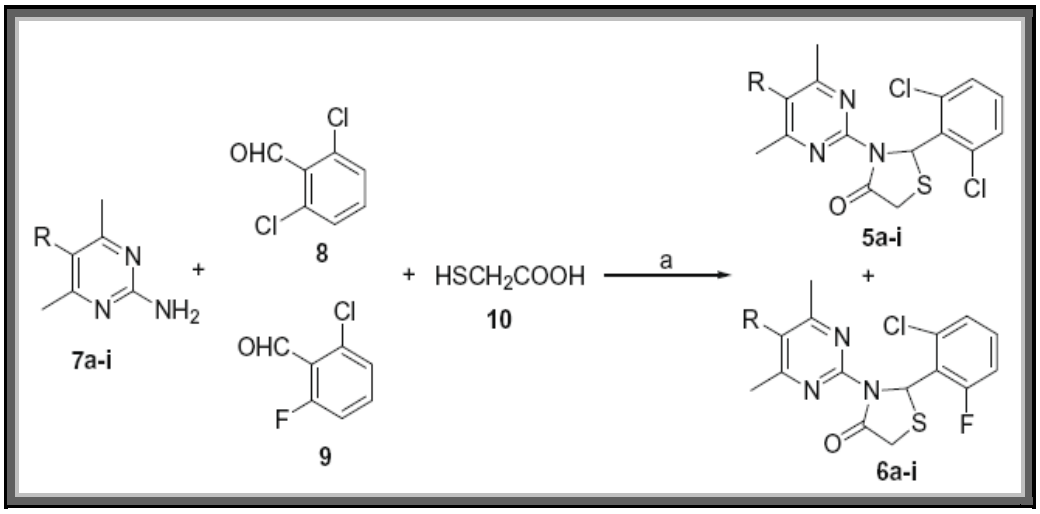
Recently Dandia et al. have reported a microwave-assisted three-component, regioselective one-pot cyclocondensation method for the synthesis of a series of novel spiro[indole-thiazolidinones] using an environmentally benign procedure at atmospheric pressure in an open vessel. This rapid method produces pure products in high yields within few minutes, in comparison to conventional two-step procedure [19].

Holmes et al. reported solution and polymer-supported synthesis of 4-thiazolidinones derived from amino acids [20]. A three-component condensation of an amino ester or resin bound amino acid (glycine, alanine, b-alanine, phenylalanine, and valine), an aldehyde (benzaldehyde, o-tolualdehyde, m-tolualdehyde, p-tolualdehyde,

and 3-pyridine carboxaldehyde), and a a-mercapto carboxylic acid, led to the formation of 5-membered heterocycles.

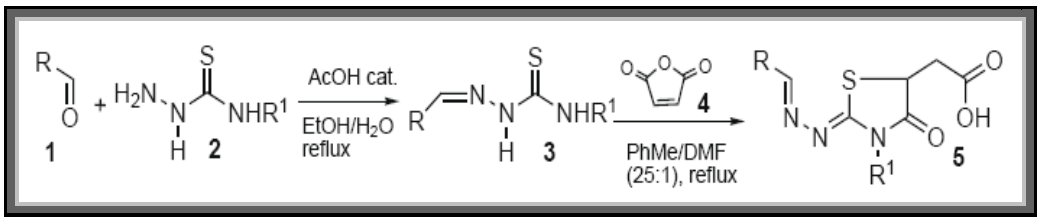
Recently Maclean et al. reported an encoded 4-thiazolidinone library on solid phase [21]. Three sets of 35 building blocks were combined by encoded split-pool synthesis to give a library containing more than 42,000 members. Building block selection was based in part on a novel small molecule follicle stimulating hormone receptor agonist hit and in part for diversity.

H.Chen et. al [22] have reported an improved microwave assisted synthesis of 2-(2,6-dihalophenyl)-3-(5-(un)substitued-4,6-dimethyl pyrimidin- 2-yl)thiazolidin-4-ones in good yields promoted by DCC (Scheme-1), and the reaction process of such intramolecular cycloamidation via the key uncyclized intermediate which they eventually screened for HIV-RT inhibitory activity.

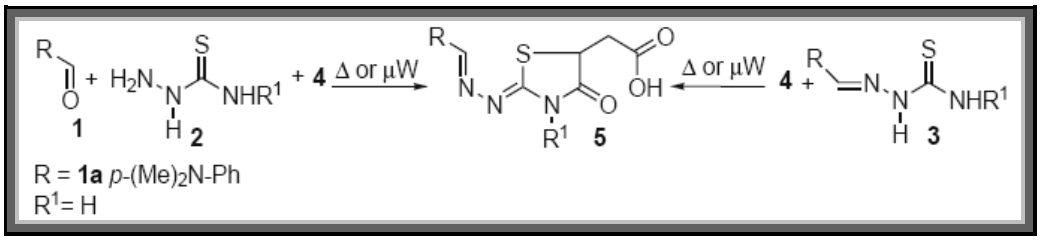


Scheme-1: Synthesis of 5 and 6 Reagents and conditions (a) (1) MW, 140 ^{0}C in sealed tube, 10 min, 7a:8:10 = 1:1:2; (2) MW, 140 ^{0}C in sealed tube, 2 equiv DCC, 5 min.

C. Saiz et. al [23] have reported an efficient tandem procedure for the synthesis of 2-hydrazolyl-4-thiazolidinones under microwave conditions. Different solvents and various reaction equivalents were explored until good isolated yields of thiazolidinones were obtained (Scheme 2). Microwave heating for the synthesis of thiazolidinone 5a resulted in a significantly better yield compared to thermal conditions (75% vs 40%). Microwave irradiation also allowed for a faster conversion. For tandem reactions, the best yields were obtained when a solvent mixture of PhMe/DMF (1:1) was used. Thiazolidinone 5a was prepared in 68% yield using a stepwise sequence and in 82% yield under tandem conditions.



Stepwise 2-hydrazolyl-4-thiazolidinone synthesis under conventional conditions.



Scheme 2: Tandem and stepwise reactions for the synthesis of 2-hydrazolyl-4-thiazolidinone.

5.1.1 *4-THIAZOLIDINONE: A BIOLOGICALLY ACTIVE SCAFOLD*

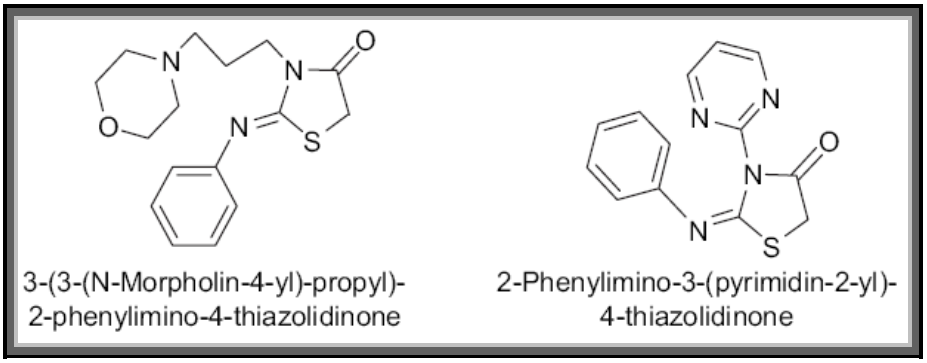
One of the main objectives of organic and medicinal chemistry is the design, synthesis and production of molecules having value as human therapeutic agents. During the past decade, combinatorial chemistry has provided access to chemical libraries based on privileged structures [24], with heterocyclic structures receiving special attention as they belong to a class of compounds with proven utility in medicinal chemistry [25]. There are numerous biologically active molecules with five membered rings, containing two hetero atoms. Thiazolidine is an important scaffold known to be associated with several biological activities [26].

The potency of the thiazolidinone moiety against various diseases is discussed at length in Chapter no. 1 pertaining to the General Introduction of this same thesis. However, the latest references for the same activities discussed in Chapter 1 are given herein viz. Ant-HIV [27], Anti Cancer [28-33], Follicle Stimulating Hormone (FSH) receptor agonist [34, 35], Anti Microbial [36-39] and Anti inflammatory [40].

The activities shown by 4-Thiazolidinone, other than those conversed in Chapter 1, have been discussed herein.

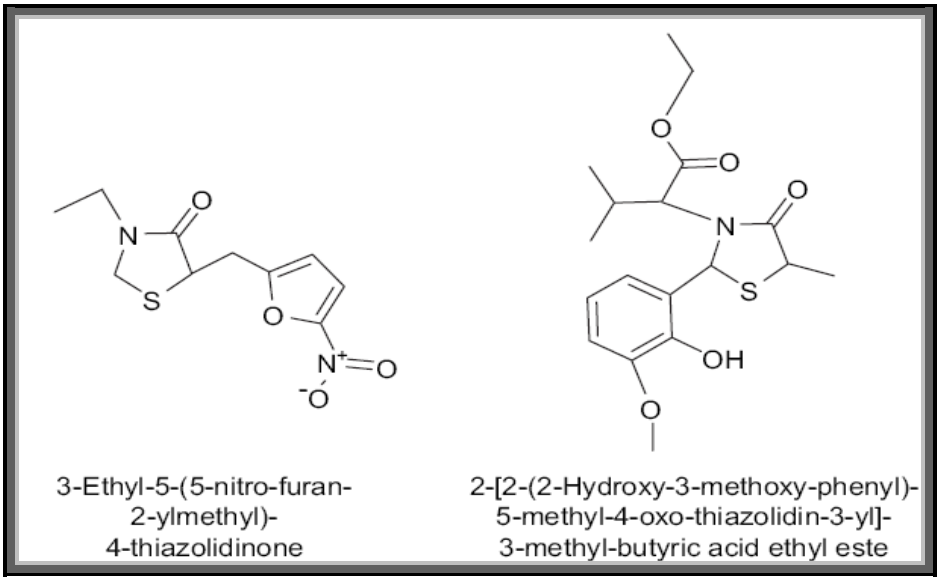
A Hypnotic Activity

Several 3-(3-(N-morpholin-4-yl-propyl)-2-(arylimino)-4-thiazolidinones [41] and 2-(arylimino)-3-(pyrimidin-2-yl)-4-thiazolidinones [42] were evaluated for their ability to potentiate pentobarbital-induced hypnosis in mice at a dose of 100 mg/kg. All thiazolidinones were found to potentiate pentobarbital sleeping time. The increase in the duration of sleep ranged from 10±3 min in untreated control to 98.6±10 min in mice pretreated with substituted thiazolidinones.



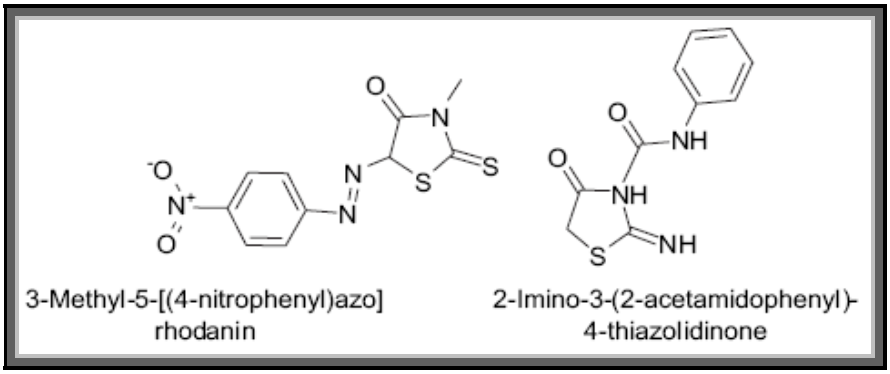
B Anti Tubercular activity

The emergence of multi-drug resistant tuberculosis, coupled with the increasing overlap of AIDS and tuberculosis pandemics has brought tuberculosis to the forefront as a major worldwide health concern. Turkevich et al. reported few 2-Imino-4-thiazolidinone derivatives as possible antitubercular compounds [43]. In other study, 5-(5-Nitrofurfurylidene)-3-ethylrhodanine has been found to be a promising tuberculostatic compound [44]. Few derivatives of 2-Imino-4-thiazolidinones have also been reported as having antitubercular activity with low toxicity. Repeated therapeutic doses were found to possess antitubercular activity comparable to streptomycin or phthivazid [45]. Kapustayak et al. has studied structure-tuberculostatic activity relationship of some 4-Thiazolidinones [46]. Another study reported chemotherapeutic effectiveness against Myobacterium tuberculosis. A few derivatives were found to inhibit the growth of human tubercle bacilli, H37Rv strain, in a concentration of 12.5 mg/mL [47]. Several other derivatives of thiazolidinones have also been found to inhibit the growth of Myobacterium tuberculosis H37Rv strain [48, 49]. In an attempt to find new inhibitors of the enzymes in the essential rhamnose biosynthetic pathway, a virtual library of 2,3,5 trisubstituted-4-thiazolidinones was created.



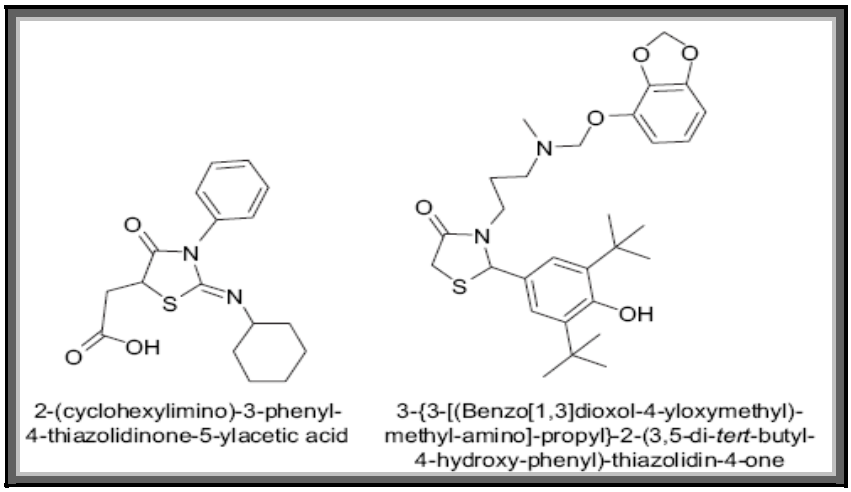
C Anthelmintic Activity

3-Methyl-5-[(4-nitrophenyl)azo]rhodanin, nitrodan, was reported as a potent anthelmintic compound [50-52] which was effective when administered in feed against Hymenolepsis nana and Syphacia obvelata infections in mice, Asceridia galli infections in chickens, and Toxocera canis, Ancylostoma caninum, and Uncinaria stenocephala infections in dogs, pigs and horses. 2-Imino-3-(2-acetamidophenyl)-4-thiazolidinone derivatives have been found to be effective in vitro against horse Strongyloids at concentration of 10±3-10±6 M [53]. Various 2-Thiono-3-substituted-5-[(2-methyl-4-nitrophenyl) azo]-4-thiazolidinones and 2-Thiono-3-methyl-5-[(2,4-dinitrophenyl)azo]-4-thiazolidinone as potent anthelmintic agents, which were not only effective alone but also showed activity with other parasiticides [54, 55].



D Cardiovascular Activity

Cardiovascular effects of a series of 2-Cyclopentyl/(cyclohexylimino)-3-aryl-4-thiazolidinone-5-ylacetic acids on adult cats of either sex were reported [56]. All substituted 4-Thiazolidinones induced hypotension of varying degree. The duration of hypotensive activity observed with most of these compounds was less than 15 min.

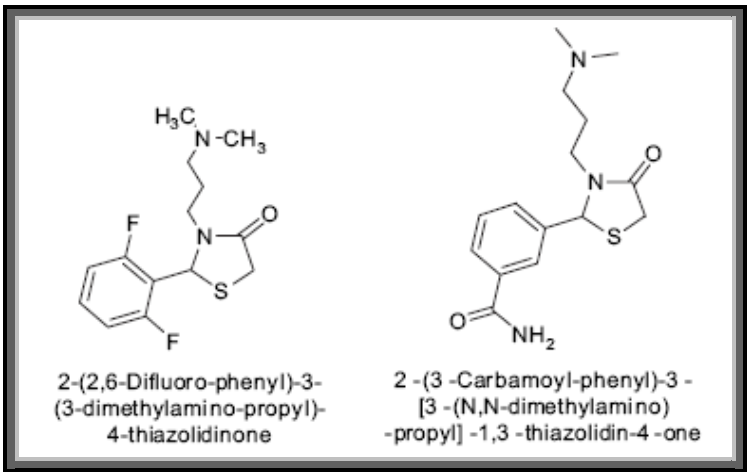


Suzuki et al. examined the effects of CP-060S (3-{3-[(Benzo[1,3]dioxol-4-yloxymethyl)-methyl-amino]-propyl}-2-(3,5-di-tert-butyl-4-hydroxy-phenyl)-4-thiazolidinone) on cardiac function and myocardial oxygen consumption (MVO2) in anesthetized dogs [57]. CP-060S (10-300 mg/kg IV) decreased heart rate, increased aortic flow and decreased mean blood pressure in a dose-dependent manner. The PR (pulse rate) interval was significantly prolonged by administration of CP-060S (300 mg/kg IV). It increased coronary blood flow in a dose-dependent manner (10-300 mg/kg IV). Left ventricular end-diastolic pressure and maximal first derivative of left ventricular pressure were not significantly affected. CP- 060S (10-300 mg/kg IV) increased coronary sinus blood flow and decreased arteriovenous oxygen difference and MVO2 in a dose-dependent manner. Its effect on cardiac function and MVO2 are qualitatively similar to those of diltiazem, a typical Ca-channel blocker.

E Antihistaminic activity (H1-antagonist)

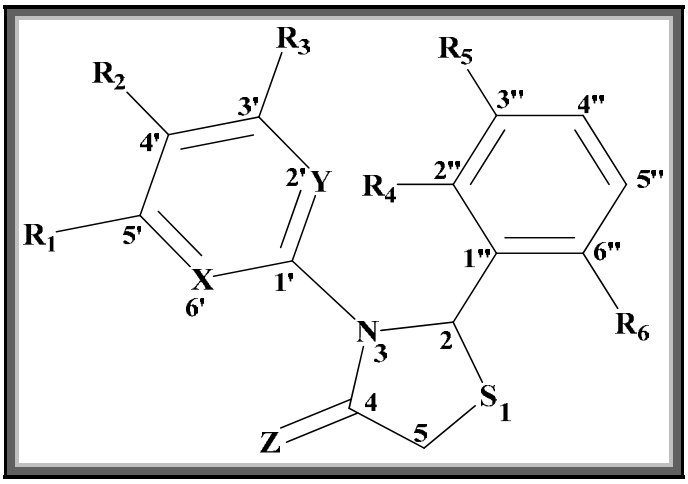
Thiazolidinones are known to show their action on histamine receptors. The geometrical similarity between 2-Aryl-3-[3-(N,N-dimethylamino)propyl]-1,3-thiazolidin-4-ones and different histamine (H1) antagonists such as bamipine, clemastine, cyproheptadine, triprolidine, promethazine, chlorpheniramine, and carbinoxamine [58, 59] prompted Diurno et al. to evaluate these compounds for antihistaminic activity [60]. Singh et al. have investigated the antihistaminic (H1-antagonist) activity of 2,3-disubstituted thiazolidin-4-ones and concluded that the hydrophobic substitution at the 4-position of the phenyl ring and cumulative negative

polar effects of all the substituents in the phenyl group are advantageous for antihistaminic activity [61, 62].



In another study, Diurno et al. synthesized, characterized and evaluated a new series of 2-(Substituted-phenyl)-3-[3-(N,N-dimethylamino)-propyl]-1,3-thiazolidin-4-ones for their capacity to inhibit the contraction induced by histamine on guinea pig ileum [63]. 2-(3-Carbamoyl-phenyl)-3-[3-(N,N-dimethylamino)-propyl]-1,3-thiazolidin-4-one and derivatives of series as free bases were converted into the corresponding hydrochlorides for the pharmacological assays. The H1-antihistaminic activity of the synthesized compounds was evaluated in vitro by measuring their ability to inhibit the histamine-induced contractions of isolated guinea pig ileum [64]. Results show that whenever the phenyl moiety of the 4-thiazolidinones interacts with a complementary area of the H1-receptor, the p interaction was enhanced by hydrophobic substituents increasing the HOMO energy and is affected by the size of the 4-alkyl substituent. These studies have highlighted the importance of overall hydrophobicity of the compounds in deciding the antihistaminic activity [65, 66].

V. Ravichandran et.al [67] have predicted the Anti-HIV activity for Thiazolidin-4-ones using 3D-QSAR approach. The basic structure for their studies is given below.



The observations arrived based on the 3D-QSAR calculations are as under.

- The electron withdrawing groups such as F, Cl, Br, CN etc., on 3', 2", 6" will increase the Anti-HIV activity.
- The electron withdrawing group at position 4' has detrimental effect on Anti HIV activity.
- If 2', 4', 2", 3", 6" have bulky groups then they will impart negative impact on the activity.
- Smaller electronegative groups at 2", 6" will increase the activity.
- Hydrophobic groups at position 3', 2", 6" augment the Anti-HIV activity.
- Hydrophobic groups at position, 4', 3", and 4" are not favorable for biological activity.
- Hydrogen bond acceptors on position no. 3', 2", 6" will increase the activity.
- Hydrogen bond donors at 4' is a favorable condition whereas the same at position no 2" and 6" will have a detrimental effect on the activity.

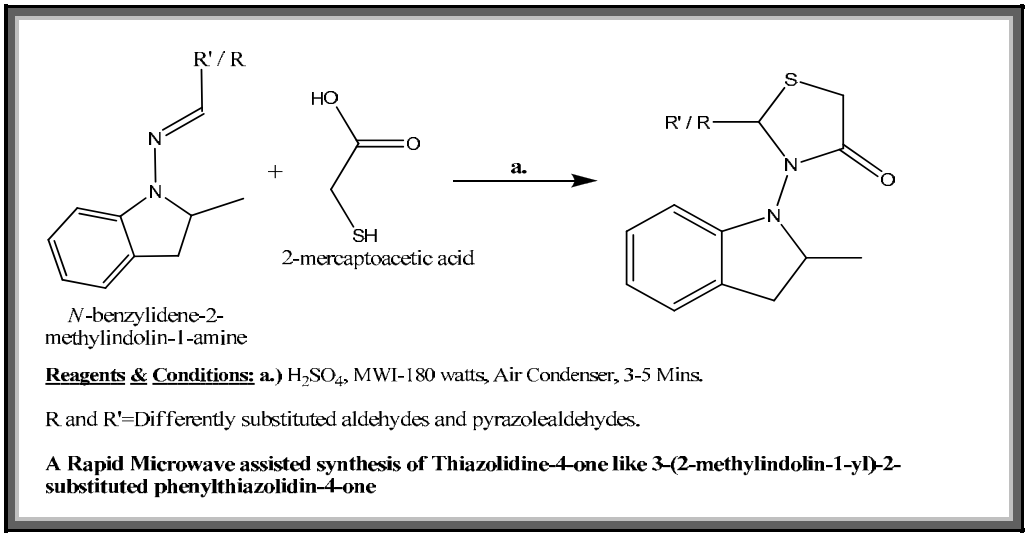
5.2 AIM OF CURRENT WORK

Thiazolidinones have been extensively explored in the past decades for various therapeutic activities using a Fragment Based Drug Discovery (FBDD) approach. Moreover, the indole moiety, a privileged structure has its own advantages when it comes to biological activity. The Mannich reactions carried out with the indole moiety in our laboratoy has shown promising anti cancer results[a]. Also the literature revealed that the thiazolidine-4-one have shown promising results in Anti HIV activity.

In the current work we intended to synthesize various 4-Thiazolidinones appended with 2-methyl indoline as a core fragment and also to study their biological activity.

[a] Joshipura, D, N; Shah, A, K. Ph.D. Thesis-2009, Department of Chemistry, Saurashtra University, Rajkot

5.3 REACTION SCHEME



N-benzylidene-2-methylindolin-1-amine

Reagents & Conditions: a.) H_2SO_4, MWI-180 watts, Air Condenser, 3-5 Mins.

R and R'=Differently substituted aldehydes and pyrazolealdehydes.

A Rapid Microwave assisted synthesis of Thiazolidine-4-one like 3-(2-methylindolin-1-yl)-2-substituted phenylthiazolidin-4-one

5.3.1 *PHYSICAL DATA TABLE*

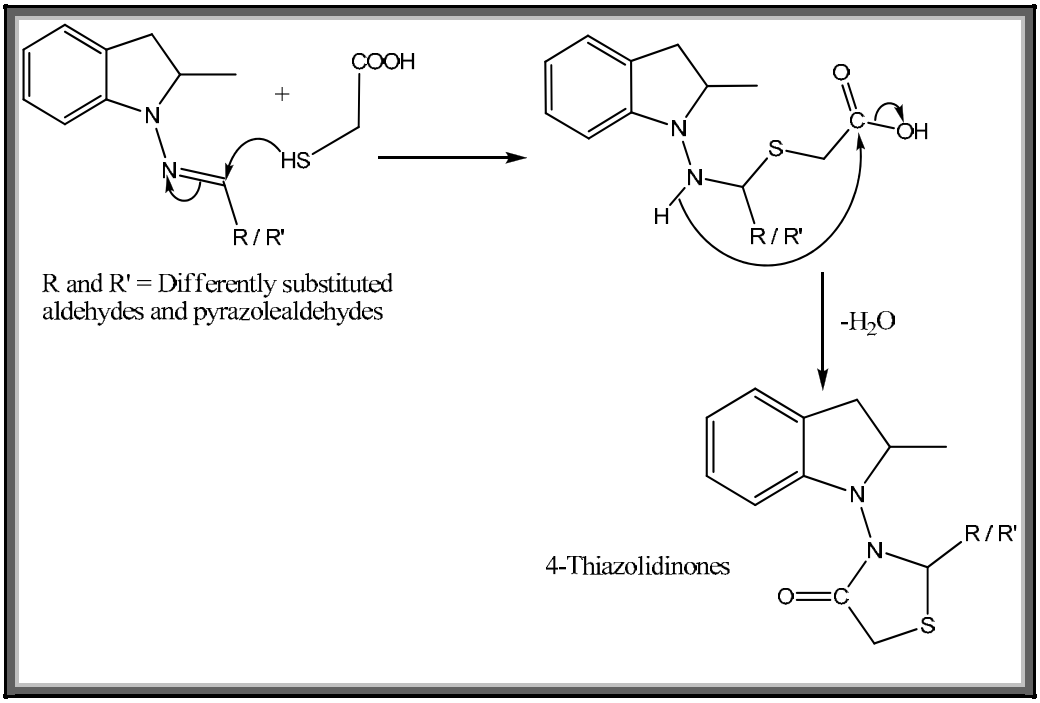
Code	R / R'	M. F.	M. W.	M. P. ^{0}C	Time (min)	Yield %	R_f
TZD-2-1-01	**R** = H	$C_{18}H_{18}N_2OS$	310.41	178-180	5.20	52	0.54
TZD-2-1-02	**R** = 3-Cl	$C_{18}H_{17}ClN_2OS$	344.86	190-192	4.50	64	0.50
TZD-2-1-03	**R** = 3,4-OCH_3	$C_{20}H_{22}N_2O_3S$	370.47	206-208	6.30	55	0.52
TZD-2-1-06	**R** = 3-NO_2	$C_{18}H_{17}N_3O_3S$	355.41	174-176	4.30	75	0.55
TZD-2-1-07	**R** = 4-Cl	$C_{18}H_{17}ClN_2OS$	344.86	208-210	5.00	70	0.54
TZD-2-1-10	**R** = 4-NO_2	$C_{18}H_{17}N_3O_3S$	355.41	212-214	5.30	64	0.58
TZD-1-1-01	**R'** = H	$C_{27}H_{24}N_4OS$	452.57	218-220	6.00	66	0.48
TZD-1-1-02	**R'** = 4-Cl	$C_{27}H_{23}ClN_4OS$	487.02	222-224	6.30	55	0.45
TZD-1-1-03	**R'** = 4-NO_2	$C_{27}H_{23}N_5O_3S$	497.57	214-216	6.10	70	0.50
TZD-1-1-09	**R'** = 4-Br	$C_{27}H_{23}BrN_4OS$	531.47	242-244	6.40	60	0.52

TLC solvent system for R_f : Toluene:Ethyl acetate - 7:3.

Microwave Irradiation: 180 Watts.

5.4 PLAUSIBLE REACTION MECHANISM

5.4.1 *Formation Of 4-Thiazolidinone from N-Benzylidene-2-methylindolin-1-amine*



The reaction proceeds by the attack of mercapto acetic acid upon the C=N group, with the HS-CH_2-COOH adding to the carbon atom followed by capture of the proton by nitrogen and subsequent cyclization. During the reaction an uncyclized intermediate is formed in few cases. In many instances 4-Thiazolidinones can conveniently be prepared by refluxing the mixture of thioglycolic acid and the Schiff base in benzene, dry ether or ethanol.

The nucleophilic attack of the mercaptoacetic acid anion will take place on the carbon of azomethine which has got a positive character; while it is evident that the nitrogen has the negative character. Simultaneous removal of water that forms in the reaction helps in condensation and determination of the reaction time.

5.5 EXPERIMENTAL

5.5.1 *MATERIALS AND METHODS*

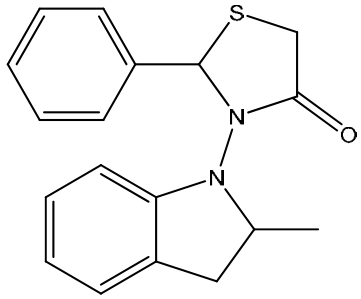
Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. All the reactions were carried out in Samsung MW83Y Microwave Oven which was locally modified for carrying out chemical reactions. IR spectra were recorded in Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

5.5.2 *GENERAL PROCEDURE: 3-(2-Methylindolin-1-yl)-2-substituted phenyl thiazolidin-4-ones*

A neat fusion reaction has been carried out in the presence of strong acid such as sulfuric acid to prepare the thiazolidine-4-one. 0.005 moles of N-substituted benzylidene-2-methylindoline-1-amine was taken in an Erlenmeyer flask. Slurry was prepared using 5 to 10 times i.e. 0.05 moles of Thioglycolic Acid which served as the solvent for the reaction as well hence we call it a neat fusion. Very few drops of sulfuric acid were added in the reaction mixture to catalyze the reaction. The reaction mixture was then subjected to microwave irradiation for a specific time (see Physical data Table) at low power (180 W) with an air condenser attached to the reaction assembly. The progress of the reaction was monitored by TLC examination at an interval of every 10 seconds. On completion of reaction, it was cooled at room temperature and poured onto ice water which afforded the crude product. Sodium bicarbonate was added to maintain the pH at 6-7. The organic mass was extracted using ethyl acetate thrice. The combined organic extracts were then washed thrice with demineralized water to remove traces of acids from the reaction. After separation, sodium sulphate was put in the combined organic extracts to remove any traces of water and then vacuum distilled. The product thus obtained was filtered, washed with cold water, dried, and recrystallized using DMF.

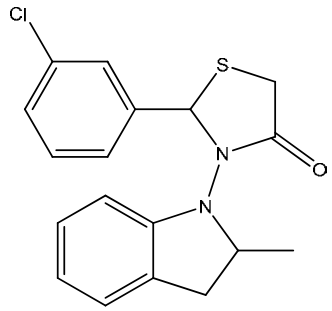
5.6 ANALYTICAL DATA

5.6.1 3-(2-Methylindolin-1-yl)-2-phenylthiazolidin-4-one (TZD-2-1-01)



Yield: 52%; M.P.- 178-180 ºC; IR (cm^{-1}): 1615 (Ring stretching for Indoline), 1414 (Ring stretching modes for thiazolidinone ring), 875-660 (C-H out of plane bending for Thiazolidinone ring, several bands observed), 2972-2935 (C-H asymmetric stretching for R-CH_3), 2855 (C-H symmetric stretching for R-CH_3), 1447 (C-H asymmetric bending for R-CH_3), 1382 (C-H symmetric bending for R-CH_3), 3080-3000 (C-H stretching frequency for aromatic region), 1590 (C-C skeletal stretching of phenyl nucleus), 1238 (C-H in plane bending for phenyl ring), 710 (C-C out of plane bending for mono substituted benzene ring), 2925 (C-H stretching frequency for ketone), 1750 (C=O stretching frequency for ketone in 5 membered saturated ring), 1080-1025 (C-N stretching frequency for tertiary amine); MS: *m/z:* 310.11; Anal. Calcd. for $C_{18}H_{18}N_2OS$: C, 69.65; H, 5.84; N, 9.02; O, 5.15, S, 10.33; Found: C, 69.61; H, 5.80; N, 9.00; O, 5.12; S, 10.28.

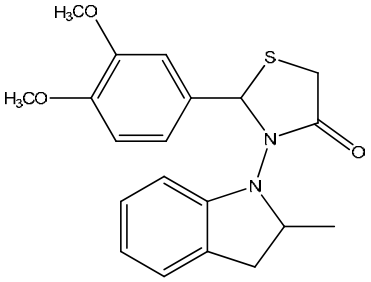
5.6.2 2-(3-Chlorophenyl)-3-(2-methylindolin-1-yl)thiazolidin-4-one (TZD-2-1-02)



Yield: 64%; M.P.- 190-192 ºC; IR (cm^{-1}): 1600 (Ring stretching for Indoline), 1393 (Ring stretching modes for thiazolidinone ring), 860-640 (C-H out of plane bending for Thiazolidinone ring, several bands observed), 2945-2915 (C-H asymmetric stretching for R-CH_3), 2838 (C-H symmetric stretching for R-CH_3), 1430 (C-H asymmetric bending for R-CH_3), 1359 (C-H symmetric bending for R-CH_3), 3095-3005 (C-H stretching frequency for aromatic region), 1575 (C-C skeletal stretching of phenyl nucleus), 1216 (C-H in plane bending for phenyl ring), 705 (C-H out of plane bending for 1,3-Disubstituted benzene ring), 2905 (C-H stretching frequency for ketone), 1730 (C=O stretching frequency for ketone in 5 membered saturated ring), 1066-1007 (C-N stretching frequency for tertiary amine), 757 (C-Cl stretching frequency for mono chlorinated aromatic

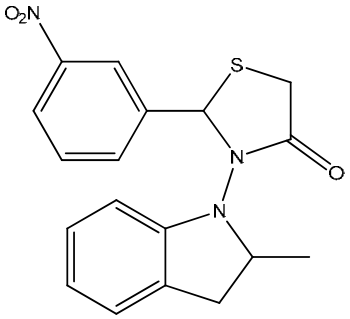
compounds); MS: *m/z:* 344.08; Anal. Calcd. for $C_{18}H_{17}ClN_2OS$: C, 62.69; H, 4.97; Cl, 10.28; N, 8.12; O, 4.64; S, 9.30; Found: C, 62.63; H, 4.94; Cl, 10.25; N, 8.10; O, 4.61; S, 9.23

5.6.3 2-(3,4-Dimethoxyphenyl)-3-(2-methylindolin-1-yl)thiazolidin-4-one (TZD-2-1-03)



Yield: 55 %; M.P.- 206-208 ºC; IR (cm^{-1}): 1611 (Ring stretching for Indoline), 1409 (Ring stretching modes for thiazolidinone ring), 859-637 (C-H out of plane bending for Thiazolidinone ring, several bands observed), 2958-2915 (C-H asymmetric stretching for R-CH_3), 2861 (C-H symmetric stretching for R-CH_3), 1444 (C-H asymmetric bending for R-CH_3), 1388 (C-H symmetric bending for R-CH_3), 3069-3019 (C-H stretching frequency for aromatic region), 1597 (C-C skeletal stretching of phenyl nucleus), 1233 (C-H in plane bending for phenyl ring), 887 (C-H out of plane bending for 1,2,4-trisubstituted benzene ring), 2925 (C-H stretching frequency for ketone), 1744 (C=O stretching frequency for ketone in 5 membered saturated ring), 1089-1030 (C-N stretching frequency for tertiary amine), 3110 (C-H stretching for aryl-alkyl ether); MS: *m/z:* 370.14; Anal. Calcd. for $C_{20}H_{22}N_2O_3S$: C, 64.84; H, 5.99; N, 7.56; O, 12.96; S, 8.66; Found: C, 64.80; H, 5.94; N, 7.51; O, 12.93; S, 8.62.

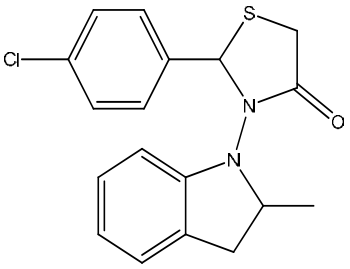
5.6.4 3-(2-Methylindolin-1-yl)-2-(3-nitrophenyl)thiazolidin-4-one (TZD-2-1-06):



Yield: 75 %; M.P.- 174-176 ºC; IR (cm^{-1}): 1617 (Ring stretching for Indoline), 1409 (Ring stretching modes for thiazolidinone ring), 866-680 (C-H out of plane bending for Thiazolidinone ring, several bands observed), 2968-2931 (C-H asymmetric stretching for R-CH_3), 2852 (C-H symmetric stretching for R-CH_3), 1438 (C-H asymmetric bending for R-CH_3), 1373 (C-H symmetric bending for R-CH_3), 3094-3015 (C-H stretching frequency for aromatic region), 1579 (C-C skeletal stretching of phenyl nucleus), 1223 (C-H in plane bending for phenyl ring), 717 (C-C out of plane bending for 1,3-Disubstituted benzene ring), 2921 (C-H stretching frequency for ketone), 1746 (C=O stretching frequency for

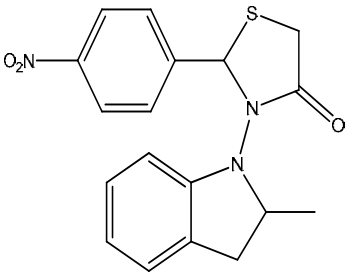
ketone in 5 membered saturated ring), 1083-1021 (C-N stretching frequency for tertiary amine), 1539-1527 (NO_2 asymmetric stretching frequency for aromatic Nitro group), 1308 (NO_2 symmetric stretching for aromatic NO_2 group); MS: *m/z:* 355.10; Anal. Calcd. for $C_{18}H_{17}N_3O_3S$: C, 60.83; H, 4.82; N, 11.82; O, 13.50; S, 9.02; Found: C, 60.79; H, 4.78; N, 11.79; O, 13.45; S, 9.00.

5.6.5 2-(4-Chlorophenyl)-3-(2-methylindolin-1-yl)thiazolidin-4-one (TZD-2-1-07)



Yield: 70%; M.P.- 208-210 ºC; IR (cm^{-1}): 1603 (Ring stretching for Indoline), 1417 (Ring stretching modes for thiazolidinone ring), 873-657 (C-H out of plane bending for Thiazolidinone ring, several bands observed), 2975-2915 (C-H asymmetric stretching for R-CH_3), 2826 (C-H symmetric stretching for R-CH_3), 1430 (C-H asymmetric bending for R-CH_3), 1378 (C-H symmetric bending for R-CH_3), 3083-3017 (C-H stretching frequency for aromatic region), 1563 (C-C skeletal stretching of phenyl nucleus), 1229 (C-H in plane bending for phenyl ring), 826 (C-H out of plane bending for 1,4-Disubstituted benzene ring), 2917 (C-H stretching frequency for ketone), 1732 (C=O stretching frequency for ketone in 5 membered saturated ring), 1064-1006 (C-N stretching frequency for tertiary amine), 723 (C-Cl stretching for monochlorinated aromatic compounds); MS: *m/z:* 344.08; Anal. Calcd. for $C_{18}H_{17}ClN_2OS$: C, 62.69; H, 4.97; Cl, 10.28; N, 8.12; O, 4.64; S, 9.30; Found: C, 62.66; H, 4.95; Cl, 10.25; N, 8.09; O, 4.61; S, 9.26.

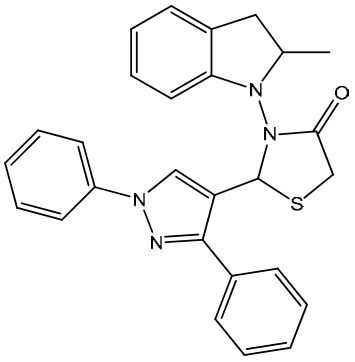
5.6.6 3-(2-Methylindolin-1-yl)-2-(4-nitrophenyl)thiazolidin-4-one (TZD-2-1-10)



Yield: 64 %; M.P.- 212-214 ºC; IR (cm^{-1}): 1606 (Ring stretching for Indoline), 1402 (Ring stretching modes for thiazolidinone ring), 871-650 (C-H out of plane bending for Thiazolidinone ring, several bands observed), 2960-2922 (C-H asymmetric stretching for R-CH_3), 2850 (C-H symmetric stretching for R-CH_3), 1433 (C-H asymmetric bending for R-CH_3), 1375 (C-H symmetric bending for R-CH_3), 3088-3005 (C-H stretching frequency for aromatic region), 1583 (C-C skeletal stretching of phenyl nucleus), 1224 (C-H in plane bending for phenyl ring), 831 (C-H

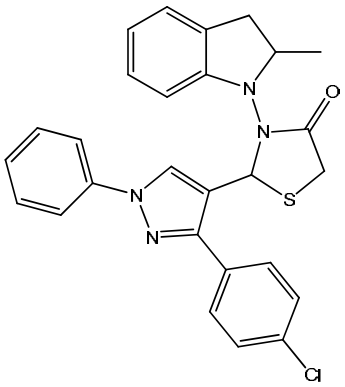
out of plane bending for 1,4-Disubstituted benzene ring), 2914 (C-H stretching frequency for ketone), 1739 (C=O stretching frequency for ketone in 5 membered saturated ring), 1074-1016 (C-N stretching frequency for tertiary amine), 1531-1525 (NO_2 asymmetric stretching frequency for aromatic Nitro group), 1303 (NO_2 symmetric stretching for aromatic NO_2 group); ^{1}H NMR (DMSO-d_6) δ ppm: 1.27-1.28 (d, 3H, H_1), 4.47-4.52 (m, 1H, H_2), 2.69-2.74 (d, 1H, H_3), 3.39-3.46 (q, 1H, H_3), 7.05-7.07 (d, 1H, H_4, *J*=8 *Hz*), 7.13-7.16 (m, 2H, H_5 & H_7), 6.79-6.83 (m, 1H, H_6), 7.37 (s, 1H, H_8), 7.64-7.66 (d, 2H, H_9 & H_{12}, J value= *8 Hz*), 8.09-8.11 (d, 2H, H_{10} & H_{11}, J value= *8 Hz*), 5.37 (s, 2H, H_{13}); MS: *m/z:* 355.10; Anal. Calcd. for $C_{18}H_{17}N_3O_3S$: C, 60.83; H, 4.82; N, 11.82; O, 13.50; S, 9.02 Found: C, 60.78; H, 4.77; N, 11.78; O, 13.46; S, 9.00.

5.6.7 3-(2-Methylindolin-1-yl)-2-(1,3-diphenyl-1H-pyrazol-4-yl)thiazolidin-4-one-(TZD-1-1-01)



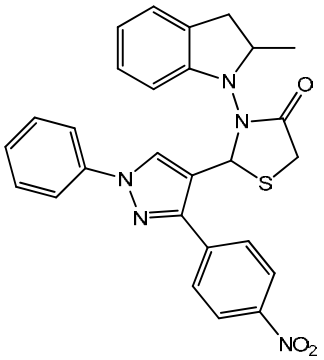
Yield: 66 %; M.P.- 218-220 ºC; IR (cm^{-1}): 1612 (Ring stretching for Indoline), 1645 (Ring stretching for Pyrazole), 1537 (Ring stretching modes for thiazolidinone ring), 885 (C-H out of plane bending for Thiazolidinone ring, several bands observed), 2963 (C-H asymmetric stretching for R-CH_3), 1429 (C-H asymmetric bending for R-CH_3), 3128-3047 (C-H stretching frequency for aromatic region), 1224 (C-H in plane bending for phenyl ring), 703 (C-C out of plane bending for mono substituted benzene ring), 2918 (C-H stretching frequency for ketone), 1731 (C=O stretching frequency for ketone in 5 membered saturated ring), 1453 (CH_2 bending for –CH_2-C=O group), 1017 (C-N stretching frequency for tertiary amine); MS: *m/z:* 452.17; Anal. Calcd. for $C_{27}H_{24}N_4OS$: C, 71.65; H, 5.35; N, 12.38; O, 3.54; S, 7.09 Found: C, 71.62; H, 5.31; N, 12.33; O, 3.51; S, 7.06.

5.6.8 2-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(2-methylindolin-1-yl)thiazolidin-4-one (TZD-1-1-02):



Yield: 55 %; M.P.- 222-224 ºC; IR (cm^{-1}): 1608 (Ring stretching for Indoline), 1659 (Ring stretching for Pyrazole), 1522 (Ring stretching modes for thiazolidinone ring), 894 (C-H out of plane bending for Thiazolidinone ring, several bands observed), 2964 (C-H asymmetric stretching for R-CH_3), 1434 (C-H asymmetric bending for R-CH_3), 3127-3055 (C-H stretching frequency for aromatic region), 1225 (C-H in plane bending for phenyl ring), 835 (C-H out of plane bending for 1,4-Disubstituted benzene ring), 2917 (C-H stretching frequency for ketone), 1738 (C=O stretching frequency for ketone in 5 membered saturated ring), 1453 (CH_2 bending for –CH_2-C=O group), 1013 (C-N stretching frequency for tertiary amine), 722 (C-Cl stretching for mono chlorinated aromatic compounds); MS: *m/z:* 486.13; Anal. Calcd. for $C_{27}H_{23}ClN_4OS$: C, 66.59; H, 4.76; Cl, 7.28; N, 11.50; O, 3.29; S, 6.58; Found: C, 66.56; H, 4.72; Cl, 7.23; N, 11.46; O, 3.25; S, 6.55.

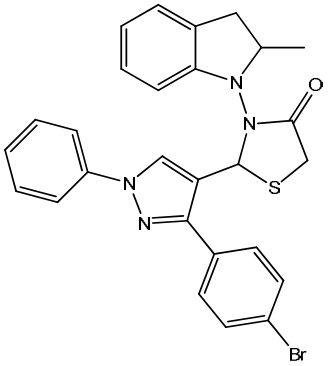
5.6.9 3-(2-Methylindolin-1-yl)-2-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)thiazolidin-4-one (TZD-1-1-03):



Yield: 70 %; M.P.- 214-216 ºC; IR (cm^{-1}): 1606 (Ring stretching for Indoline), 1654 (Ring stretching for Pyrazole), 1527 (Ring stretching modes for thiazolidinone ring), 898 (C-H out of plane bending for Thiazolidinone ring, several bands observed), 2966 (C-H asymmetric stretching for R-CH_3), 1437 (C-H asymmetric bending for R-CH_3), 3126-3053 (C-H stretching frequency for aromatic region), 1228 (C-H in plane bending for phenyl ring), 837 (C-H out of plane bending for 1,4-Disubstituted benzene ring), 2922 (C-H stretching frequency for ketone), 1737 (C=O stretching frequency for ketone in 5 membered saturated ring), 1450 (CH_2 bending for –CH_2-C=O group), 1016 (C-N stretching frequency for tertiary amine), 1332 (NO_2 symmetric stretching for aromatic NO_2 group); ^{1}H NMR (DMSO-d_6) δ ppm: 1.37-1.39 (d, 3H, H_1), 4.49-4.53 (m, 1H, H_2), 2.78-2.83 (d, 1H, H_3), 3.48-3.54 (q, 1H, H_3),

7.06-7.08 (d, 1H, H_4, *J*=8 *Hz*), 7.14-7.22 (m, 2H, H_5 & H_7), 6.84-6.88 (t, 1H, H_6), 7.62 (s, 1H, H_8), 7.36-7.40 (t, 1H, H_9), 7.50-7.55 (t, 2H, H_{10} & H_{14}), 8.34-8.38 (m, 3H, H_{11}, H_{12}, H_{13}), 7.82-7.85 (d, 2H, H_{15} & H_{18}, J *value=11.6 Hz*), 8.01-8.04 (d, 2H, H_{16} & H_{17}, *J Value=13.6*), 5.45 (s, 2H, H_{19}); MS: *m/z:* 497.15; Anal. Calcd. for $C_{27}H_{23}N_5O_3S$: C, 65.17; H, 4.66; N, 14.08; O, 9.65; S, 6.44; Found: C, 65.13; H, 4.62; N, 14.02; O, 9.61; S, 6.40.

5.6.10 2-(3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(2-methylindolin-1-yl)thiazolidin-4-one (TZD-1-1-09):



Yield: 60 %; M.P.- 242-244 ºC; IR (cm^{-1}): 1612 (Ring stretching for Indoline), 1667 (Ring stretching for Pyrazole), 1537 (Ring stretching modes for thiazolidinone ring), 882 (C-H out of plane bending for Thiazolidinone ring, several bands observed), 2960 (C-H asymmetric stretching for R-CH_3), 1431 (C-H asymmetric bending for R-CH_3), 3120-3055 (C-H stretching frequency for aromatic region), 1229 (C-H in plane bending for phenyl ring), 831 (C-H out of plane bending for 1,4-Disubstituted benzene ring), 2927 (C-H stretching frequency for ketone), 1735 (C=O stretching frequency for ketone in 5 membered saturated ring), 1458 (CH_2 bending for –CH_2-C=O group), 1013 (C-N stretching frequency for tertiary amine), 537 (C-Br stretching for aromatic ring); MS: *m/z:* 532.08; Anal. Calcd. for $C_{27}H_{23}BrN_4OS$: C, 61.02; H, 4.36; Br, 15.03; N, 10.54; O, 3.01; S, 6.03; Found: C, 60.99; H, 4.32; Br, 15.01; N, 10.52; O, 3.00; S, 6.01.

5.7 SPECTRAL DISCUSSION

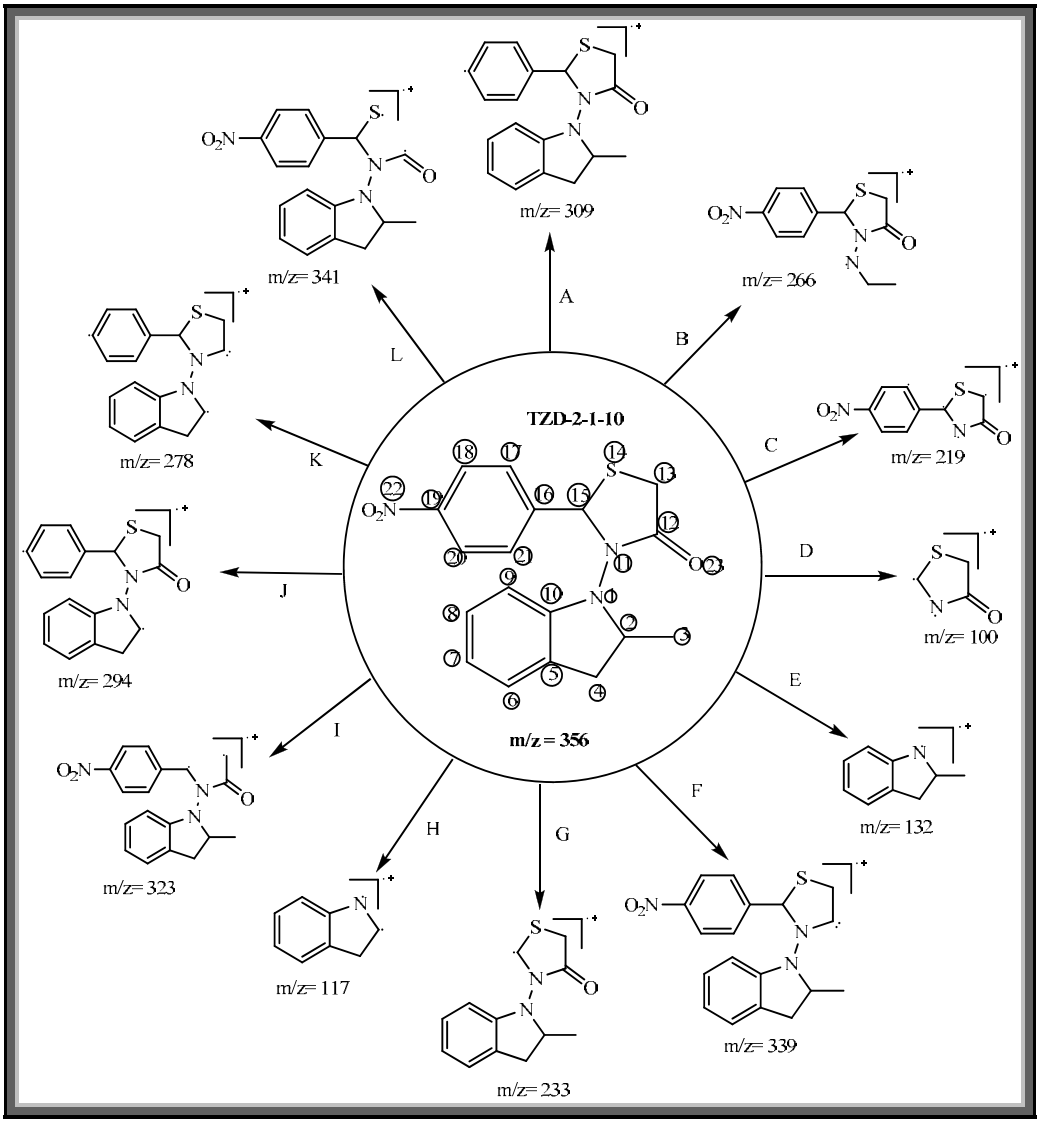
5.7.1 *IR SPECTRAL STUDY*

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. The characteristic bands of ring stretching for indoline were observed at 1600-1500 cm^{-1}. The ring stretching mode for thiazolidinone gave frequency in the range of 1540-1400 cm^{-1} while the C-H out of plane bending for thiazolidinone gave several characteristic bands around 900-650 cm^{-1}. The pyrazole ring showed ring stretching bands around 1650-1380 cm^{-1}. The C-H asymmetric stretching for methyl moiety was seen around 2972-2952 cm^{-1}, similarly the C-H symmetric stretching for methyl moiety gave characteristic bands near 2885-2860 cm^{-1}. The asymmetric bending frequencies of methyl were observed in the range of 1475-1450 cm^{-1} while the symmetric bending frequencies gave the characteristic bands near 1383-1377 cm^{-1}. The C-H aromatic stretching frequencies were observed between 3100-3000 cm^{-1} and the in plane bending frequencies of the same were found at 1250-950 cm^{-1}. The out of plane bending frequencies for aromatic region were observed around 830-700 cm^{-1}. The C-H stretching frequencies for the ketone function gave characteristic bands at 2925-2850 cm^{-1}, while it's C=O stretching frequency was observed at 1750-1740 cm^{-1}. The C-N stretching frequencies for tertiary amine were seen at 1350-1020 cm^{-1}. The C-Halogen stretching frequencies for aromatic rings were observed around 750-500 cm^{-1}. The above mentioned respective frequencies suggest the correct formation of the desired products.

5.7.2 *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. The probable Mass fragmentation pattern for the representative compound of each series is discussed below.

5.7.2.1 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF TZD-2-1-10*

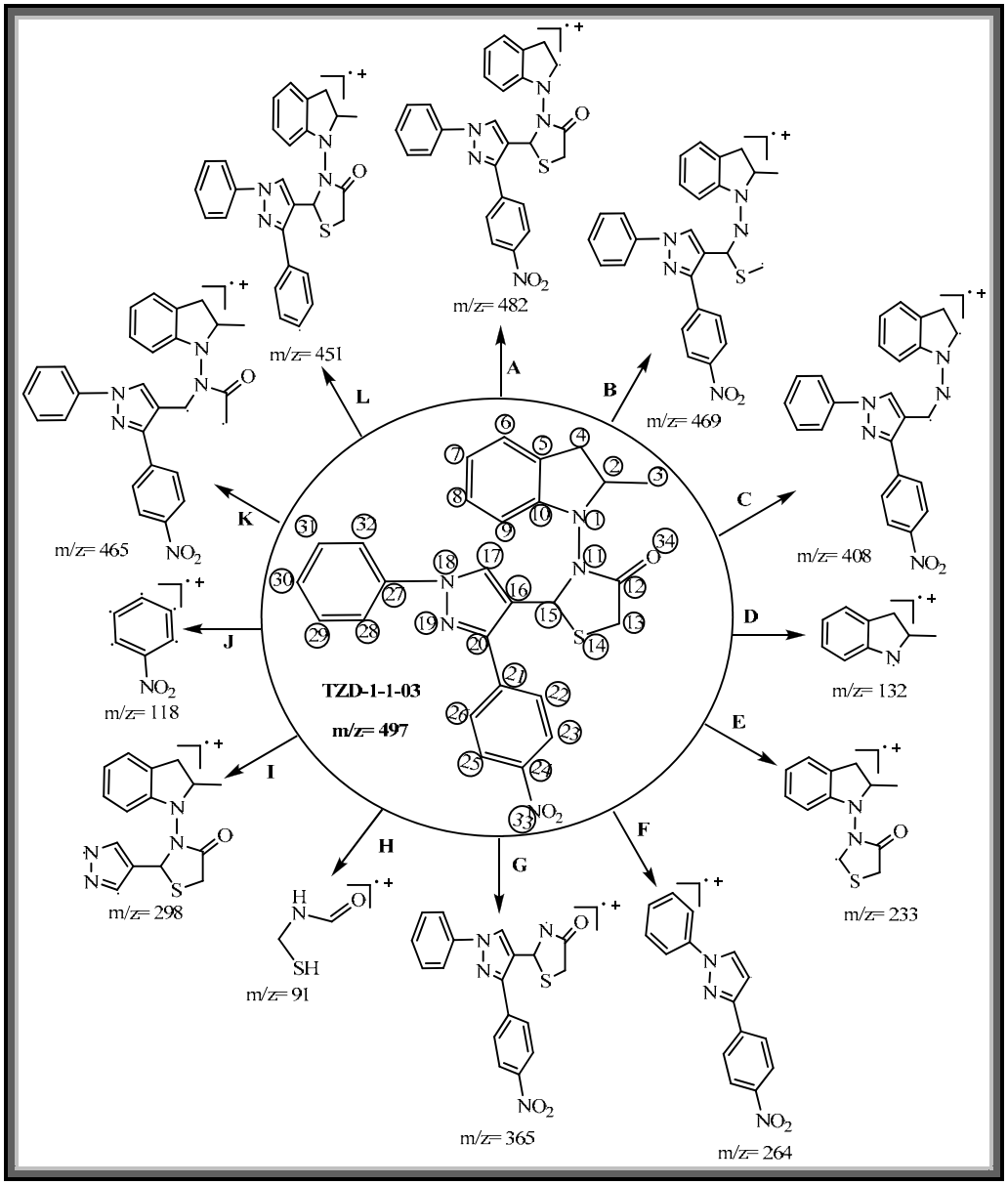


3-(2-Methylindolin-1-yl)-2-(4-nitrophenyl) thiazolidin-4-one (TZD-2-1-10)

1. The target compound showed the characteristic molecular ion peak 356 *m/z*.
2. The bond cleavage between C_{19}-N_{22} generated a molecular ion which corresponds to a characteristic peak at 309 *m/z* **(A).**
3. A bond cleavage between C_2-C_4 and N_1-C_{10} generated a molecular ion which corresponds to a characteristic peak at 266 *m/z* **(B)**.
4. Bond cleavages between N_1-N_{11} generated a molecular ion which corresponds to a characteristic peak at 219 *m/z* **(C)**.
5. Bond cleavages between N_1-N_{11} and C_{15}-C_{16} generated a molecular ion which corresponds to a characteristic peak at 100 *m/z* **(D)**.

6. Bond cleavages between N_1-N_{11} generated another molecular ion which corresponds to a characteristic peak at 132 *m/z* **(E)**.
7. Bond cleavages between C_{12}-O_{23} generated a molecular ion which corresponds to a characteristic peak at 339 *m/z* **(F)**.
8. Bond cleavages between C_{15}-C_{16} generated a molecular ion which corresponds to a characteristic peak at 233 *m/z* **(G)**.
9. Bond cleavages between N_1-N_{11} and C_2-C_3 generated a molecular ion which corresponds to a characteristic peak at 117 *m/z* **(H)**.
10. Bond cleavages between C_{13}-S_{14}, S_{14}-C_{15}, generated a molecular ion which corresponds to a characteristic peak at 323 *m/z* **(I)**.
11. Bond cleavages between C_2-C_3, C_{19}-N_{22} generated a molecular ion which corresponds to a characteristic peak at 294 *m/z* **(J)**.
12. Bond cleavages between C_2-C_3, C_{12}-O_{23} and C_{19}-N_{22} generated a molecular ion which corresponds to a characteristic peak at 278 *m/z* **(K)**.
13. Bond cleavage between C_{12}-C_{13} and C_{13}-C_{14} generated a molecular ion which corresponds to a characteristic peak at 341 *m/z* **(L)**.

5.7.2.2 *PLAUSIBLE MASS FRAGMENTATION PATTERN OF TZD-1-1-03*



3-(2-Methylindolin-1-yl)-2-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl) -thiazolidin-4-one (TZD-1-1-03)

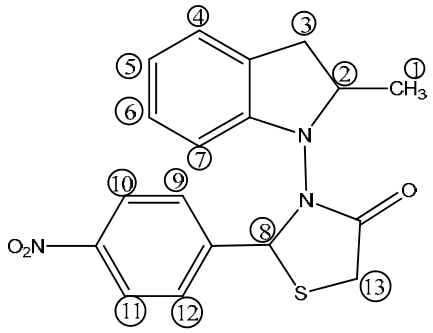
1. The target compound shows the desired characteristic molecular ion peak of 497 *m/z*.
2. The bond cleavage between C_2-C_3 generated a molecular ion which corresponds to a characteristic peak at 482 *m/z* **(A)**.
3. The bond cleavage between N_{11}-C_{12} and C_{12}-C_{13} generated another molecular ion which corresponds to a characteristic peak at 469 *m/z* **(B)**.

4. The bond cleavage between N_{11}-C_{12}, S_{14}-C_{15} and C_2-C_3 generated a molecular ion which corresponds to a characteristic peak at 408 *m/z* **(C)**.
5. The bond cleavages between N_1-N_{11} generated a molecular ion which corresponds to a characteristic peak at 132 *m/z* **(D)**.
6. The bond cleavages between C_{15}-C_{16} generated a molecular ion which corresponds to a characteristic peak at 233 *m/z* **(E)**.
7. The bond cleavages between C_{15}-C_{16} generated another molecular ion which corresponds to a characteristic peak at 264 *m/z* **(F)**.
8. The bond cleavages between N_1-N_{11} generated another molecular ion which corresponds to a characteristic peak at 365 *m/z* **(G)**.
9. The bond cleavages between N_1-N_{11}, C_{15}-C_{16}, C_{12}-C_{13}, & C_{13}-S_{14} generated a molecular ion which corresponds to a characteristic peak at 91 *m/z* **(H)**.
10. The bond cleavage between N_{18}-C_{27}, and C_{20}-C_{21} generated a molecular ion which corresponds to a characteristic peak at 298 *m/z* **(I)**.
11. Bond cleavages between C_{20}-C_{21} generated a molecular ion which corresponds to a characteristic peak at 118 *m/z* **(J)**.
12. Bond cleavages between C_{13}-S_{14}, and S_{14}-C_{15}, generated a molecular ion which corresponds to a characteristic peak at 465 *m/z* **(K)**.
13. Bond cleavage between C_{24}-N_{33} generated a molecular ion which corresponds to a characteristic peak at 451 *m/z* **(L)**.

5.7.3 *^{1}H-NMR SPECTRAL STUDY*

^{1}H-NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in DMSO solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from H-NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. The spectral interpretation can be discussed as under.

3-(2-Methylindolin-1-yl)-2-(4-nitrophenyl) thiazolidin-4-one (TZD-2-1-10)

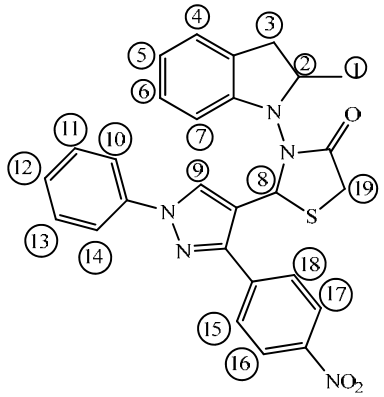


1. The three protons of the methyl function has a single proton in its vicinity thus showing a characteristic strong doublet between 1.27-1.28 δ ppm.
2. The proton no. 2 is surrounded by several protons like the three methyl protons and two geminal protons on the other carbon. This will split its signal into a multiplet which is seen in the NMR spectrum between 4.47-4.52 δ ppm.
3. As seen in the previous chapters there are two geminal protons at 3 position. Both these geminal protons show different signals for each of them one of them shows a characteristic doublet between 2.69-2.74 δ ppm where as the other proton shows a quartet between 3.39-3.46 δ ppm as expected.
4. The proton no.4 shows a characteristic doublet in the aromatic region of 7.05 7.07 δ ppm and its J value was calculated to be 8 Hz which clearly suggests that it is ortho coupled to any other proton. On observing the structure it is quite evident that proton no. 4 is ortho to proton no.5 hence the above mentioned doublet is definitely for proton no.4.
5. The proton no. 5 and 7 are seen in the NMR spectrum as a multiplet in the aromatic region between 7.13 -7.16 δ ppm. The multiplet is seen due to the fact that first proton no. 5 is ortho coupled to two protons i.e. proton no. 4 and 6 while it is meta coupled to proton no. 7. On the other hand the protonno.7 is also ortho coupled to proton o.6 but is meta coupled to proton on.5 hence all these effects give rise to a multiplet and hence we assign it to protons 5 and 7.
6. The proton no. 6 is again surrounded and coupled to a number of protons. It is ortho coupled to protons 5 and 7 while it is meta coupled to proton no.4. Thus it shows a multiplet for its single proton in the aromatic region between 6.79 6.83 δ ppm.

7. The proton no. 8 is not surrounded by any other proton hence it should normally show a singlet. Moreover, on three sides it is surrounded by a Nitrogen atom, a sulphur atom as well as a phenyl nucleus which are all electron withdrawing in nature. This will deshield the proton to a large extent forcing it to give a signal in the down filed region. On observing the NMR spectrum, a singlet at 7.37 δ ppm is observed which is assigned to proton no.8 without any doubt.
8. On looking at the structure it can be assumed that the proton no. 9 and 12 are having similar kind of chemical environment which will lead to a single signal for both these protons and moreover as they are ortho coupled to other set of protons, a sharp doublet should be observed in the aromatic region. This is evidently seen in the spectrum as a sharp doublet between 7.64-7.66 δ ppm. The J value for this doublet was found out to be 8 Hz. Hence this doublet is assigned to proton nos. 9 and 12.
9. Similarly, proton nos. 10 and 11 are also chemically equivalent due to similar environment only difference being that as they are near to the electron withdrawing Nitro group their signal would shift towards a bit downfield region. As they are ortho coupled to proton nos. 9 and 12, the spectra reveals another doublet in the aromatic region between 8.09-8.11 δ ppm and the calculated J value again found to be 8 Hz. Hence without any doubt this doublet is assigned to proton nos. 10 and 11.
10. The methylene protons at position 13 are also not having any other protons in its vicinity hence their signal will not split giving us a characteristic singlet at 5.37 δ ppm. The main reason for this signal to shift downfield is that this proton is surrounded by sulphur on one side and a ketone group on the other side which again are both electron withdrawing in nature which will deshield the protons thus forcing them to go downfield as compared to any other proton in isolation.

Thus, by assigning all the peaks as per the above given justifications and by calculating the J value for the respective protons the proposed structure of TZD-2-1-10 is confirmed.

3-(2-Methylindolin-1-yl)-2-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl) - thiazolidin-4-one (TZD-1-1-03)



1. The proton no. 1 is a methyl proton having one proton in its vicinity hence a strong doublet for these three proton is observed in the NMR spectrum between 1.37-1.39 δ ppm.
2. The proton no. 2 is a single proton surrounded by three protons on one side and two geminal protons on the other side hence it is evident that a multiplet signal should be observed for this proton between 4.49-4.53 δ ppm in the NMR spectrum.
3. As discussed above the two geminal protons will show a doublet and a quartet respectively. The characteristic doublet is seen in the NMR spectrum at 2.78 2.83 δ ppm while the characteristic quartet of the other geminal twin is observed between 3.48-3.54 δ ppm.
4. The proton no. 4 is a single proton which is ortho coupled to proton no. 5. On observing the spectra a characteristic doublet is found in the aromatic region between 7.06-7.08 δ ppm and by the means of the J value which was calculated to be 8 Hz, it can easily be assigned to proton no. 4.
5. The proton nos. 5 and 7 are seen in the NMR spectrum again as a multiplet for two protons in the aromatic region between 7.14-7.22 δ ppm which is due to the various coupling effects from their neighboring protons viz. proton no. 4 as well as proton no.6
6. The proton no. 6 is a single proton having two protons in its direct vicinity which will split its signal into a triplet. This characteristic triplet for proton no. 6 is observed between 6.84-6.88 δ ppm in the NMR spectrum.

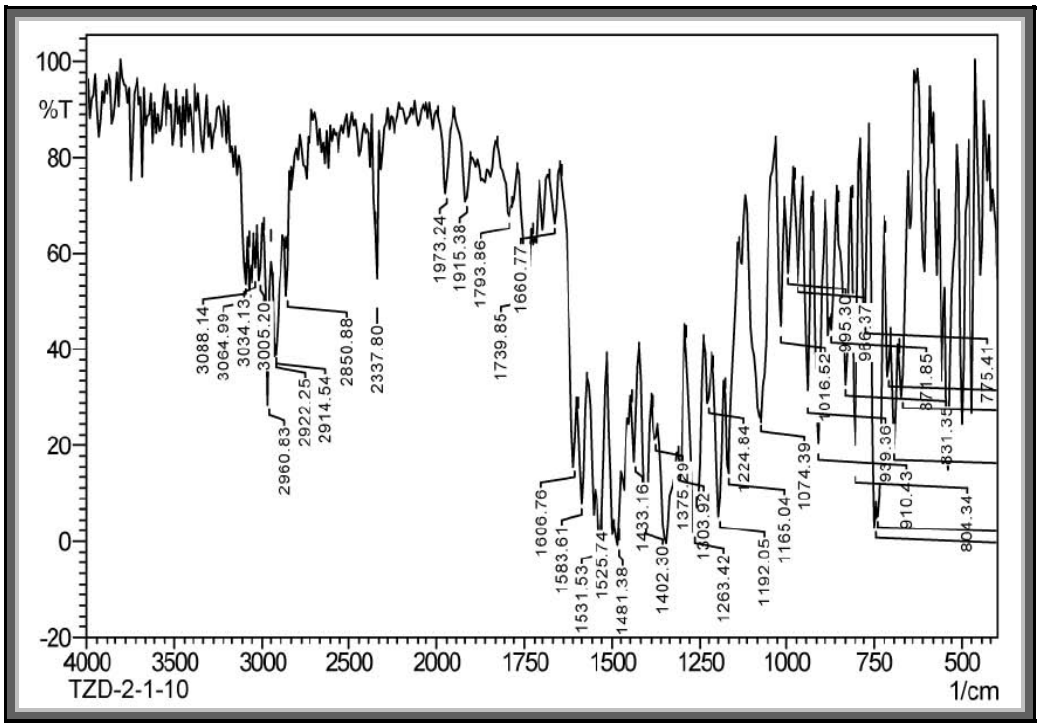
7. The proton no. 8 does not have any other proton in its vicinity. Also, as there are two electron withdrawing groups like Nitrogen as well as Sulphur in its direct contact its proton will get highly deshielded and would be seen at much downfield region. This characteristic strong singlet is observed in the NMR spectrum at 7.62 δ ppm which is assigned to proton no. 8 without any doubts.
8. Under normal circumstances the proton no. 9 should give a singlet as there are no other protons in its direct vicinity. But, this singlet is not found in the NMR spectrum. Instead, a triplet is found at 7.36-7.40 δ ppm which accounts for a single proton. This triplet is assigned to proton no. 9 because of the fact that there is a rotation of the single bond between the two nitrogen atoms of Indoline and thiazolidinone moieties. Due to these rotations there will be field effect interactions between proton no. 9 and proton no7 or proton no.9 and proton no.1. These combined field effects on proton no.9 will give rise to a complex triplet which should otherwise have shown a simple singlet. Hence this triplet found in the NMR spectrum accounting for a single proton is assigned to proton no.9 without any further doubts.
9. The proton nos. 10 and 14 show a characteristic triplet in the aromatic region between 7.50-7.55 δ ppm this is due to the fact that they both have two protons ortho coupled to it and one proton is meta coupled to it respectively.
10. A multiplet accounting for 3 protons is assigned to proton nos. 11, 12, and 13 as they too will not only couple with each other but will also couple with proton nos. 10 and 14. These coupling effects will give rise to a multiplet. This multiplet is seen in the NMR spectrum between 8.34- 8.38 δ ppm which is assigned to proton nos. 11, 12, and 13.
11. Again on observing the structure, proton no 15 and 18 are chemically equivalent as their chemical environments are similar. Hence they will show a doublet for 2 protons which is seen in the NMR spectrum between 7.82-7.85 δ ppm and the J values were calculated to be 11.6 Hz. This high J value suggests that they are ortho coupled to another set of protons.

12. Similarly, proton nos. 16 and 17 are also chemically equivalent and will show a doublet the only difference is that as they are near to the electron withdrawing Nitro group their protons would be deshielded giving a doublet signal in a bit downfield region as compared the signals from proton no. 15 and 18. This doublet is seen in the NMR spectrum between 8.01 to 8.04 δ ppm and its J value was calculated to be 13.6 Hz which is in accordance with the structure as they are ortho coupled to protons nos. 15 and 18. Hence this doublet between 8.01 to 8.04 δ ppm is assigned to proton nos. 16 and 17.
13. The proton no. 19 is the methylene proton of the thiazolidinone motif which is surrounded by a ketone group on one side and a sulphur group on the other side. Thus, its signals should be a singlet which must be found at a downfield region which is exactly the case a sharp singlet at 5.45 δ ppm is observed in the NMR spectrum which is assigned to proton no.19.

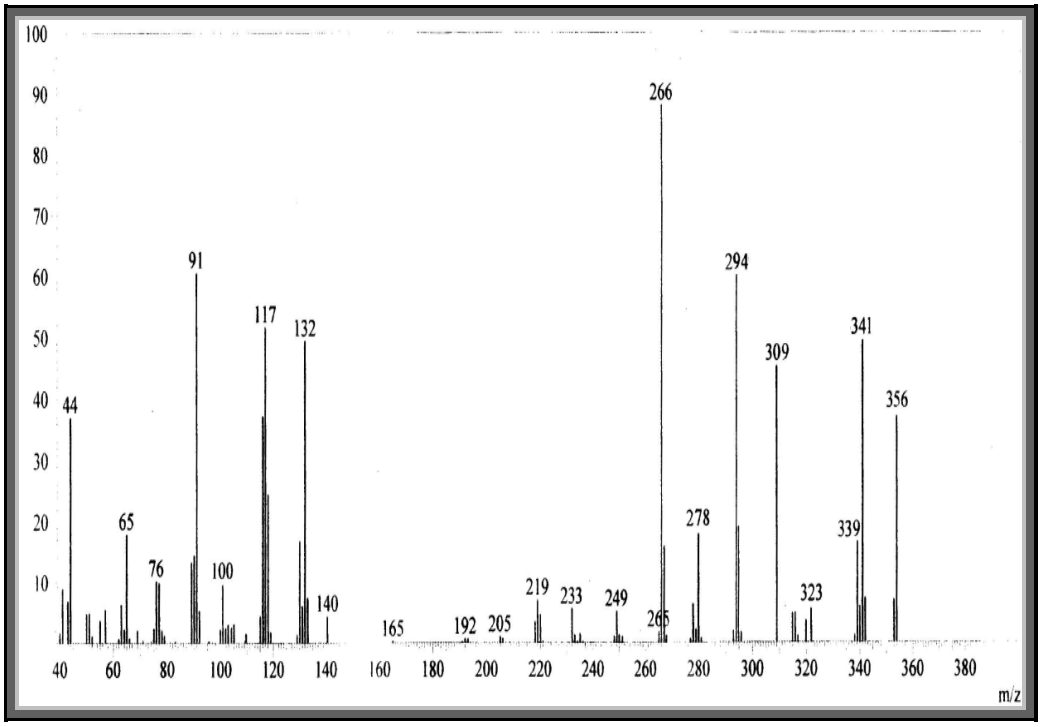
Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton it can clearly be suggested that the proposed structure for compound no. TZD-1-1-03 has been confirmed.

5.8 SPECTRAL REPRESENTATIONS OF THE COMPOUNDS

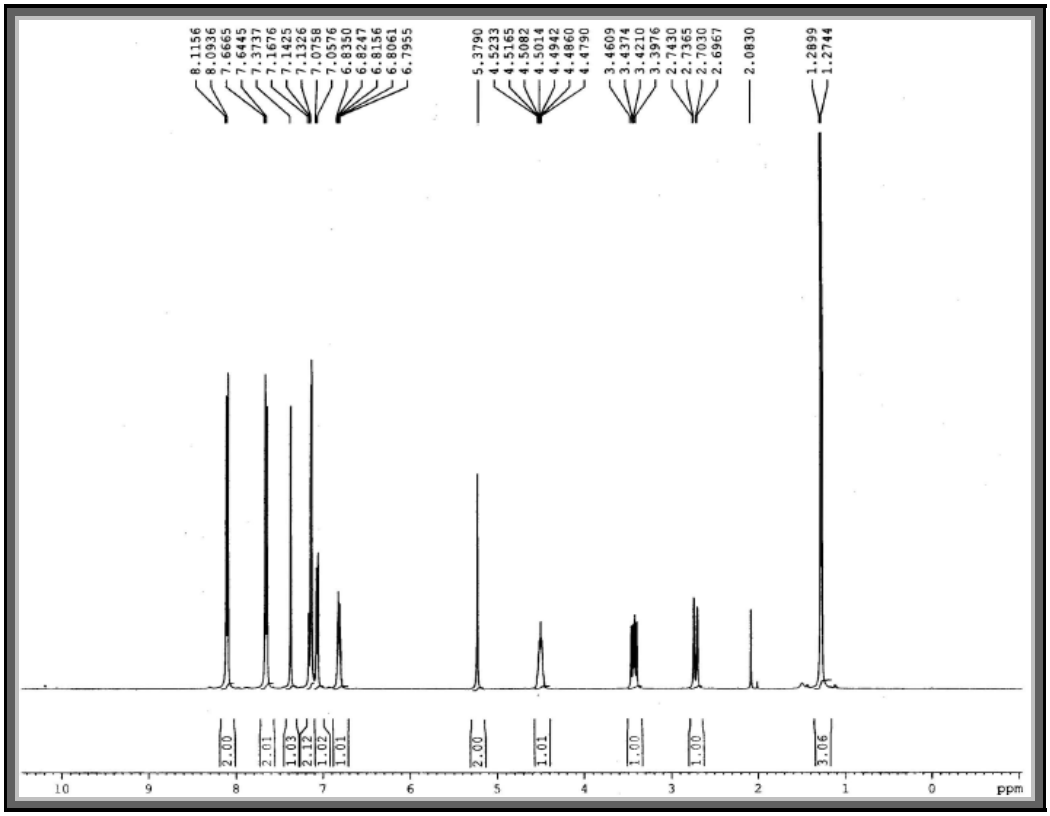
5.8.1 *IR SPECTRUM OF TZD-2-1-10*



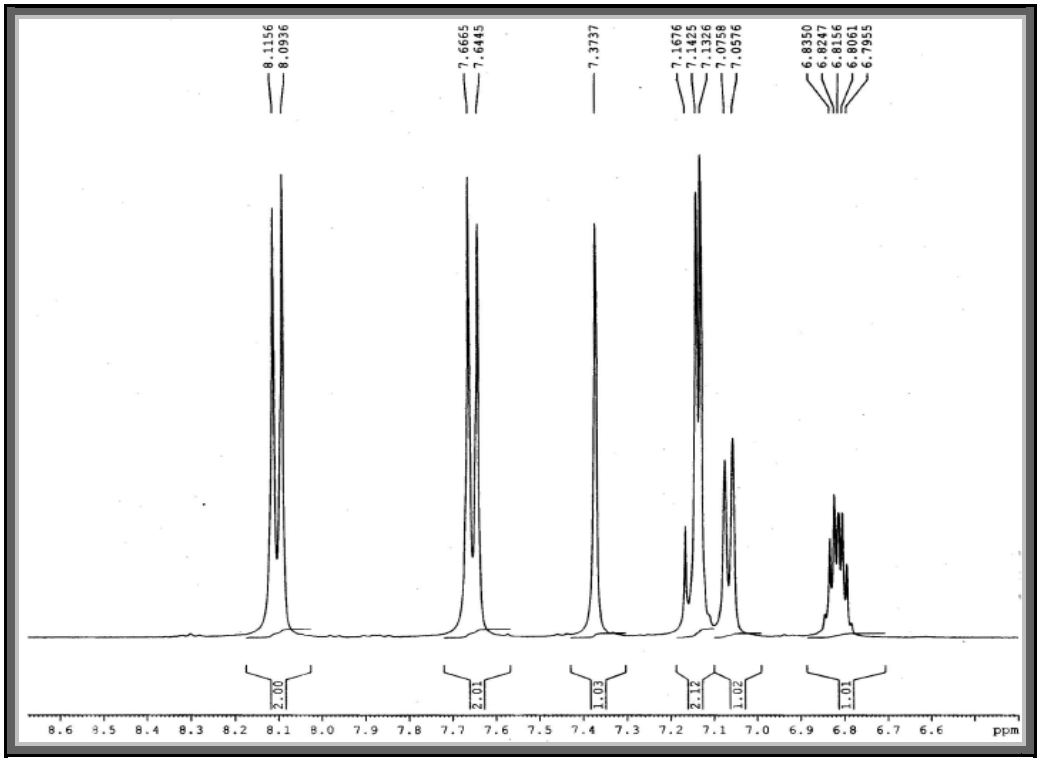
5.8.2 *MASS SPECTRUM OF TZD-2-1-10*



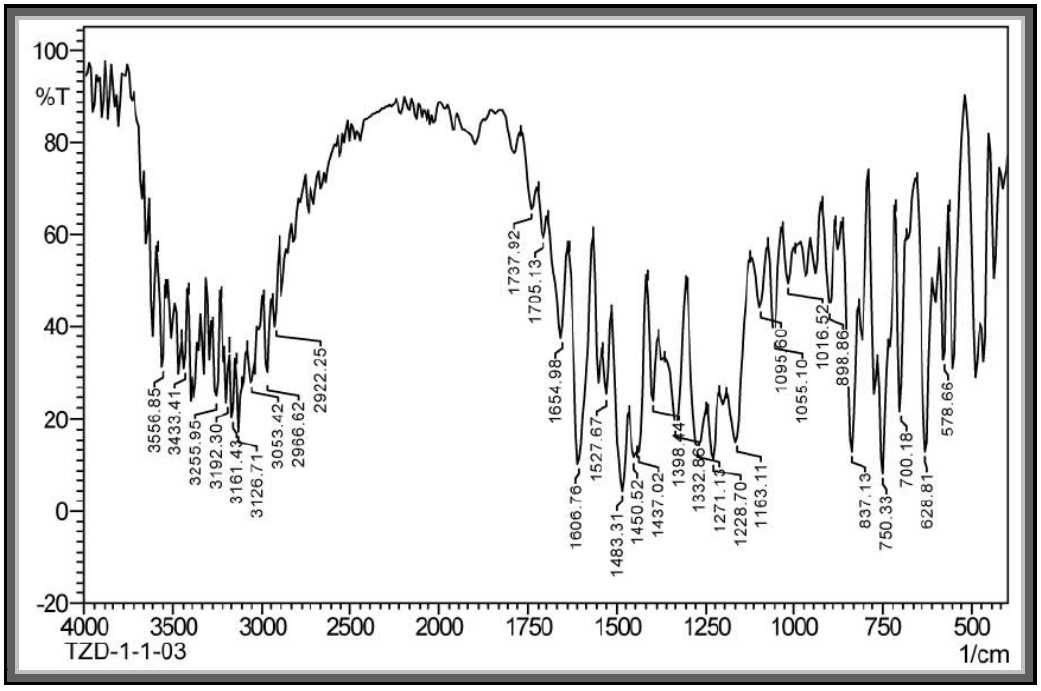
5.8.3 *^{1}H-NMR SPECTRUM OF TZD-2-1-10*



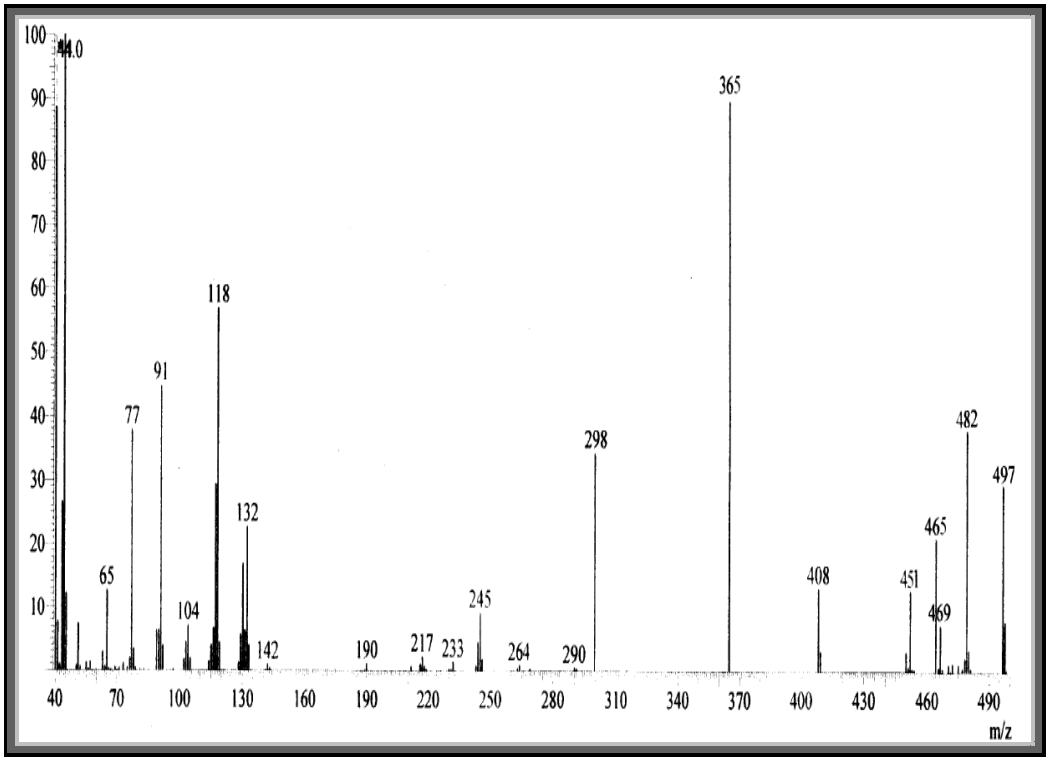
5.8.3.1 *EXPANDED ^{1}H-NMR SPECTRUM OF TZD-2-1-10*



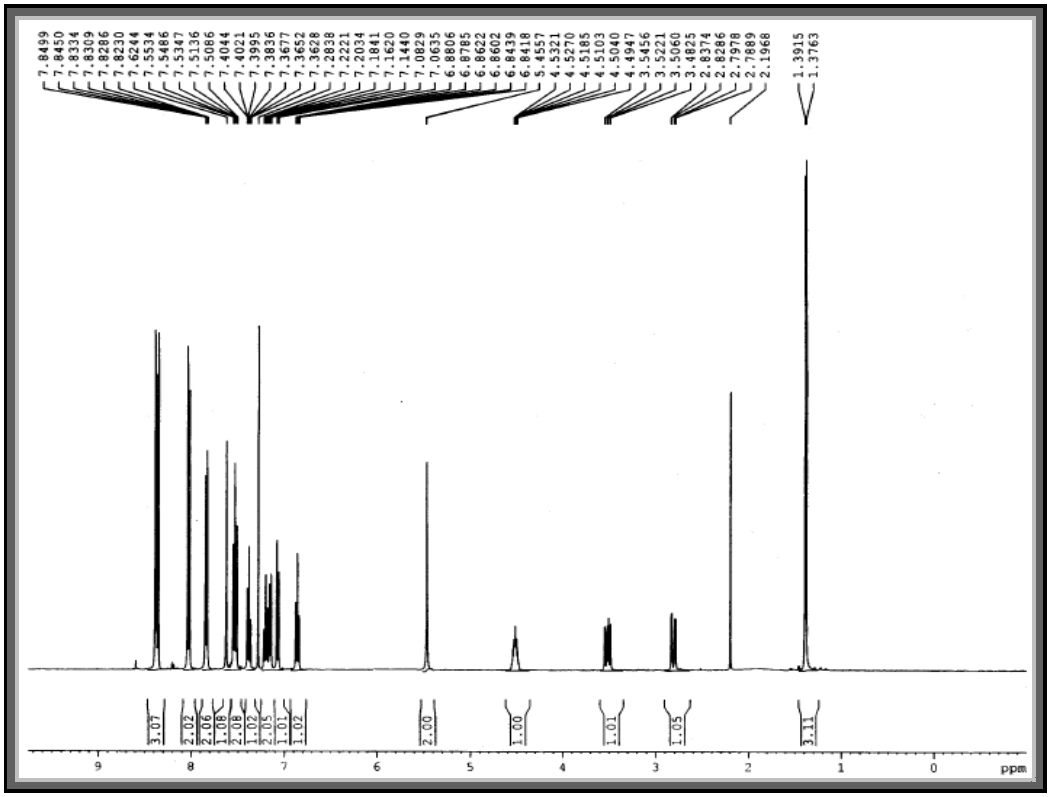
5.8.4 *IR SPECTRUM OF TZD-1-1-03*



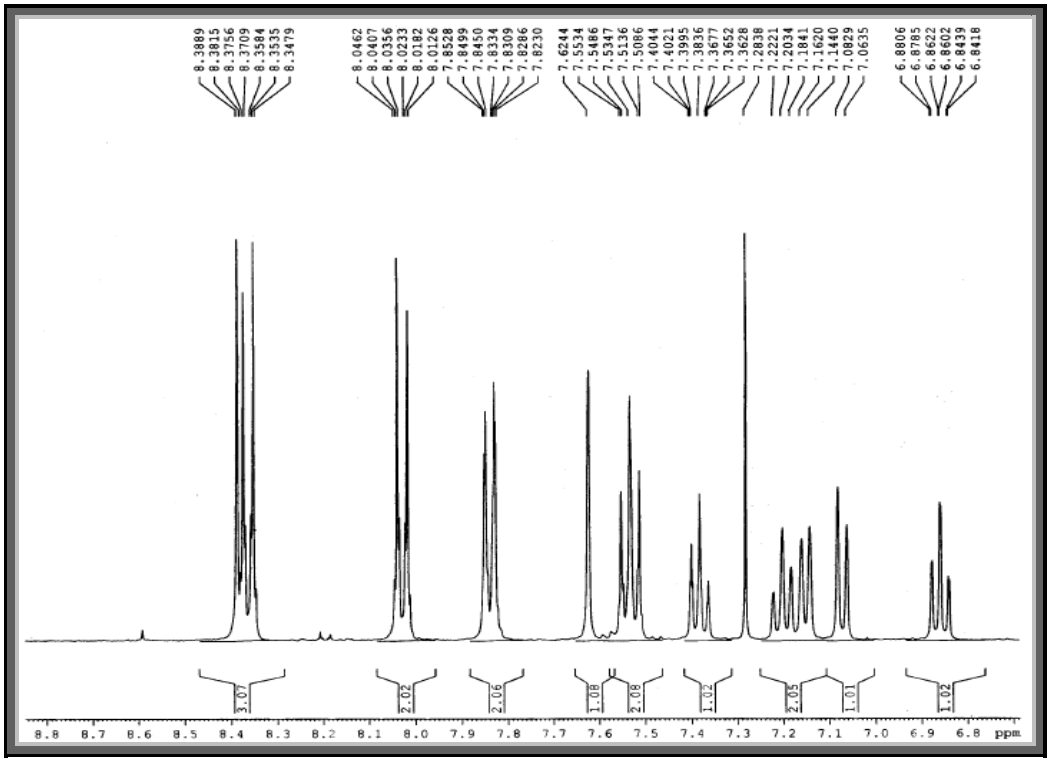
5.8.5 *MASS SPECTRUM OF TZD-1-1-03*



5.8.6 *^{1}H-NMR SPECTRUM OF TZD-1-1-03*



5.8.6.1 *EXPANDED ^{1}H-NMR SPECTRUM OF TZD-1-1-03*



5.9 RESULTS AND DISCUSSIONS

This chapter deals with the 3-(2-methylindolin-1-yl) thiazolidin-4-one core structure. The importance of 2-Methylindoline-1-amine as a privileged structure as well as the biological significance of thiazolidinones has been discussed at length in the chapter no. 1 of this thesis and also in this chapter which served as the rational for the synthesis of these kinds of compounds. Moreover, this chapter has quite a lot of interesting features in terms of synthesis methodology as well as biological activity study. Previously, the thiazolidinone moiety was prepared by refluxing the Schiff base with the thioglycolic acid for longer time in presence of various different solvents as well as catalysts. In the recent years, many new synthetic methodologies haven been used such as synthesis using Ionic liquids, synthesis using better catalysts such as DCC and HBTU etc. and also using Microwave irradiation.

The synthesis has been taken up using Microwave irradiation over other methods owing to their advantages such as reduction in time, higher yields as well as hassle free work up procedures on one hand and on the other hand it is a small step forward in our endeavor towards the sustainable development through implementing the newer research work using eco friendly processes.

5.10 CONCLUSION

The synthesized compounds have been screened for their Anti viral (HIV-I and HIV-II) activities which have been discussed in Chapter no.6.

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CHAPTER – 6

BIOLOGICAL ACTIVITY STUDY OF NEWLY SYNTHESIZED COMPOUNDS

6.1 INTRODUCTION

The bioactive heterocyclic compounds synthesized in the earlier chapters were evaluated for their Anti-HIV activity. The biological screening protocols as well as the results of the Anti-HIV activity study are reported in this chapter.

HIV-1 (human immunodeficiency virus type-1) is the pathogenic retrovirus and causative agent of AIDS or AIDS-related complex (ARC) [1, 2]. When a viral RNA is translated into a polypeptide sequence, it is assembled in a long polypeptide chain, which includes several individual proteins namely, reverse transcriptase, protease, integrase, etc. Before these enzymes become functional, they must be cut from the longer polypeptide chain. Acquired immune deficiency syndrome (AIDS) is a formidable pandemic that is still wreaking havoc world wide. The catastrophic potential of this virally caused disease may not have been fully realized. The causative moiety of the disease is human immunodeficiency virus (HIV), which is a retrovirus of the lentivirus family [3]. The three viral enzymes namely reverse transcriptase, protease and integrase encoded by the gag and gagepol genes of HIV play an important role in the virus replication cycle. Among them, viral reverse transcriptase (RT) catalyzes the formation of proviral DNA from viral RNA, the key stage in viral replication. Its central role in viral replication makes RT a prime target for anti-HIV therapy [4].

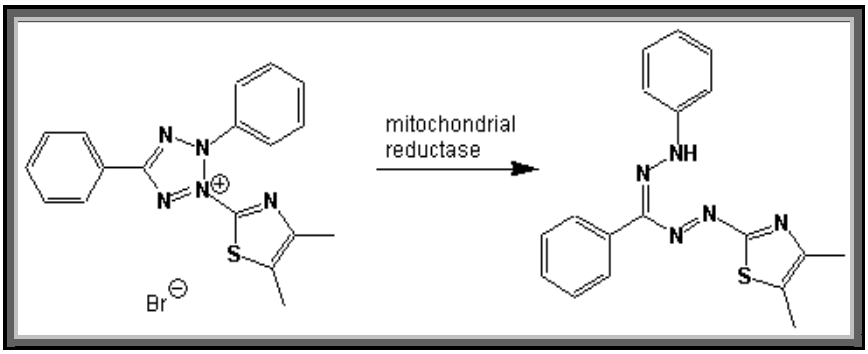
From the early beginning of the AIDS pandemic in early 1980s, there was an urgent need for an efficacious screening system to discover new antiretrovirals for the treatment of HIV-infected individuals. The development and characterization of new antivirals depend on appropriate screening assays. A rapid and automated tetrazolium-based colorimetric assay and scoring for cytopathogenicity caused by the virus are the most frequently used fast screening tools to identify compounds with anti-HIV activity [5-7]. Fluorimetric assays based on the expression of Green Fluorescent Protein (GFP), either carried out with the viral genome (instead of the nef gene) or expressed as a reporter gene from the HIV LTR in engineered T-cell lines [8], have been described to analyze HIV replication and screen for the inhibitors of HIV-1 transcription [9, 10].

6.2 METHODOLOGY

The prepared samples were evaluated by following the protocol [11a] published in the journal "Nature Protocol" which describes how to efficiently evaluate compounds for their anti-HIV activity using the human T-lymphotropic virus type I (HTLV-I)-transformed MT-4 cell line [12]. The method is based on HIV-induced cytopathogenic effect (CPE) and measures the degree of cell killing on HIV infection. Given the multiplicity of infection used in this assay, only very few, if any, cells remain viable 5 d after infection. Inhibition of viral replication by anti-HIV compounds will consequently enhance cell survival, which can be assessed spectrophotometrically via the in situ reduction of 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a blue formazan product [13]. The key advantage of this technique is that by this method not only direct inhibitors of HIV replication but also compounds protecting infected cells from HIV-induced apoptosis can be discovered.

The MTT assay and the MTS assay are the laboratory test methods and standard colorimetric assays (an assay which measures changes in color) for measuring the activity of enzymes that reduce MTT or MTS + PMS to Formazan, giving a purple color. It can also be used to determine cytotoxicity of potential medicinal agents and other toxic materials, since those agents would result in cell toxicity and therefore metabolic dysfunction and therefore decreased performance in the assay. Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in living cells. A solubilization solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The absorption maximum is dependent on the solvent employed.

[a] Personal communication with Prof. Christophe Pannecouque, Rega Institute of Medical Research, Leuven, Belgium



In this protocol, stock solutions of compounds are made in DMSO (generally at 10 mg ml^{-1}). Final DMSO concentration in the test should not exceed 1% (vol/vol). Solutions of compounds in water, PBS or medium can be used without limitations. However, for the use of other solvents, compatibility with the assay and cells should be checked beforehand.

6.2.1 *MATERIALS*

6.2.1.1 *Reagents*

- MT-4 cells can be obtained from institutions such as MRC or NIH.
- Complete medium': RPMI-1640 medium with 20 mM HEPES buffer (Life Technologies), supplemented with 10% (vol/vol) heat-inactivated FCS, 2 mML-glutamine, 0.1% sodium bicarbonate and 20 mg ml^{-1} gentamicin
- Virus stock
- A solution of 30 ml Triton X-100 and 2 ml methanesulfonic acid or 2 ml concentrated hydrochloric acid in 500 ml isopropanol

6.2.1.2 *Equipments*

- Titertek multidrop dispenser
- Biomek 3000 robot
- Microplate washer
- Multiskan Ascent reader
- An invert light microscope (magnification: ocular X 10 and objective X 20)

6.2.1.3 ***Reagent Setup***

MTT 7.5 $mgml^{-1}$ of MTT in PBS should be prepared as follows: put 10 g MTT (powder) in a 2-liter bottle, add 1.333ml of PBS and cover the bottle with tin foil (to protect against light). Sonicate until MTT is dissolved (this will take 2 or 3 h). From time to time, take the bottle out of the bath and shake it manually. If MTT does not dissolve after 3 h, leave it like this. Place the bottle in the dark at room temperature for 1 or 2 h to cool it down (as sonication would have elevated the temperature). Filter the solution over an easy flow filter (0.22 mm cellulose acetate membrane for tissue culture applications) under reduced pressure. Store the filtered MTT solution in dark-brown plastic bottles (±150 ml). Do not fill the bottles completely as the aqueous solution will expand during the freezing process. Place the bottles of cleared MTT inside a -20 ^{0}C freezer (this solution can stored for >1 year under these conditions).

6.2.1.4 ***Preparation of the Stock Solution for the test compounds***

Homogeneous aliquots (solution, suspension or emulsion) of the test compounds have to be prepared. In most cases, the product will be added as a solution, but the use of suspensions or emulsions is not prohibitive. The most commonly used solvents are DMSO, water, buffer solutions (e.g., PBS) and, when necessary, a mixture of these solvents. In case a compound requires another solvent or solvent mixture, one can easily perform a test making a serial dilution of the compound in medium in the format of the stock titration plate but making the serial dilutions of the solvent instead of the virus stock. It should be kept in mind that NMP dissolves plastics like polystyrene and, therefore, stock solutions of compounds in NMP should be prepared in glassware.

6.2.2 *Procedure*

6.2.2.1 *Growing Virus Stock*[b,c]

- Microscopically inspect the quality of the cells to be used.
- Place the required number of cells in a 50-ml tube (e.g., for MT-4, 300,000 cells per ml of final culture; for C8166 cells, 200,000 cells per ml of final culture).
- Add PBS to the tube to obtain a final volume of 40 ml (this is used to wash the cells).
- Pellet the cells by centrifugation (5 min, 220g at room temperature). Discard the supernatant by pouring off.
- Resuspend the cells by adding the required volume of an adequate culture in the complete medium.
- Add the required volume of virus stock to the cell suspension. For preparation of the virus stock, see below. The final dilution that is used in the CPE/MTT screening is 1:3,581. Therefore, to make 40 ml of a new stock, add 11 µl (40 ml / 3,581) of the previous virus stock in a total volume of 40 ml to 12 X 10^6 (=300,000 cells per ml X 40 ml) MT-4 cells.
- Transfer the treated cells in an appropriate culture flask.
- Incubate at 37 ^{0}C, 5% CO_2 ($\geq$ 95% relative humidity (RH)).
- Microscopically inspect the cell culture everyday for the presence of cytopathogenic effect, for example, for HIV-1 strain IIIB on MT-4 cells, usually nearly full-blown CPE is observed after 3–4 d of cell culture (mostly no CPE is observed during the first 2 d).
- Transfer the content of the culture flask to a 50-ml tube.
- Pellet the cells by centrifugation (5 min, 220g).
- Carefully label the required number of cryotubes.

[b] A cycle of freezing and thawing a virus stock greatly affects its titer and should therefore be avoided. When assessing the titer of a virus stock, an aliquot should be used that has been frozen and stored in identical conditions as the whole batch of that virus stock.

[c] It should be noted that the amount of inoculum used to infect cells to produce a virus stock is critical. Although one would expect that bigger the input of inoculum the faster a high-titer stock is obtained, one has to take into consideration that besides the cytopathogenic effect, the virus also has a cytotoxic effect

- Dispense the supernatant (virus stock) in aliquots of 0.5 or 1 ml in cryotubes[d].

6.2.2.2 *Titration of the virus stock*

- Cultivate the infected MT-4 cells (or C8166 cells) in a humidified atmosphere (≥ 95% RH) at 37 ^{0}C and 5% CO2 in air. Subcultivate the cells every 3–4 d. Seed the cells at 6 X 10^5 cells per ml (2.5 X 10^5 cells per ml for C8166 cells) before starting the experiment. To do this, count the cells, pellet by centrifugation (5 min, 220g) and resuspend in complete medium.
- Fill flat-bottomed, 96-well microtiter plates with 100 µl of complete medium.
- Add virus stock in 25-µl volume to the six middle cups (2B–G) of the microtiter plate. The actual volume in the cups 2B–G is 125 µl.
- Make nine serial, fivefold dilutions of the wells 2B–G using the Biomek 3000 robot or a multichannel pipette. Proceed as follows: take 25 µl out of column 2 and add to column 3, mix, decontaminate tips with bleach and change tips, transfer 25 µl to the following column and proceed further up to column 10. Do not add virus to column 11, as this will be used as the uninfected control.
- Centrifuge exponentially growing MT-4 cells (or C8166 cells) for 5 min at 220g and discard the supernatant by pouring into bleach solution.
- Resuspend the cells at 6 X 10^5 cells per ml (2.5 X 10^5 cells per ml for C8166 cells) in complete medium in a flask that is connected to an autoclaved dispensing cassette of a Titertek multidrop dispenser (or use a multichannel pipette).
- Dispense 50 ml of cell suspension to the microtiter plate wells except for the outer rows (columns 1 and 12, rows A and H). (Note: fill column 12 with cells when using the multidrop dispenser because there can be a variation in the dispensed volume) and 50 µl of complete medium. To keep the cell suspension homogeneous, the flask should be lightly magnetically stirred. However, a caveat to this is that to avoid damage to the cells, preferentially use a ‘bone-shaped’ magnetic stirrer that, by making less contact with the bottom of the flask, results in less ‘grinding’ of the cells, or swirl regularly.

[d] Store the cryotubes at -80 ^{0}C. A virus can be stored for >10 years in these conditions without marked loss of infectivity. However, it is recommended to titrate a virus stock before use after prolonged storage.

This step results in a final serial dilution of the virus stock of 1:10 to 1:3.90 X 10^6.

- Fill the outer rows (columns 1 and 12, rows A and H) with 100 µl of medium.
- Incubate the microtiter plates in a humidified atmosphere (≥ 95% RH) at 37 ^{0}C and 5% CO_2 in air for 5 d.
- After the 5-d incubation, examine the cells microscopically for eventual HIV induced CPE. A well is scored positive if any trace of CPE is observed. The 50% Cell Culture Infective Dose ($CCID_{50}$) value is calculated using the Reed and Muench method. The calculation is as follows:

  $M = inv\ log\{X_1 + [(X_2 - X_1)((Y_1 - 50) / Y_1 - Y_2)]\}$ where,

  Y_1 = percent of wells scored positive closest to, but higher than, 50% at a certain virus dilution,

  Y_2 = percent of wells scored positive closest to, but lower than, 50% at a certain virus dilution,

  X_1 = the log (dilution of the virus where **Y_1** was observed),

  X_2 = the log (dilution of the virus where **Y_2** was observed) and

  M = dilution of virus stock for 1 $CCID_{50}$
- Dissolve the test compounds in the most appropriate solvent. Attention should be paid to limit the dilution steps in the pre-dilution process of the compound solution to a maximum of 1:100[e].

[e] Depending on the stability of the compounds, the stock solutions can either be frozen at -20 ^{0}C, stored at 4 ^{0}C or kept at room temperature and, if necessary, protected from light. Cooled samples should be allowed to reach room temperature before opening the lid of the recipient/vessel as otherwise moisture will condense and mix with the solvent (e.g., DMSO). As a caveat to this, compounds that are not soluble in water, for example, many non-nucleoside reverse transcriptase inhibitors (NNRTI's) will lead to precipitation or crystallization of the compound.

6.2.2.3 ***Assessing the Anti-HIV activity and the Cytotoxicity of compounds***

- Cultivate MT-4 (or C8166) cells in a humidified atmosphere (≥ 95% RH) at 37 ^{0}C and 5% CO2. Subcultivate the cells every 3–4 d, seeding at 6 X 10^5 cells per ml (2.5 X 10^5 cells per ml for C8166 cells). Count the cells, pellet by centrifugation (5 min, 220g) and resuspend in complete medium.
- Fill flat-bottomed, 96-well microtiter plates with 100 µl of complete medium.
- Add stock solutions (10 X highest final test concentration) of compounds in 25 µl volumes to the six middle cups of the second column of the microtiter plate (2B–G). The actual volume in the cups 2B–G is 125 µl.
- Make nine serial, fivefold dilutions of the compound directly in the microtiter plates using the Biomek 3000 robot or a multichannel pipette. Proceed as follows: take 25 µl out of column 2 and add it to column 3, mix, take 25 µlout of column 3 and repeat the dilution up to column 10. Leave column 11 without drug, as control.
- Add 50 µl of HIV at 100–300 $CCID_{50}$ and medium, respectively, to the upper (rows B–D) and lower parts (rows E–G) of the microtiter tray.
- Centrifuge exponentially growing MT-4 or C8166 cells for 5 min (220g) and discard the supernatant.
- Resuspend the MT-4 cells at 6 X 10^5 cells per ml (or C8166 cells at 2.5 X 10^5 cells per ml) in complete medium in a flask that is connected with an autoclavable dispensing cassette of a Titertek multidrop dispenser (or use a multichannel pipette).
- Dispense 50 µl of cell suspension to the microtiter plate wells except for the outer rows (column 1 and rows A and H). Fill column 12 with cells as well when using the multidrop because there can be a variation in the dispensed volume. To keep the cell suspension homogeneous, the flask should be lightly magnetically stirred. However, to avoid damage to the cells, preferentially use a double-ended magnetic stir bar that makes minimal contact with the bottom of the flask resulting in less ‘grinding’ effect on the cells, or swirl regularly. Fill the wells in the outer row with an additional 100 µl of medium (or PBS).
- Incubate the plates at 37 ^{0}C in a humidified atmosphere of 5% CO_2 in air.

- Five days after infection, spectrophotometrically examine the viability of HIV (upper part of the plate) - and mock (lower part of the microtiter plate)-infected cells by the MTT method [f].

6.2.2.4 *MTT Assay*

- Add 20 µl of MTT (7.5 mg ml^{-1}) solution (warmed to 37 ^{0}C) to each well except the outer row) of the microtiter plates using the Titertek multidrop dispenser (or a multichannel pipette).
- Incubate the trays at 37 ^{0}C in a CO_2 incubator for 1 h.
- Remove a constant volume of medium (e.g., 150 µl) from each cup using a multichannel pipette or a microplate washer (Biotek EL404, a double, aspiration and dispensing, 96-channel washer, should be used as the brusque movement of the plate or of the manifold head of an 8- or 12-channel washer will lead to suspension of the cell clusters and subsequent aspiration of part of the cells, if not all) without disturbing the MT-4 or C8166 cell clusters containing the formazan crystals.
- Lyse the cells (and infectious virus) and solubilize the formazan crystals by adding 100 µl of the acidified Triton X-100 isopropanol solution to each cup using the microplate washer (or a multichannel pipette).
- Complete dissolution of the formazan crystals by placing the plates on a vibrating platform shaker for 10 min.
- Read the absorbances in an eight-channel computer-controlled Multiskan Ascent reader and stacker at two wavelengths (540 and 690 nm). Subtract the absorbance measured at 690 nm from the absorbance at 540 nm to eliminate the effects of scattering by cell debris. Use the first column wells for background subtraction because they contain all reagents except for cells, virus and compounds.

[f] A strict scheme of cell cultivation (subcultivating the cells every 3–4 d, seeding at 6 X 10^5 cells per ml for MT-4 cells and 2.5 X 10^5 cells per ml for C8166 cells) is necessary to avoid overgrowth of the uninfected cells and decrease of cellular viability after 5-d incubation.

- Calculate 50% Cytotoxic Concentration (CC_{50}) and 50% Inhibitory Concentration (IC_{50}); *"**CC_{50} is the concentration of compound that reduced the absorbance (OD_{540}) of the mock-infected control sample by 50%."***

- Calculate the protection achieved by the compounds in HIV-infected cells by the following formula:

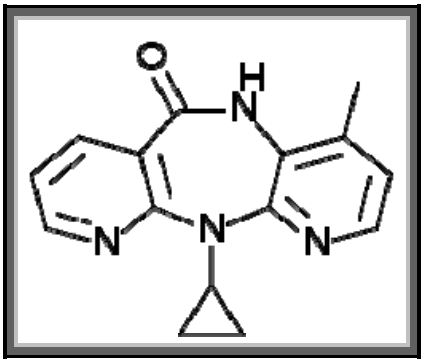
  $((OD_T)_{HIV} - (OD_C)_{HIV}) / ((OD_C)_{mock} - (OD_C)_{HIV})$ X 100, where

  $(OD_T)_{HIV}$ is the OD measured with a given concentration of the test compound in the HIV-infected cells;
  $(OD_C)_{HIV}$ is the OD measured for the control untreated, HIV-infected cells (column 11), which stands for 100% infection-related CPE; and
  $(OD_C)_{mock}$ is the OD measured for the control untreated, mock-infected cells (column 11), which stands for 0% infection-related CPE.

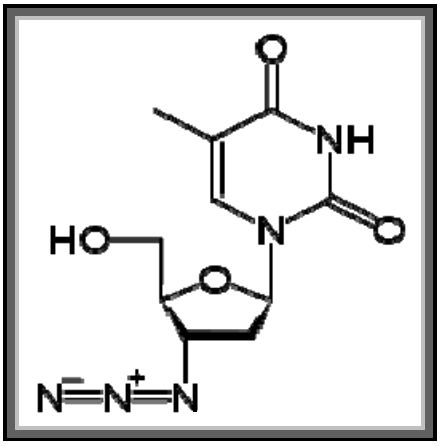
  "The concentration achieving 50% protection according to the above formula is defined as IC_{50}."

All the compounds have been screened against two HIV strains namely III_B i.e. HIV-1 strain where as the other is ROD which is a HIV-2 strain. The ***IC_{50}*** and ***CC_{50}*** values were arrived at by using the above mentioned methodology. The results of the screening of these compounds were compared with the results of the standard Anti-HIV drugs namely Nevirapine, Retrovir, Dideoxycitidine, and Didanosine the structures of which are given below.

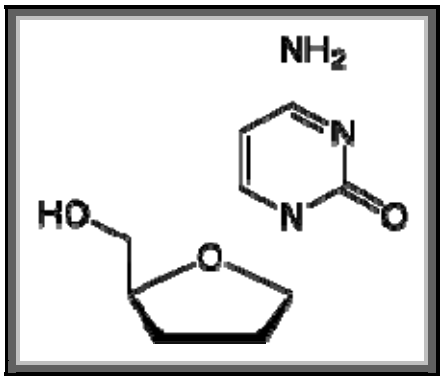
Nevirapine



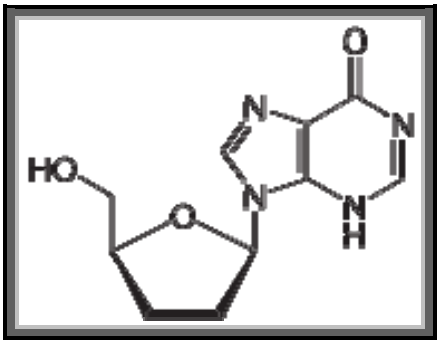
Retrovir / Zidovudine



Dideoxycitidine



Didanosine



Data table: Results for Anti-HIV activity against 2 strains HIV-I (III_B) and HIV-II (ROD)													
Code	**Strain**	**Exp_no**	**IC_{50}** (µg/ml)	**CC_{50}** (µg/ml)	**SI**	**Max. Prot.** (%)	**Appr.**	**Av. IC_{50}** (µg/ml)	**SD**	**Av. CC_{50}** (µg/ml)	**SD**	**SI**	**Remarks**
SHA/NB-001	III_B	P3.4935	> 14.4	= 14.4	< 1	9	1	> 15.43		15.43	1.79	<1	
		P3.4940	> 14.4	= 14.4	< 1	9	1						
	ROD	P3.4936	> 17.5	= 17.5	< 1	5	1	> 15.43		15.43	1.79	<1	
		P3.4941	> 25	> 25	X 1	1	1						
SHA/NB-002	III_B	P3.4935	> 4.03	= 4.03	< 1	8	1	> 9.81		9.81	4.48	< 1	Cryst. Observ. At 125 µg / ml
		P3.4940	> 8.49	= 8.49	< 1	3	1						
	ROD	P3.4936	> 13.2	= 13.2	< 1	2	2	> 9.81		9.81	4.48	< 1	
		P3.4941	> 13.5	= 13.5	< 1	1	1						
SHA/NB-003	III_B	P3.4935	> 0.379	= 0.379	< 1	3	1	> 0.62		0.62	0.56	< 1	
		P3.4940	> 0.293	= 0.293	< 1	0	1						
	ROD	P3.4936	> 0.344	= 0.344	< 1	0	1	> 0.62		0.62	0.56	< 1	
		P3.4941	> 1. 45	= 1.45	< 1	0	1						
SHA/NB-004	III_B	P3.4935	> 0.174	= 0.174	< 1	8	1	> 0.34		0.34	0.16	< 1	Cryst. Observ. At 125 µg / ml
		P3.4940	> 0.414	= 0.414	< 1	0	1						
	ROD	P3.4936	> 0.25	= 0.25	< 1	1	1	> 0.34		0.34	0.16	< 1	
		P3.4941	> 0.539	= 0.539	< 1	0	1						
SHA/NB-005	III_B	P3.4935	> 0.511	= 0.511	< 1	9	1	> 1.17		1.17	0.73	< 1	
		P3.4940	> 1.69	= 1.69	< 1	0	1						
	ROD	P3.4936	> 0.569	= 0.569	< 1	3	1	> 1.17		1.17	0.73	< 1	
		P3.4941	> 1.89	= 1.89	< 1	5	1						
SHA/NB-006	III_B	P3.4935	> 11.2	= 11.2	< 1	5	1	> 11.10		11.10	1.05	< 1	
		P3.4940	> 12.1	= 12.1	< 1	5	1						
	ROD	P3.4936	> 10	= 10	< 1	2	1	> 11.10		11.10	1.05	< 1	
		P3.4941	> 25	> 25	X 1	10	1						
SHA/NB-007	III_B	P3.4935	> 47.3	= 47.3	< 1	8	1	> 59.63		59.63	15.02	< 1	Cryst. Observ. At 125 µg / ml
		P3.4940	> 57.3	= 57.3	< 1	2	1						
	ROD	P3.4936	> 52.6	= 52.6	< 1	7	1	> 59.63		59.63	15.02	< 1	
		P3.4941	> 81.3	= 81.3	< 1	9	1						
SHA/NB-008	III_B	P3.4935	> 42.9	= 42.9	< 1	10	1	> 58.73		58.73	16.20	< 1	Cryst. Observ. At 25 µg / ml
		P3.4940	> 67.2	= 67.2	< 1	8	1						
	ROD	P3.4936	> 47.6	= 47.6	< 1	7	1	> 58.73		58.73	16.20	< 1	
		P3.4941	> 77.2	= 77.2	< 1	8	1						

Code	Strain	Exp_no	IC_{50} (µg/ml)	CC_{50} (µg/ml)	SI	Max. Prot. (%)	Appr.	Av. IC_{50} (µg/ml)	SD	Av. CC_{50} (µg/ml)	SD	SI	Remarks
SHA/NB-009	III_B	P3.4935	> 15.6	= 15.6	< 1	6	1	> 28.58		28.58	14.35	< 1	
		P3.4940	> 35.1	= 35.1	< 1	16	1						
	ROD	P3.4936	> 17.9	= 17.9	< 1	13	1	> 28.58		28.58	14.35	< 1	
		P3.4941	> 45.7	= 45.7	< 1	22	1						
SHA/NB-010	III_B	P3.4935	> 38.5	= 38.5	< 1	8	1	> 49.75		49.75	11.49	< 1	
		P3.4940	> 57.6	= 57.6	< 1	5	1						
	ROD	P3.4936	> 41.4	= 41.4	< 1	6	1	> 49.75		49.75	11.49	< 1	
		P3.4941	> 61.5	= 61.5	< 1	3	1						
SHA/NB-011	III_B	P3.4935	> 15.1	= 15.1	< 1	9	1	> 14.07		14.07	0.90	< 1	
		P3.4940	> 13.6	= 13.6	< 1	0	1						
	ROD	P3.4936	> 13.5	= 13.5	< 1	6	1	> 14.07		14.07	0.90	< 1	
		P3.4941	> 25	> 25	X 1	4	1						
SHA/NB-012	III_B	P3.4935	> 75	= 75	< 1	19	1	> 69.75		69.75	7.22	< 1	
		P3.4940	> 76.6	= 76.6	< 1	12	1						
	ROD	P3.4936	> 61.6	= 61.6	< 1	4	1	> 69.75		69.75	7.22	< 1	
		P3.4941	> 65.8	= 65.8	< 1	4	1						
SHA/NB-013	III_B	P3.4935	> 12.8	= 12.8	< 1	9	1	> 11.61		11.61	1.58	< 1	
		P3.4940	> 9.82	= 9.82	< 1	0	1						
	ROD	P3.4936	> 12.2	= 12.2	< 1	5	1	> 11.61		11.61	1.58	< 1	
		P3.4941	> 25	> 25	X 1	0	1						
SHA/NB-014	III_B	P3.4935	> 10.2	= 10.2	< 1	3	1	> 12.93		12.93	2.70	< 1	
		P3.4940	> 15.6	= 15.6	< 1	12	1						
	ROD	P3.4936	> 13	= 13	< 1	6	1	> 12.93		12.93	2.70	< 1	
		P3.4941	> 25	> 25	X 1	3	1						
SHA/NB-015	III_B	P3.4935	> 13.1	= 13.1	< 1	6	1	> 13.97		13.97	1.95	< 1	
		P3.4940	> 16.2	= 16.2	< 1	1	1						
	ROD	P3.4936	> 12.6	= 12.6	< 1	6	1	> 13.97		13.97	1.95	< 1	
		P3.4941	> 25	> 25	X 1	4	1						
SHA/NB-016	III_B	P3.4935	> 13.7	= 13.7	< 1	5	1	> 14.30		14.30	1.49	< 1	Cryst. Observ. At 125 µg / ml
		P3.4940	> 16	= 16	< 1	8	1						
	ROD	P3.4936	> 13.2	= 13.2	< 1	7	1	> 14.30		14.30	1.49	< 1	
		P3.4941	> 25	> 25	X 1	5	1						

Code	Strain	Exp_no	IC_{50} (µg/ml)	CC_{50} (µg/ml)	SI	Max. Prot. (%)	Appr.	Av. IC_{50} (µg/ml)	SD	Av. CC_{50} (µg/ml)	SD	SI	Remarks
SHA/BN-001	III_B	P3.4935	> 9.83	= 9.83	< 1	6	1	> 6.27		6.27	4.39	< 1	Cryst. Observ. At 125 µg / ml
		P3.4940	> 2.27	= 2.27	< 1	4	1						
	ROD	P3.4936	> 2.67	= 2.67	< 1	2	1	> 6.27		6.27	4.39	< 1	
		P3.4941	> 10.3	= 10.3	< 1	0	1						
SHA/BN-002	III_B	P3.4935	> 37.1	= 37.1	< 1	6	1	> 55.83		55.83	16.35	< 1	Cryst. Observ. At 125 µg / ml
		P3.4940	> 68.2	= 68.2	< 1	16	1						
	ROD	P3.4936	> 47.2	= 47.2	< 1	8	1	> 55.83		55.83	16.35	< 1	
		P3.4941	> 70.8	= 70.8	< 1	7	1						
SHA/BN-004	III_B	P3.4935	> 16.6	= 16.6	< 1	6	1	> 25.48		25.48	10.59	< 1	
		P3.4940	> 31.1	= 31.1	< 1	6	1						
	ROD	P3.4936	> 16.6	= 16.6	< 1	6	1	> 25.48		25.48	10.59	< 1	
		P3.4941	> 37.6	= 37.6	< 1	0	1						
SHA/BN-005	III_B	P3.4935	> 0.729	= 0.729	< 1	7	1	> 1.15		1.15	0.54	< 1	Cryst. Observ. At 125 µg / ml
		P3.4940	> 1.5	= 1.5	< 1	2	1						
	ROD	P3.4936	> 0.658	= 0.658	< 1	3	1	> 1.15		1.15	0.54	< 1	
		P3.4941	> 1.72	= 1.72	< 1	3	1						
SHA/BN-007	III_B	P3.4935	> 17.8	= 17.8	< 1	3	1	> 32.25		32.25	15.40	< 1	Cryst. Observ. At 125 µg / ml
		P3.4940	> 35.5	= 35.5	< 1	0	1						
	ROD	P3.4936	> 23.2	= 23.2	< 1	3	1	> 32.25		32.25	15.40	< 1	
		P3.4941	> 52.5	= 52.5	< 1	2	1						
SHA/NmNB-008	III_B	P3.4935	> 41.6	= 41.6	< 1	1	1	> 55.55		55.55	13.00	< 1	Cryst. Observ. At 25 µg / ml
		P3.4940	> 67.5	= 67.5	< 1	16	1						
	ROD	P3.4936	> 47.4	= 47.4	< 1	7	1	> 55.55		55.55	13.00	< 1	
		P3.4941	> 65.7	= 65.7	< 1	8	1						
SHA/NmNB-009	III_B	P3.4935	> 63.5	= 63.5	< 1	4	1	> 63.95		63.95	1.18	< 1	
		P3.4940	> 65.5	= 65.5	< 1	10	1						
	ROD	P3.4936	> 62.7	= 62.7	< 1	19	1	> 63.95		63.95	1.18	< 1	
		P3.4941	> 64.1	= 64.1	< 1	12	1						
SHA/NmNB-010	III_B	P3.4935	> 74.1	= 74.1	< 1	12	1	> 86.33		86.33	13.61	< 1	
		P3.4940	> 101	= 101	< 1	37	1						
	ROD	P3.4936	> 83.9	= 83.9	< 1	16	1	> 86.33		86.33	13.61	< 1	
		P3.4941	> 125	> 125	X 1	45	1						

Code	Strain	Exp_no	IC_{50} (µg/ml)	CC_{50} (µg/ml)	SI	Max. Prot. (%)	Appr.	Av. IC_{50} (µg/ml)	SD	Av. CC_{50} (µg/ml)	SD	SI	Remarks
SHA/NmNB-013	III_B	P3.4935	> 49	= 49	< 1	20	1	≥ 20.70		≥ 49.00			
		P3.4940	= 20.7	> 125	> 6	58	1						
	ROD	P3.4936	> 56.6	= 56.6	< 1	23	1	≥ 84.50		≥ 49.00			
		P3.4941	= 84.5	> 125	> 1	66	1						
SHA/NmNB-015	III_B	P3.4935	> 44.9	= 44.9	< 1	4	1	> 57.05		57.05	8.81	< 1	Cryst. Observ. At 125 µg / ml
		P3.4940	> 64.6	= 64.6	< 1	10	1						
	ROD	P3.4936	> 56.4	= 56.4	< 1	34	1	> 57.05		57.05	8.81	< 1	
		P3.4941	> 62.3	= 62.3	< 1	24	1						
SHA/NmNB-016	III_B	P3.4935	> 45.7	= 45.7	< 1	4	1	> 57.25		57.25	7.84	< 1	
		P3.4940	> 61.5	= 61.5	< 1	0	1						
	ROD	P3.4936	> 59.1	= 59.1	< 1	10	1	> 57.25		57.25	7.84	< 1	
		P3.4941	> 62.7	= 62.7	< 1	2	1						
SHA/GAA-005	III_B	P3.4935	> 72.3	= 72.3	< 1	5	1	> 70.60		70.60	2.71	< 1	
		P3.4940	> 69.7	= 69.7	< 1	6	1						
	ROD	P3.4936	> 67.2	= 67.2	< 1	16	1	> 70.60		70.60	2.71	< 1	
		P3.4941	> 73.2	= 73.2	< 1	20	1						
SHA/GAA-008	III_B	P3.4935	> 24.2	= 24.2	< 1	4	1	> 39.60		39.60	16.54	< 1	Cryst. Observ. At 125 µg / ml
		P3.4940	> 54.2	= 54.2	< 1	2	1						
	ROD	P3.4936	> 26.4	= 26.4	< 1	2	1	> 39.60		39.60	16.54	< 1	
		P3.4941	> 53.6	= 53.6	< 1	0	1						
SHA/GAA-009	III_B	P3.4935	> 62.7	= 62.7	< 1	5	1	> 62.65		62.65	2.57	< 1	
		P3.4940	> 59	= 59	< 1	5	1						
	ROD	P3.4936	> 64.5	= 64.5	< 1	13	1	> 62.65		62.65	2.57	< 1	
		P3.4941	> 64.4	= 64.4	< 1	17	1						
SHA/GAA-010	III_B	P3.4935	> 65.9	= 65.9	< 1	14	1	> 70.33		70.33	7.37	< 1	
		P3.4940	> 67.9	= 67.9	< 1	32	1						
	ROD	P3.4936	> 66.2	= 66.2	< 1	29	1	> 70.33		70.33	7.37	< 1	
		P3.4941	> 81.3	= 81.3	< 1	5	1						

Code	Strain	Exp_no	IC_{50} (µg/ml)	CC_{50} (µg/ml)	SI	Max. Prot. (%)	Appr.	Av. IC_{50} (µg/ml)	SD	Av. CC_{50} (µg/ml)	SD	SI	Remarks
SHA/NAISB-001	III_B	P3.4935	> 10.7	= 10.7	< 1	2	2	> 12.20		12.20	1.12	< 1	Cryst. Observ. At 25 µg / ml
		P3.4940	> 12.9	= 12.9	< 1	1	1						
	ROD	P3.4936	> 13.2	= 13.2	< 1	4	1	> 12.20		12.20	1.12	< 1	
		P3.4941	> 12	= 12	< 1	2	1						
SHA/NAISB-002	III_B	P3.4940	> 10.5	= 10.5	< 1	6	1	> 15.00		15.00	4.46	< 1	
		P3.4942	> 13.2	= 13.2	< 1	3	1						
	ROD	P3.4941	> 15.3	= 15.3	< 1	4	1	> 15.00		15.00	4.46	< 1	
		P3.4943	> 21	= 21	< 1	4	1						
SHA/NAISB-003	III_B	P3.4940	> 69.5	= 69.5	< 1	0	1	> 81.55		81.55	11.29	< 1	Cryst. Observ. At 25 µg / ml
		P3.4942	> 78.3	= 78.3	< 1	3	1						
	ROD	P3.4941	> 81.8	= 81.8	< 1	12	1	> 81.55		81.55	11.29	< 1	
		P3.4943	> 96.6	= 96.6	< 1	17	1						
SHA/NAISB-004	III_B	P3.4940	> 10.2	= 10.2	< 1	16	1	> 11.58		11.58	1.57	< 1	Cryst. Observ. At 125 µg / ml
		P3.4942	> 13.5	= 13.5	< 1	35	1						
	ROD	P3.4941	> 12.2	= 12.2	< 1	20	1	> 11.58		11.58	1.57	< 1	
		P3.4943	> 10.4	= 10.4	< 1	40	1						
SHA/NAISB-005	III_B	P3.4940	> 12.9	= 12.9	< 1	7	1	> 13.48		13.48	0.89	< 1	
		P3.4942	> 14.8	= 14.8	< 1	4	1						
	ROD	P3.4941	> 13.2	= 13.2	< 1	12	1	> 13.48		13.48	0.89	< 1	
		P3.4943	> 13	= 13	< 1	10	1						
SHA/NAISB-006	III_B	P3.4940	> 60	= 60	< 1	7	1	> 42.03		42.03	22.49	< 1	Cryst. Observ. At 25 µg / ml
		P3.4942	> 19	= 19	< 1	5	1						
	ROD	P3.4941	> 62.6	= 62.6	< 1	9	1	> 42.03		42.03	22.49	< 1	
		P3.4943	> 26.5	= 26.5	< 1	19	1						
SHA/NAISB-007	III_B	P3.4940	> 17.1	= 17.1	< 1	6	1	> 22.08		22.08	12.15	< 1	Cryst. Observ. At 125 µg / ml
		P3.4942	> 14.2	= 14.2	< 1	6	1						
	ROD	P3.4941	> 40.2	= 40.2	< 1	10	1	> 22.08		22.08	12.15	< 1	
		P3.4943	> 16.8	= 16.8	< 1	9	1						
SHA/NAISB-008	III_B	P3.4940	> 115	= 115	< 1	6	1	> 109		≥ 109		< or X 1	Cryst. Observ. At 25 µg / ml
		P3.4942	> 109	= 109	< 1	13	1						
	ROD	P3.4941	> 125	> 125	X 1	11	2	> 109		≥ 109		< or X1	
		P3.4943	> 125	> 125	X 1	4	1						

Code	Strain	Exp_no	IC_{50} (µg/ml)	CC_{50} (µg/ml)	SI	Max. Prot. (%)	Appr.	Av. IC_{50} (µg/ml)	SD	Av. CC_{50} (µg/ml)	SD	SI	Remarks
SHA/NAISB-009	III_B	P3.4940	> 10.1	= 10.1	< 1	14	1	> 12.50		12.50	2.07	< 1	Cryst. Observ. At 125 µg / ml
		P3.4942	> 14.8	= 14.8	< 1	6	1						
	ROD	P3.4941	> 11.6	= 11.6	< 1	13	1	> 12.50		12.50	2.07	< 1	
		P3.4943	> 13.5	= 13.5	< 1	9	1						
SHA/NAISB-010	III_B	P3.4940	= 16.4	> 125	> 8	73	3	15.10	1.84	> 125.00		> 8	Cryst. Observ. At 5 µg / ml
		P3.4942	= 13.8	> 125	> 9	102	1						
	ROD	P3.4941	= 52.9	> 125	> 2	72	3	40.30	17.82	> 125.00		> 3	
		P3.4943	= 27.7	> 125	> 5	121	3?						
SHA/NAISB-011	III_B	P3.4940	> 125	> 125	X 1	1	1	> 125.00		> 125.00		X 1	Cryst. Observ. At 25 µg / ml
		P3.4942	> 125	> 125	X 1	13	1						
	ROD	P3.4941	> 125	> 125	X 1	3	1	> 125.00		> 125.00		X 1	
		P3.4943	> 125	> 125	X 1	6	1						
SHA/NAISB-012	III_B	P3.4940	> 17.2	= 17.2	< 1	8	1	≥ 2.52		15.75	1.14	≤ 6	Cryst. Observ. At 125 µg / ml
		P3.4942	= 2.52	= 14.7	= 6	81	1						
	ROD	P3.4941	= 2.66	= 15	= 6	74	1	2.24	0.60	15.75	1.14	7	
		P3.4943	= 1.81	= 16.1	= 9	116	1						
SHA/NAISB-013	III_B	P3.4940	> 76.4	= 76.4	< 1	16	1	> 80.53		80.53	20.96	< 1	Cryst. Observ. At 125 µg / ml
		P3.4942	> 60.4	= 60.4	< 1	25	1						
	ROD	P3.4941	> 75.3	= 75.3	< 1	18	1	> 80.53		80.53	20.96	< 1	
		P3.4943	> 110	= 110	< 1	45	1						
SHA/NAISB-015	III_B	P3.4940	> 80.4	= 80.4	< 1	7	1	> 65.48		65.48	30.86	< 1	Cryst. Observ. At 25 µg / ml
		P3.4942	> 20.6	= 20.6	< 1	19	1						
	ROD	P3.4941	> 89.7	= 89.7	< 1	16	1	> 65.48		65.48	30.86	< 1	
		P3.4943	= 23.4	= 71.2	= 3	52	1						
SHA/NAISB-018	III_B	P3.4940	> 110	= 110	< 1	7	1	> 110.00		≥ 110.00		< or X1	Cryst. Observ. At 25 µg / ml
		P3.4942	> 125	> 125	X 1	5	1						
	ROD	P3.4941	> 117	= 117	< 1	11	1	> 110.00		≥ 110.00		< or X 1	
		P3.4943	> 125	> 125	X 1	19	1						
SHA/NAISB-019	III_B	P3.4940	> 98.1	= 98.1	< 1	10	1	> 98.10		≥ 98.10		< or X 1	
		P3.4942	> 110	= 110	< 1	20	1						
	ROD	P3.4941	> 125	> 125	X 1	12	1	> 98.10		≥ 98.10		< or X 1	
		P3.4943	> 125	>125	X 1	21	1						

Code	Strain	Exp_no	IC_{50} (µg/ml)	CC_{50} (µg/ml)	SI	Max. Prot. (%)	Appr.	Av. IC_{50} (µg/ml)	SD	Av. CC_{50} (µg/ml)	SD	SI	Remarks
SHA/NAISB-020	III_B	P3.4940	> 17.7	= 17.7	< 1	18	1	> 24.98		24.98	8.49	< 1	Cryst. Observ. At 125 µg / ml
		P3.4942	> 20.7	= 20.7	< 1	18	1						
	ROD	P3.4941	> 37	= 37	< 1	7	1	> 24.98		24.98	8.49	< 1	
		P3.4943	> 24.5	= 24.5	< 1	25	1						
SHA/NAIPAL-001	III_B	P3.4942	> 54	= 54	< 1	17	1	> 65.67		65.67	11.55	< 1	Cryst. Observ. At 125 µg / ml
	ROD	P3.4941	> 77.1	= 77.1	< 1	7	1	> 65.67		65.67	11.55	< 1	
		P3.4943	> 65.9	= 65.9	< 1	18	1						
SHA/NAIPAL-002	III_B	P3.4940	> 60.9	= 60.9	< 1	13	1	> 69.43		69.43	16.62	< 1	Cryst. Observ. At 125 µg / ml
		P3.4942	> 73.4	= 73.4	< 1	16	1						
	ROD	P3.4941	> 52.6	= 52.6	< 1	23	1	> 69.43		69.43	16.62	< 1	
		P3.4943	> 90.8	= 90.8	< 1	36	1						
SHA/NAIPAL-003	III_B	P3.4940	> 119	= 119	< 1	6	1	> 125		> 125		X 1	Cryst. Observ. At 25 µg / ml
		P3.4942	> 125	> 125	X 1	4	1						
	ROD	P3.4941	> 125	> 125	X 1	9	1	> 125		> 125		X 1	
		P3.4943	> 125	> 125	X 1	8	1						
SHA/NAIPAL-004	III_B	P3.4940	> 12.6	= 12.6	< 1	7	1	> 12.55		12.55	0.66	< 1	
		P3.4942	> 13	= 13	< 1	9	1						
	ROD	P3.4941	> 11.6	= 11.6	< 1	9	1	> 12.55		12.55	0.66	< 1	
		P3.4943	> 13	= 13	< 1	11	1						
SHA/NAIPAL-008	III_B	P3.4940	> 125	> 125	X 1	14	1	> 125.00		> 125.00		X 1	Cryst. Observ. At 25 µg / ml
		P3.4942	> 125	> 125	X 1	8	1						
	ROD	P3.4941	> 125	> 125	X 1	4	1	> 125.00		> 125.00		X 1	
		P3.4943	> 125	> 125	X 1	8	1						
SHA/NAIPAL-009	III_B	P3.4940	> 125	> 125	X 1	3	1	> 125.00		> 125.00		X 1	Cryst. Observ. At 125 µg / ml
		P3.4942	> 125	> 125	X 1	2	1						
	ROD	P3.4941	> 125	> 125	X 1	31	1	≥ 112.00		> 125.00		> or X1	
		P3.4943	= 112	> 125	> 1	53	1						
SHA/TZD-1-1-001	III_B	P3.4940	> 71.7	= 71.7	< 1	11	1	> 74.73		74.73	4.74	< 1	Cryst. Observ. At 125 µg / ml
		P3.4942	> 79.3	= 79.3	< 1	7	1						
	ROD	P3.4941	> 69.7	= 69.7	< 1	9	1	> 74.73		74.73	4.74	< 1	
		P3.4943	> 78.2	= 78.2	< 1	25	1						

Code	Strain	Exp_no	IC_{50} (µg/ml)	CC_{50} (µg/ml)	SI	Max. Prot. (%)	Appr.	Av. IC_{50} (µg/ml)	SD	Av. CC_{50} (µg/ml)	SD	SI	Remarks
SHA/TZD-1-1-002	III_B	P3.4940	> 73.8	= 73.8	< 1	12	1	> 78.33		78.33	6.98	< 1	
		P3.4942	> 80.1	= 80.1	< 1	8	1						
	ROD	P3.4941	> 72	= 72	< 1	6	1	> 78.33		78.33	6.98	< 1	
		P3.4943	= 21.1	= 87.4	= 4	55	1						
SHA/TZD-1-1-003	III_B	P3.4940	> 12.7	= 12.7	< 1	7	1	> 13.83		13.83	1.43	< 1	
		P3.4942	> 12.5	= 12.5	< 1	9	1						
	ROD	P3.4941	> 14.8	= 14.8	< 1	13	1	> 13.83		13.83	1.43	< 1	
		P3.4943	> 15.3	= 15.3	< 1	9	1						
SHA/TZD-1-1-009	III_B	P3.4940	> 71.9	= 71.9	< 1	1	1	> 69.33		69.33	9.69	< 1	
		P3.4942	> 73.6	= 73.6	< 1	7	1						
	ROD	P3.4941	> 55.1	= 55.1	< 1	8	1	> 69.33		69.33	9.69	< 1	
		P3.4943	> 76.7	= 76.7	< 1	8	1						
SHA/TZD-2-1-001	III_B	P3.4940	> 62.7	= 62.7	< 1	6	1	> 68.33		68.33	5.51	< 1	
		P3.4942	> 72.7	= 72.7	< 1	6	1						
	ROD	P3.4941	> 64.5	= 64.5	< 1	6	1	> 68.33		68.33	5.51	< 1	
		P3.4943	> 73.4	= 73.4	< 1	17	1						
SHA/TZD-2-1-002	III_B	P3.4940	> 97.2	= 97.2	< 1	16	1	> 89.35		89.35	14.75	< 1	Cryst. Observ. At 125 µg / ml
		P3.4942	> 71.7	= 71.7	< 1	23	1						
	ROD	P3.4941	> 105	= 105	< 1	19	1	> 89.35		89.35	14.75	< 1	
		P3.4943	> 83.5	= 83.5	< 1	30	1						
SHA/TZD-2-1-003	III_B	P3.4940	> 22.9	= 22.9	< 1	11	1	> 33.40		33.40	13.45	< 1	
		P3.4942	> 26.1	= 26.1	< 1	6	1						
	ROD	P3.4941	> 52.8	= 52.8	< 1	13	1	> 33.40		33.40	13.45	< 1	
		P3.4943	> 31.8	= 31.8	< 1	14	1						
SHA/TZD-2-1-006	III_B	P3.4940	> 19.7	= 19.7	< 1	34	1	> 51.73		51.73	23.08	< 1	Cryst. Observ. At 125 µg / ml
		P3.4942	> 50	= 50	< 1	50	1						
	ROD	P3.4941	= 13.3	= 69	= 5	78	1	14.65	1.91	51.73	23.08	4	
		P3.4943	= 16	= 68.2	= 4	66	2						
SHA/TZD-2-1-007	III_B	P3.4940	> 51.9	= 51.9	< 1	6	1	> 65.23		65.23	9.55	< 1	
		P3.4942	> 67.6	= 67.6	< 1	4	1						
	ROD	P3.4941	> 66.8	= 66.8	< 1	14	1	≥ 23.90		65.23	9.55	≤ 3	
		P3.4943	= 23.9	= 74.6	= 3	51	1						

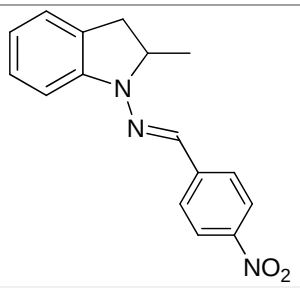
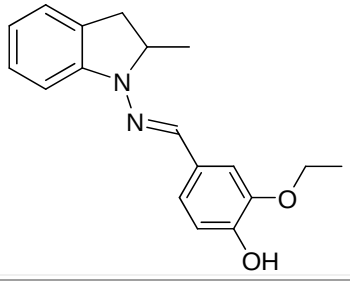
Code	Strain	Exp_no	IC_{50} (µg/ml)	CC_{50} (µg/ml)	SI	Max. Prot. (%)	Appr.	Av. IC_{50} (µg/ml)	SD	Av. CC_{50} (µg/ml)	SD	SI	Remarks
SHA/TZD-2-1-010	III_B	P3.4940	= 76.6	> 125	> 2	67	1	62.55	19.87	> 125.00		> 2	Cryst. Observ. At 125 µg / ml
		P3.4942	= 48.5	> 125	> 3	99	1						
	ROD	P3.4941	> 125	> 125	X 1	44	1	≥ 46.50		> 125.00		< or X 3	
		P3.4943	= 46.5	> 125	> 3	96	1						
BOE/BIRG587 = Nevirapine	**III_B**	**P3.4801**	**= 0.0626**	**> 4**	**> 64**	**94**	**1**	**0.050**	**0.011**	**> 4.00**		**> 80**	
		P3.4803	**= 0.047**	**> 4**	**> 85**	**100**	**1**						
		P3.4807	**= 0.0523**	**> 4**	**> 76**	**123**	**1**						
		P3.4809	**= 0.0371**	**> 4**	**> 108**	**118**	**1**						
	ROD		**> 4**	**> 4**	**< 1**	**0**	**1**	**> 4.00**		**> 4.00**		**< 1**	
DDN/AZT = Azidothymidine, zidovudine, Retrovir ©	**III_B**	**P3.4801**	**= 0.00147**	**> 25**	**> 17003**	**100**	**1**	**0.0022**	**0.0011**	**> 25.00**		**> 11587**	
		P3.4803	**= 0.00199**	**> 25**	**> 12587**	**117**	**1**						
		P3.4807	**= 0.00141**	**> 25**	**> 17762**	**121**	**1**						
		P3.4809	**= 0.00376**	**> 25**	**> 6644**	**94**	**1**						
	ROD	**P3.4795**	**= 0.00034**	**> 25**	**> 73258**	**104**	**2**	**0.00094**	**0.0005**	**> 25.00**		**> 26731**	
		P3.4797	**= 0.00153**	**> 25**	**> 16308**	**94**	**1**						
		P3.4802	**= 0.00094**	**= 19.1**	**= 20367**	**98**	**1**						
		P3.4808	**= 0.00093**	**> 25**	**> 26778**	**100**	**1**						
DDN/DDC= Dideoxy citidine	**III_B**	**P3.4786**	**= 0.126**	**> 20**	**> 159**	**157**	**1**	**0.16**	**0.12**	**> 20.00**		**> 127**	
		P3.4790	**= 0.0774**	**> 20**	**> 258**	**95**	**1**						
		P3.4794	**= 0.0994**	**> 20**	**> 201**	**122**	**1**						
		P3.4796	**= 0.328**	**> 20**	**> 61**	**99**	**1**						
	ROD	**P3.4787**	**= 0.123**	**> 20**	**> 162**	**109**	**1**	**0.19**	**0.11**	**> 20.00**		**> 108**	
		P3.4791	**= 0.165**	**> 20**	**> 122**	**73**	**2**						
		P3.4795	**= 0.104**	**> 20**	**> 193**	**106**	**2**						
		P3.4797	**= 0.348**	**> 20**	**> 58**	**93**	**1**						
DDN/DDI = Dideoxyinosine, Didanosine.	**III_B**	**P3.4788**	**= 2.58**	**> 50**	**> 19**	**141**	**2**	**2.09**	**0.68**	**> 50.00**		**> 24**	
		P3.4792	**= 1.1**	**> 50**	**> 46**	**140**	**1**						
		P3.4801	**= 2.5**	**> 50**	**> 20**	**109**	**1**						
		P3.4807	**= 2.19**	**> 50**	**> 23**	**134**	**1**						
	ROD	**P3.4789**	**= 3.04**	**> 50**	**> 16**	**95**	**1**	**3.78**	**1.22**	**> 50.00**		**> 13**	
		P3.4793	**= 4.04**	**> 50**	**> 12**	**93**	**1**						
		P3.4802	**= 5.38**	**> 50**	**> 9**	**83**	**1**						
		P3.4808	**= 4.26**	**> 50**	**> 12**	**102**	**1**						

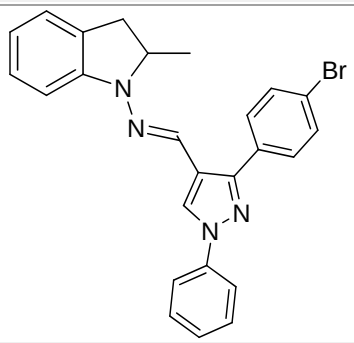
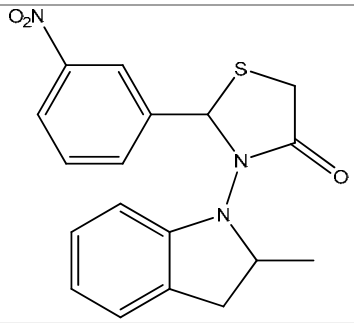
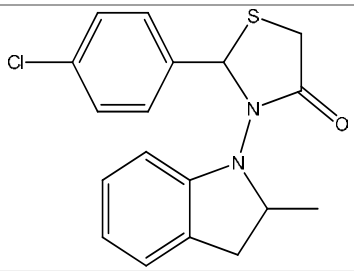
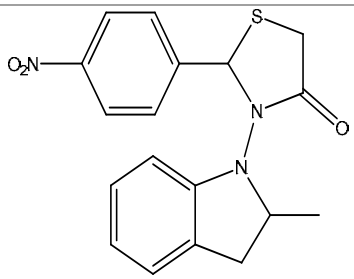
6.3 RESULTS AND DISCUSSIONS

The screening of the synthesized compounds by the above mentioned protocol for the Anti-HIV activity gave some interesting results. On studying the Selectivity Index (SI) it was observed that few compounds and their class yielded moderate to low anti-HIV activity. On observing the data set very closely it also revealed that some of the compounds possess a good average IC_{50} values or encouraging average CC_{50} values but their Selectivity Index was below 1 and therefore they could not be considered moderately active.

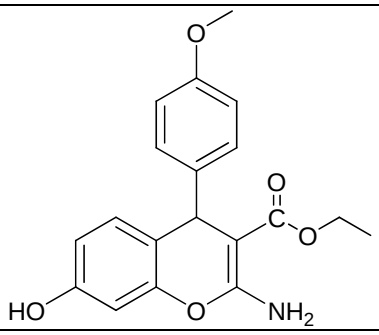
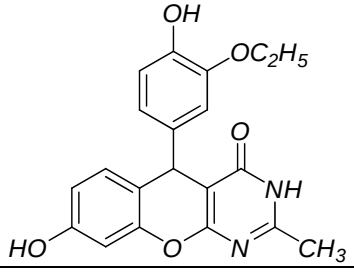
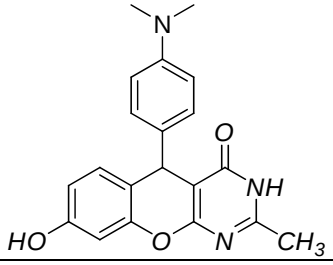
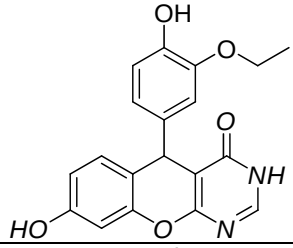
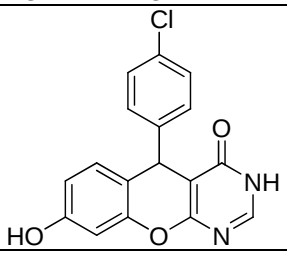
6.4 CONCLUSION

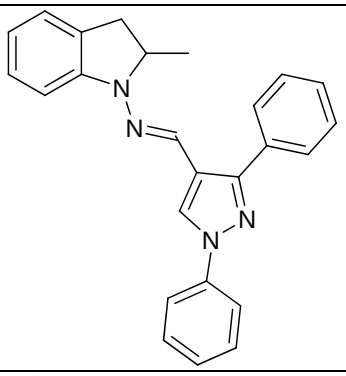
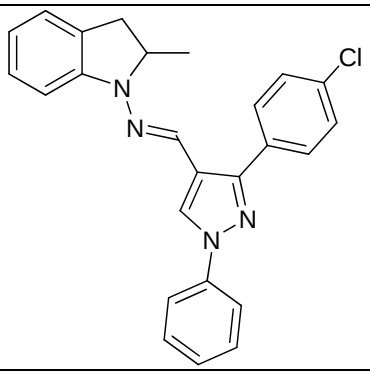
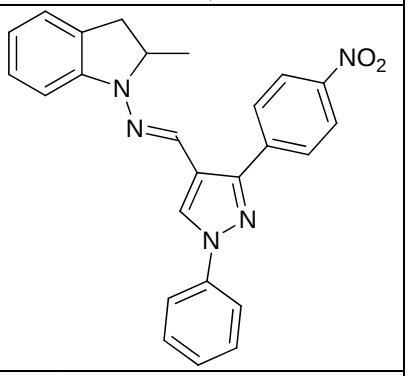
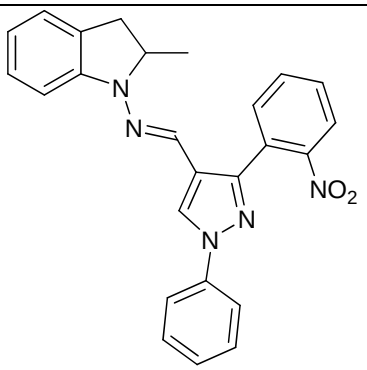
Thus, all the compounds of each class covered under this thesis were screened for their Anti-HIV activity against two HIV strains out of which 22 compounds comprising of 7 different classes have shown good average CC_{50} as well as average IC_{50} values but as their selectivity index was very low they could not be considered as moderately active. While there were other 6 compounds which gave a moderate to good Selectivity Index. The following table demonstrates the compounds which came out moderately active against either both the strains or any one of the two strains based upon the Selectivity Index value.

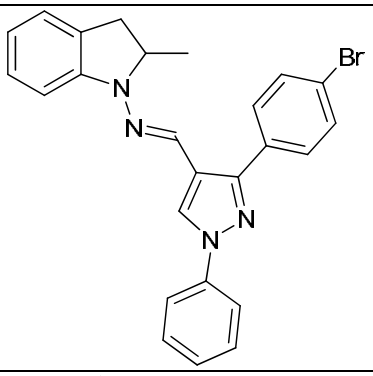
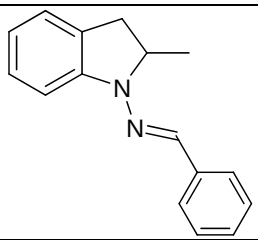
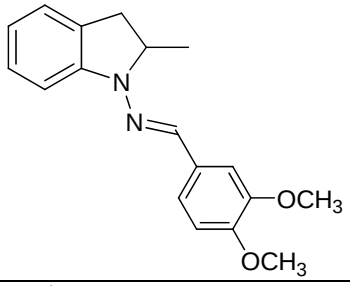
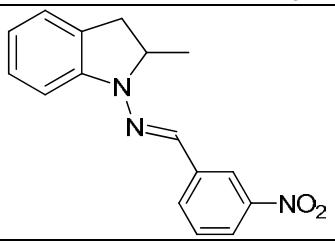
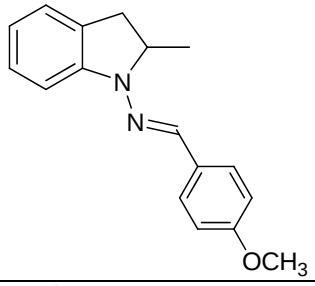
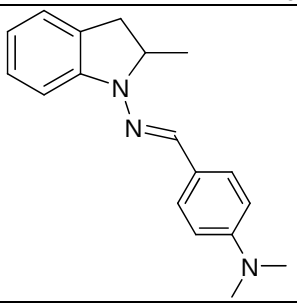
Sr. No.	Code Name	Strain	Selectivity Index (SI)	Structure
1	NAISB-010	III_B	> 8	
		ROD	> 3	
2	NAISB-012	III_B	≤ 6	
		ROD	= 7	

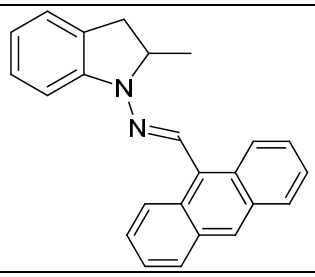
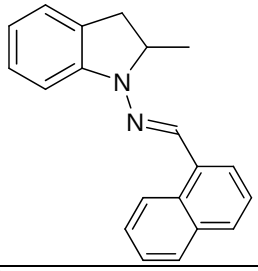
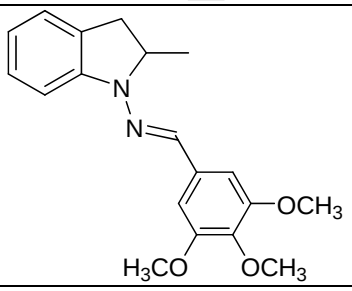
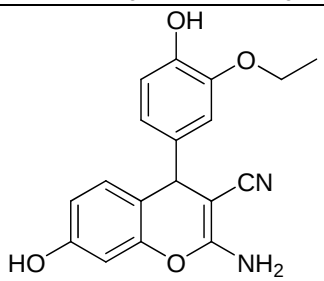
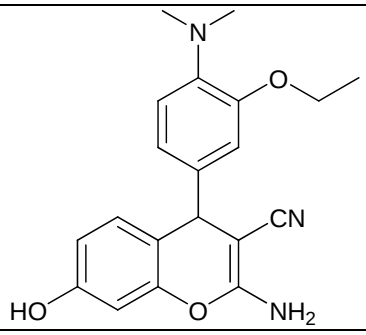
3	NAIPAL-009	ROD	> or X1	
4	TZD-2-1-006	ROD	= 4	
5	TZD-2-1-007	ROD	≤ 3	
6	TZD-2-1-010	III_B	> 2	
		ROD	< or X 3	

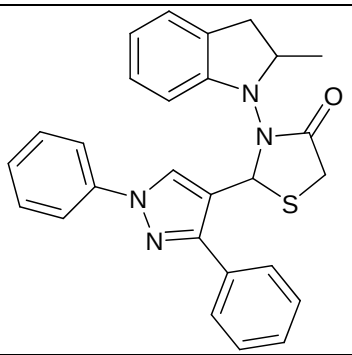
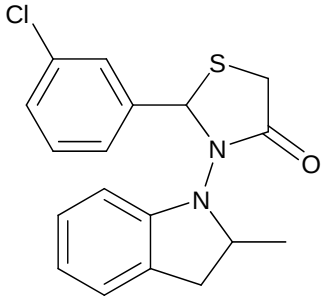
The list of the 22 compounds which demonstrated encouraging IC_{50} and CC_{50} values but whose selectivity index was low is given below

Sr. No.	Series	Code	Structure
1	BN	BN-002	
2	GAA	GAA-007	
3	GAA	GAA-008	
4	NmNB	NmNB-008	
5	NmNB	NmNB-15	

6	NAIPAL	NAIPAL-001	
7	NAIPAL	NAIPAL-002	
8	NAIPAL	NAIPAL-003	
9	NAIPAL	NAIPAL-008	

10	NAIPAL	NAIPAL-009	
11	NAISB	NAISB-001	
12	NAISB	NAISB-003	
13	NAISB	NAISB-006	
14	NAISB	NAISB-008	
15	NAISB	NAISB-011	

16	NAISB	NAISB-013	
17	NAISB	NAISB-015	
18	NAISB	NAISB-018	
19	NB	NB-007	
20	NB	NB-008	

21	TZD-1-1	TZD-1-1-001	
22	TZD2-1	TZD-2-1-002	

Thus as discussed above in results and discussion, 6 moieties have come out moderately active as shown by their SI values which will help us study them further. On the basis of the above interesting results new synthetic programmes to develop more active molecules could be designed.

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SUMMARY

The work represented in the thesis entitled ***"Synthesis and Pharmacological Studies of Some New Chemical Entities"*** is divided into six chapters which can be summarized as under.

Chapter – 1 comprises of the General Introduction of the complete work covered in this thesis. It serves to build a foundational platform justifying the importance and need to carry out such a kind of work. Starting from the word *Sustainability,* its meaning and importance, it explores the important aspects of Green chemistry. It the same lines it also deals with the topic of Microwave Assisted Organic Synthesis (MAOS) stating its importance as well as its expanse of application in the field of synthetic organic chemistry. Also, Aqua Mediated Organic Synthesis (AMOS) has been conversed under this chapter citing its importance as well as its mode of action in synthetic organic chemistry as to how does water expedite the organic reactions.

Further on this chapter explores the biological significance as well as importance of pyrimidine as well as thiazolidinone moieties so as to build a strong background for those type of molecules that have been synthesized in this thesis.

Chapter-2 deals with the Aqua mediated as well as Microwave assisted synthesis of *2-Amino-7-hydroxy-4-(substituted phenyl)-4H-chromene-3-carbonitrile* derivatives and characterization thereof. Initially, it discusses the importance of Benzopyran as the privileged structure and then goes on to implicate the importance of 2-Amino-4H-chromene derivatives. The previous synthetic attempts have also been discussed in this chapter before coming on to the actual synthetic aspect of the chapter. The thorough literature survey revealed that recently a series of 4-Aryl-4H chromene derivatives were found out to be apoptosis inducers apart from the other significant biological activities. The section B of this chapter deals with the synthesis and characterization of Ethyl-2-amion-3-carboxylate-4-aryl-4H-chromene derivatives. Hence, a one pot rapid microwave as well as aqua mediated synthesis of such class of compounds was taken up to synthesize *2-Amino-7-hydroxy-4-(substituted phenyl)-4H-chromene-3-carbonitrile/(ethyl-3-carboxylate)* scaffolds from which a diverse range of other biologically important New Chemical Entities (NCE's) could be generated.

Chapter-3 encompasses the rapid microwave assisted synthesis of *8-Hydroxy-5-substituted phenyl-3H-chromeno-[2, 3-d]pyrimidine-4(5H)-one* derivatives using 2-Amino-7-hydroxy-4-(substituted phenyl)-4H-chromene-3-carbonitrile, the product of chapter 2 as the starting material. The literature survey revealed that inclusion of two bioactive motifs like benzopyran and pyrimidine into a single carbon skeleton has scarcely been achieved in this manner. Moreover, the Microwave assisted synthesis is a greener approach to synthesizing this class of molecules which other wise have been prepared by conventional synthetic approaches. Some reported synthetic schemes have also been discussed in the opening part of the chapter. In Section A formic acid has been used for cyclization where as in section B Acetic acid has been used to afford a 2-Methyl derivative of the aforesaid class of compounds.

Chapter-4 discusses about the rapid microwave assisted synthesis of N-2-(methyl indoline-1-yl)(Substituted phenyl)methanimine and characteri. Charting out the importance of indole nucleus the chapter starts with the introduction to the indoline systems and the previous synthetic routes thereof. To synthesize these class of compounds we had to first synthesize the N-amino-2-methyl indoline which as been discussed in the chapter. The thorough literature survey revealed to us that researches have been exploring the acid amine condensation on the N-amino function forming the biologically important Amide linkage but hardly any reference about the preparation of methanimine could be found. Hence, it was decided to take up the work to synthesize these compounds using microwave irradiation as the non conventional source of energy and a green synthetic approach. In the section B differently substituted pyrazole aldehydes were prepared as the literature survey revealed some important pyrazole durgs with two phenyl rings attached to it. Hence, again the inclusion of two bioactive motifs like 2-Methyl indoline and 1,3-Diphenylpyrazole nucleus into a single carbon skeleton which has scarcely been achieved is carried out in this chapter.

Chapter 5 covers the single step microwave assisted synthesis of 3-(2-methyl indoline-1-yl)-2-substitued phenyl thiazolidine-4-one derivatives and characterizations thereof. The importance of Thiazolidin-4-one system has aptly been discussed in both chapter 1 as well as in here including some recent synthetic strategies which have been employed to synthesize this class of compounds. The

literature survey revealed that such class of thaizolidin-4-ones is Anti-HIV active. Moreover, the inclusion of the 2-Methyl indoline function into the carbon skeleton of thiazolidine-4-one was also barely observed in the literature. Hence, the synthesis of these novel chemical entities was taken up using microwave irradiations as the non conventional source of energy.

Chapter-6 narrates the biological activity study of the synthesized compounds which have been screened for Anti-HIV activity against two HIV strains namely III_B as well as ROD. The protocol by which the activity study has been carried out is also discussed at length in the chapter before the data sets of the results are revealed. Some interesting results were obtained as six of the synthesized compounds were found out to be moderately active either against both the strains or against any single strain which was deduced upon by the selectivity index of each compound. The standard Anti-HIV drugs like Nevirapine, Zidovudine, Dideoxy citidine, as well Didanosine were used as reference standards for the screening. In comparison to their results the compounds synthesized in this thesis can be called moderate to weak anti-HIV compounds as the drugs have a significantly high selective index.

The synthesized compounds have also been sent for Anti cancer, Anti-TB as well as Multi drug reversal activity study, the results of which are still awaited.

CONFERENCES/SEMINARS/WORKSHOPS ATTENDED

- ISCB Conference ***"International Conference on the Interface of Chemistry Biology in Biomedical Research"*** at Chemistry Group, Birla Institute of Technology & Science, Pilani, India dated February, 22-24, 2008.
- DST-FIST, UGC (SAP) supported and GUJCOST Sponsored ***"National Workshop on Management and Use of Chemistry Database and Patent Literature"*** organized by GUJCOST & Dept. of Chemistry of Saurashtra University, Rajkot, (Gujarat), dated February, 27-29, 2008.
- DST-FIST, UGC (SAP) supported and GUJCOST Sponsored ***"National Conference on Selected Topics in Spectroscopy and Stereochemistry"*** organized by the Department of Chemistry, Saurashtra University, Rajkot, dated March, 18-20, 2009.
- ***"A National Workshop On Updates In Process and Medicinal Chemistry"*** jointly organized by National Facility for Drug Discovery through New Chemicals Entities Development & Instrumentation support to Small Manufacturing Pharma Enterprises and DST FIST, UGC-SAP & DST-DPRP Funded Department of Chemistry, Saurashtra University, Rajkot dated March, 3-4, 2009.
- ***"Two Days National Workshop on Patents & Intellectual Property Rights Related Updates"*** Sponsored by TIFAC & GUJCOST and Organized by DST-FIST, UGC-SAP & DST-DPRP Funded Department of Chemistry, Saurashtra University, Rajkot, dated September, 19-20, 2009.
- ***"International Seminar on Recent Developments in Structure and Ligand based Drug Design"*** jointly organized by Schrodinger LLC, USA; National Facility for Drug Discovery through New Chemicals Entities Development & Instrumentation support to Small Manufacturing Pharma Enterprises and DST FIST, UGC-SAP & DST-DPRP Funded Department of Chemistry, Saurashtra University, Rajkot, dated December, 23rd, 2009.

Paper/Poster presented at the International Conference:

- ***"Synthesis, Characterization and biological activity of some Novel Triazino Indole Derivatives."***

  Nilay Pandya, Vijay Virsodiya, Atul Manvar, Shailesh Thakrar, Shrey Parekh, ***Anamik Shah*.***

  Poster Presented at 12th ISCBC Conference "International Conference on the Interface of Chemistry and biology in Biomedical Research" At Chemistry Group, Birla Institute of Technology and Science, BITS-Pilani, Rajasthan India.