SYNTHESIS AND CHARACTERISATION OF SOME MEDICINALLY IMPORTANT SPIRO AND CONDENSED HETEROCYCLES THROUGH GREEN APPROACH

This thesis is submitted as
a fulfillment of the
Ph.D. program in Chemistry

SHUCHI MAHESHWARI
(I.D. No. 2010RCY102)

Department of Chemistry
MALAVIYA NATIONAL INSTITUTE OF TECHNOLOGY JAIPUR
January, 2015

DECLARATION

I hereby certify that the work which is being presented in this thesis entitled "**Synthesis and Characterisation of Some Medicinally Important Spiro and Condensed Heterocycles through Green Approach**" in fulfillment of the requirement of **Doctor of Philosophy** and submitted to the Malaviya National Institute of Technology, Jaipur is an authentic record of my own work carried out at the Department of Chemistry under the supervision of Dr. Jyoti Joshi, Associate Professor, Department of Chemistry, Malaviya National Institute of Technology, Jaipur and Prof. (Mrs.) Anshu Dandia, Head, Department of Chemistry, University of Rajasthan, Jaipur. The results contained in this thesis have not been submitted in part or full, to any other University or Institute for the award of any degree. The content of the thesis has been checked using software 'Plagiarism Detector'.

Shuchi Maheshwari
ID No. 2010RCY102
Department of Chemistry
MNIT, Jaipur
India

Date:

CERTIFICATE

This is to certify that the work reported in this thesis entitled "**Synthesis and Characterisation of Some Medicinally Important Spiro and Condensed Heterocycles through Green Approach**" has been carried out by **Ms. Shuchi Maheshwari** and submitted to the Malaviya National Institute of Technology, Jaipur, for the award of **Doctor of Philosophy** in Chemistry is a bonafide record of research work carried out by her under our supervision and guidance. The thesis work, in our opinion, has reached the requisite standard fulfilling the requirement for the degree of **Doctor of Philosophy**. The thesis embodies the original work done by her and to the best of our knowledge and belief, this work has not been carried out earlier.

(Dr. Jyoti Joshi)
Associate Professor
Department of Chemistry
MNIT, Jaipur
Jaipur-302017
India

(Prof. (Mrs.) Anshu Dandia)
Professor & Head
Department of Chemistry
University of Rajasthan, Jaipur
Jaipur-302004
India

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ABSTRACT

The thesis entitled "**Synthesis and Characterisation of Some Medicinally Important Spiro and Condensed Heterocycles through Green Approach**" is the study with special emphasis on the design and development of various green approaches for the synthesis of biologically potent heterocyclic compounds.

Heterocyclic compounds are well recognized for their multifaceted pharmacological behaviour. They display an array of biological activities. Many modern pharmaceuticals which are in regular therapeutic use are organic compounds and contain heterocyclic moieties. Some of these have been found to possess significant biological properties and surprisingly low toxicity towards humans.

Medicinal chemists nevertheless continue to shed light on the structure activity relationship that governs the field by describing new products and optimising structural variables. The search for novel chemical entities has largely focussed on the synthesis of heterocyclic compounds with pharmacological activities.

The present thesis has been divided into seven chapters.

Chapter 1 begins with a brief introduction to heterocyclic chemistry, organic synthesis, and description of 'Green Chemistry' including the twelve principles, alternative methods (microwave, ultrasound and infrared irradiation), reaction media (water, fluorous solvents, polyethylene glycol and ionic liquids) and catalysts (nanocatalysts and biocatalysts) for the synthesis of heterocyclic compounds. Two hundred and nine references have been given at the end of the chapter.

Chapter 2 describes a rapid and catalyst-free aqueous-mediated expedient multicomponent reaction capable of affording a combinatorial library of medicinally important pyrazolo[3,4-*e*][1,4]thiazepine derivatives in excellent yields in shorter reaction times. This new protocol provides a seven-membered ring system by the one-pot three-component approach involving isatin/acenapthaquinone/piperidines/ aromatic aldehydes, heterocyclic amines and α-mercapto carboxylic acids selectively instead of expected five-membered ring system or other possible isomers. In addition to chemo and regioselectivity, interesting results were also obtained with respect to diastereoselectivity. The reaction constitutes a sustainable and environment-friendly

approach for the development of biologically active compounds and opens a new way for creating molecular complexity with maximum simplicity.

Chapter 3 reports the chemoselective synthesis of indolo[2,3-*b*]quinoxaline derivatives by the reaction of isatins with 1,2-diamines in ethylene glycol under microwave irradiation using Cu doped CdS nanoparticles as a catalyst which have been prepared by simple aqueous chemical method. The method showed remarkable selectivity for indolo[2,3-*b*]quinoxalines over 3-imino-isatin, spirobenzimidazole, and ring-opened quinoxalinone derivatives. Cu doped CdS NPs play a dual role of catalyst as well as susceptor, and enhances the overall capacity to absorb MW in the reaction mixture. Cu doped CdS NPs have been characterized by various techniques such as FT-IR, XRD, TEM, SEM, EDAX, UV/VIS and ICP-AES.

Chapter 4 constitutes an efficient and inexpensive synthetic protocol for the synthesis of substituted-2,3-dihydroquinazolin-4(1*H*)-one derivatives in refluxing ethanol. The present methodology uses $CuFe_2O_4$ nanoparticles as a catalyst for the three-component condensation reaction of isatoic anhydride, substituted anilines and various aldehydes affording the desired products. Magnetically separable Copper ferrite nanoparticles have been characterized by various techniques such as FT-IR, XRD, SEM, EDAX and ICP-AES.

Chapter 5 deals with a direct and highly efficient protocol for the synthesis of medicinally important spirooxindole derivatives through a one-pot multi-component reaction of substituted isatins, malononitrile and cyclic ketones catalyzed by L-proline as an inexpensive catalyst in aqueous ethanol. This multicomponent reaction proceeds *via* sequential Knoevenagel condensation and Michael addition reaction to afford the desired products.

Chapter 6 describes a highly efficient methodology for the synthesis of spiro[indoline-3,4′-pyrazolo[3,4-*b*]pyridine] derivatives through a one-pot, three-component reaction of isatin, malononitrile, and 5-amino-3-methylpyrazole using 2,2,2-trifluoroethanol as a reusable green solvent under microwave irradiation.

Chapter 7 deals with the α-amylase inhibition assay, antioxidant, antimicrobial, antitubercular and antimalarial activity of the compounds synthesized against various strains.

All the synthesized sixty five compounds were tested for purity by TLC, while IR, ^{1}H NMR, ^{13}C NMR and Mass spectral studies have been used for the assignment of structure of the title compounds. The structure and relative stereochemistry of the compounds was also confirmed by single crystal X-ray diffraction.

Conclusion

The present work is an attempt to explore newer trends in the upcoming areas of 'Green Chemistry' by using sustainable approaches for devising alternative methods such as ultrasound irradiation, microwave irradiation, nanocatalysts, alternative solvents, etc. for the diversity-oriented synthesis of biologically potent heterocycles. The versatility of these techniques has been studied in terms of their synthetic utility for medicinally important heterocyclic compounds.

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LIST OF ABBREVIATIONS

DMF	:	Dimethyl Formamide
DMSO	:	Dimethyl sulfoxide
EDAX	:	Energy Dispersive Adsorption X-ray
ESI	:	Electrospray Ionization
Et	:	Ethyl
EtOH	:	Ethanol
Fig	:	Figure
FT-IR	:	Fourier Transform Infra-red
ICP-AES	:	Inductively Coupled Plasma Atomic Emission Spectroscopy
IL	:	Ionic Liquid
IR	:	Infrared
Me	:	Methyl
MeOH	:	Methanol
MNPs	:	Magnetic Nanoparticles
MW	:	Microwave
NPs	:	Nanoparticles
NMR	:	Nuclear Magnetic Resonance
PC	:	Polycarbonate
PEG	:	Polyethylene Glycol
SEM	:	Scanning Electron Microscope
TEM	:	Transmission Electron Microscope
THF	:	Tetrahydrofuran
US	:	Ultrasound
UV	:	Ultraviolet
XRD	:	X-ray Diffraction

CHAPTER 1
Introduction
and
Literature Review

1.1 INTRODUCTION

The well-being of modern society is unimaginable without the countless products of industrial organic synthesis. Chemistry is a branch of science in which the composition, structure, properties and change of matter are studied. It is primarily concerned with atoms and molecules and their interactions and transformations.[1a] Chemistry and chemical industries are continuously working to promote the longer life expectancy of the human beings, higher quality of life, improved safety, greater convenience and a better understanding of the universe. The term *synthesis* in Greek means 'to put together'.

The modern definition of organic chemistry is 'the chemistry of carbon compounds'. The term organic means "derived from living organisms". Originally, organic chemistry was limited to the study of the compounds produced from living organisms. But now, chemists have learned to make millions of original compounds, along with the synthetic versions of many organic compounds that are identical with the "natural" compounds. Further, organic compounds are composed primarily of carbon, hydrogen, oxygen, and nitrogen atoms bonded together to satisfy their valences.[1b] Synthetic organic chemistry is the art of 'building up' of complex organic molecules or compounds from readily available smaller molecules.

Organic synthesis of valuable molecules has boomed since the synthesis of urea from ammonium isocyanate in 1828 by Wohler.[1c,2] This serendipitious discovery negated the vital force theory, which stated that a substance produced by a living organism could not be formed synthetically. The discovery had an immense significance, because it showed that, in principle, all organic compounds can be synthesized in a laboratory.[3]

The efforts of synthetic organic chemists are therefore devoted not only to the synthesis of diverse compounds (diversity-oriented synthesis), but also to the total synthesis of complex organic compounds (target-oriented synthesis), and the development of new synthetic methods (method-oriented synthesis).[4-6] (Figure **1.1**)

1. Diversity-oriented synthesis (DOS) has come into sight as a powerful and efficient tool. The goal of diversity-oriented synthesis is the facile construction of structurally complex diverse compounds from simple starting materials. It features

three types of diversity: substitutional diversity, stereochemical diversity, and most importantly skeletal diversity resulting in the collection of catalyst and drug libraries.

The substitutional diversity can be achieved by combinatorial variation of building blocks; the stereochemical diversity by the use of stereocontrolled reactions. The most challenging facet of DOS, and of critical importance to its success, is the ability to incorporate skeletal diversity into a compound collection, i.e., the efficient generation of multiple molecular scaffolds from the same starting materials.

2. Target-oriented synthesis aims to prepare a specific target compound, which can be a complex organic molecule, a natural product, any designer molecule, or any drug molecule.

3. The method-oriented synthesis concerns with the improvement and the development of a synthetic procedure with the help of new reagents, catalysts, strategies and work-up procedures. As a result, the vast organic diversity is potentially rewarding which facilitates the lead generation in drug discovery process.

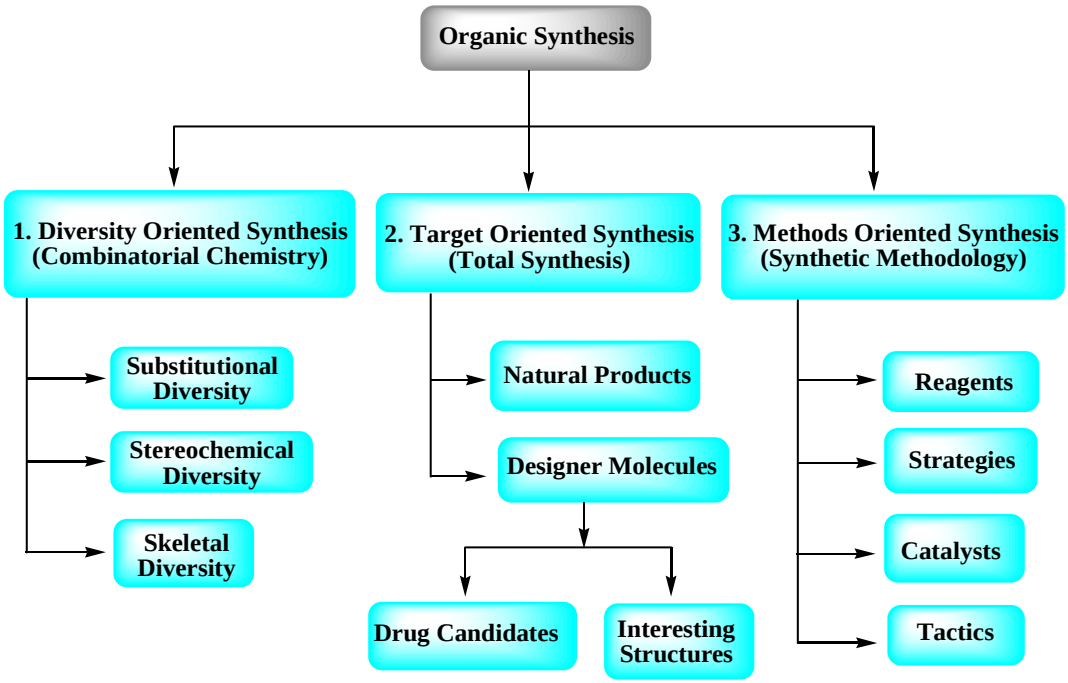


Figure 1.1: Classification of Organic Synthesis

Organic compounds containing at least one heterocyclic ring which comprises of carbon and hydrogen atoms alongwith one or more hetero-atoms as, N,

O, S, halogens, P etc. are known as heterocyclic compounds. Heterocyclic chemistry is the branch of chemistry dealing exclusively with synthesis, properties and applications of heterocyclic compounds as shown in Figure **1.2**. Heterocyclic chemistry is one of the most multifaceted and fascinating branch of organic chemistry and heterocyclic compounds comprise the largest and most diverse family of organic compounds. Many aspects of heterocyclic compounds are recognized as disciplines of general implication that intrude in almost all aspects of modern organic chemistry, medicinal chemistry and biochemistry, etc. Heterocyclic compounds offer an increased scale of structural diversity and have been established to be broadly and cost-effectively useful as therapeutic agents.[7,8]

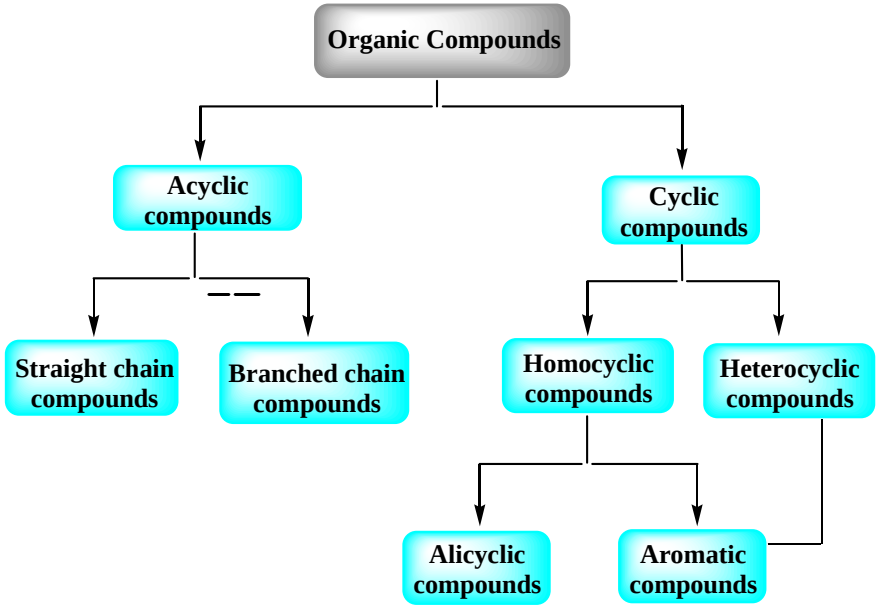


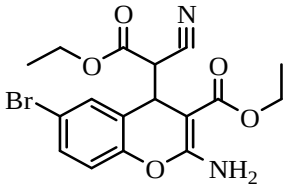
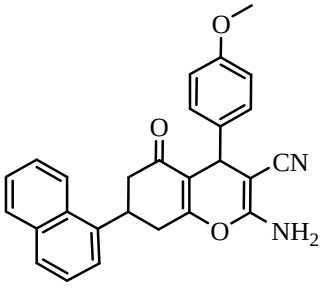
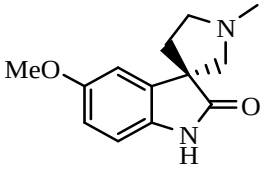
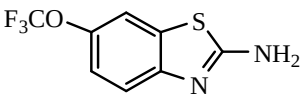
Figure 1.2: Classification of Organic compounds

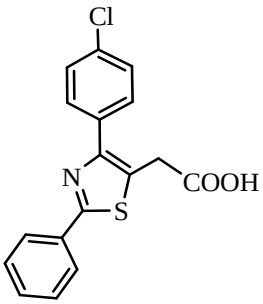
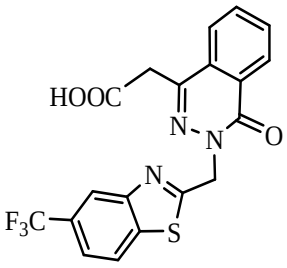
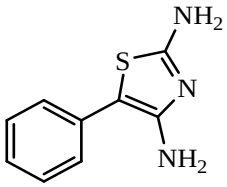
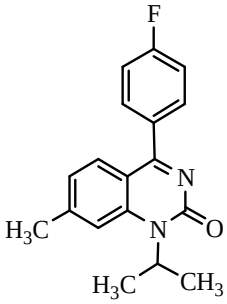
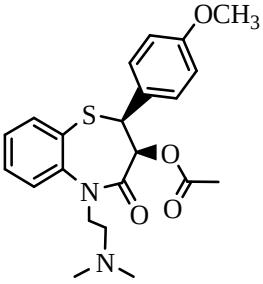
The synthesis of varity of useful heterocyclic compounds have shown their importance not only in terms of varied synthetic rutes but also on basis of physiological relavence.[9,10] Heterocycles constitute an exceedingly important class of compounds and hold a special place among pharmaceutically significant natural products and synthetic compounds. The field of heterocyclic chemistry has emerged to meet an increasing requirement of new compounds for drug discovery.[11-14] Heterocycles are among the most frequently encountered scaffolds in drugs and pharmaceutically relevant substances have shown importance due to the presence of their structural subunits which is found in most of the natural products such as

vitamins, hormones, antibiotics, and alkaloids, as well as pharmaceuticals, herbicides, dyes, etc.[15-19]

The exploration of heterocycles as privileged structure in drug discovery is, beyond doubt, one of the major areas of medicinal chemistry.[20] The wide occurrence of the heterocycles in bioactive natural products, pharmaceuticals, and agrochemicals[21,22] has made them important synthetic targets. Synthetic heterocycles act as antibacterial, antifungal, antimycobacterial, trypanocidal, anti-HIV, antileishmanial, genotoxic, antitubercular, antimalarial, herbicidal, analgesic, antiinflammatory, muscle relaxants, anticonvulsant, anticancer and lipid peroxidation inhibitory, hypnotics, antidepressant, antitumor, anthelmintic and insecticidal agents.[23-29] Heterocyclic compounds provide scaffolds on which different type of pharmacophores can arrange to yield potent and selective drugs.[30-37] (Table **1.1**)

Table 1.1: Representative heterocyclic compounds as bioactive moieties

S. No.	Name of the compound	Structure of the compound	Use of the compound
1	HA14-1		Disrupts Bax/Bcl-2 interaction and induces apoptosis of tumor cells.
2	UCPH-101		Selective non-substrate inhibitor of EAAT1.
3	Horsfiline		Horsfiline is an oxindole alkaloid extracted from *Horsfieldia superba*, which is used in traditional herbal medicine, has analgesic effects.
4	Riluzole		Riluzole is a drug which is used to treat amyotrophic lateral sclerosis. It delays the onset of tracheostomy in patients.

5	Fentiazec		Fentiazac, a novel alkanoic acid derivative, is an anti-inflammatory agent which is non-steroidal in nature has analgesic, antipyretic, and platelet-inhibitory actions.
6	Zopolrestat		Zopolrestat is a human glyoxalase I inhibitor, selectively inhibits aldo-keto reductase members AKR1B10 and AKR1B1.
7	Amiphenazole		Amiphenazole is a respiratory stimulant and is used as an antidote as well as for the treatment of respiratory failure.
8	Fluproquazone		Fluproquazone is a quinazolinone derivative with potent analgesic and antipyretic effects and also anti-inflammatory action.
9	Diltiazem		Diltiazem is a nondihydropyridine member of the class of drugs known as calcium channel blockers, used in the treatment of hypertension, angina pectoris, and arrhythmia.

Synthetic organic chemistry is perhaps the most expressive branch of the science in view of its unlimited scope. To appreciate its impact on the whole mankind, one has to look around and recognize that synthesis of organic compounds is a mainstay behind the manufacture of pharmaceuticals, high-tech materials, polymers, fertilizers, pesticides, cosmetics, and clothing.[38] (**Table 1.2**)

Table 1.2: Examples of heterocyclic compounds in our daily life

S. No.	Area	Examples of the heterocyclic compounds
1	Polymers	Polyimides, polybenzimidazoles, polybenzoxazole and polybenzothiazoles, etc.
2	Pesticides	Chlorpyrifos, Diazinon and Metaldehyde, etc.
3	Cosmetics	Derivatives of tetrahydrofuran (Ambroxid), macrocyclic compounds (Exaltolid, Ambretollid, Cervolid and Oxalacton), Poly(N-vinylpyrrolidone), Orotic acid, Zinc-pyritione and Omadin, etc.
4	Clothing	Polyester (tetrahydro-2,5-dimethyl *cis* furan and their derivatives), Fabric whiteners (imidazopyrimidines), etc.
5	High-tech materials	Anticorrosive agents (Triazoles), Optoelectronics (Spiro phenothiazine), Photographic sensitizers and developers (coumarine), Modifier for rocket propellant fuels (propyleneimine), Luminophores (pyrazoloquinolines), Light-emitting diode (1,3,5-tris(N-phenylbenzimidazol-2-yl) benzene), etc.

Thus, its significance as a research discipline extends ahead of providing a test for the state-of-the-art. It tenders the opportunity to discover and invent new areas of sciences and related disciplines in a most meticulous way to the young practitioners whose expertise may feed many peripheral areas of science and technology[39] (Figure **1.3**).

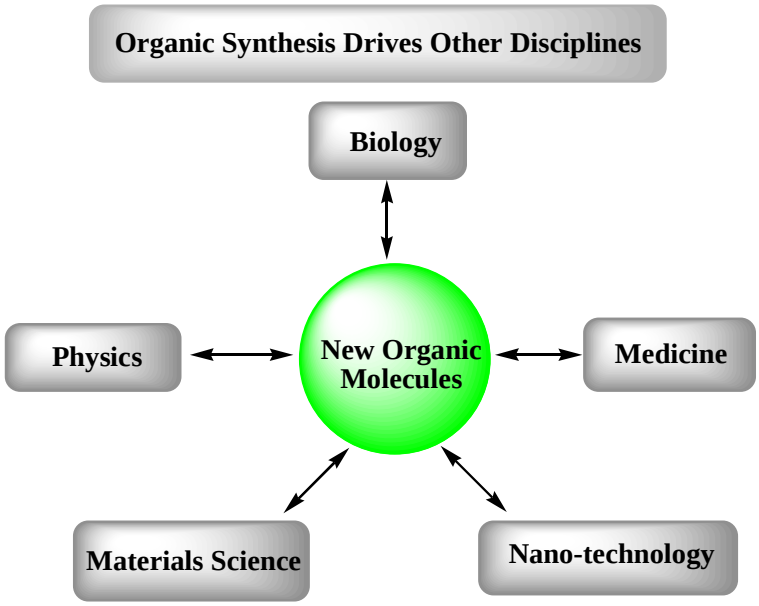


Figure 1.3: Co-relation of Organic Synthesis with other Sciences

1.2 Green chemistry

Scientists working in the field of synthetic organic chemistry have followed the foot steps of the predecusers in order to make highly complex molecules which have specific properties with very versatile chemical structures. This leads to the day-by-day discoveries of new reagents and catalysts which can enable the synthetic chemists to utilise their vision for designing the easy accessibility of complex compounds. Many challenges still lie ahead, and the solutions need to be found out not only in the field of chemistry but also in the interdisciplinary sciences of physics, biology and engineering.[40]

This has served as the motivation for the discovery of new term 'sustainable development' which involves the protection of the today's environment for taking care of the needs of future generation. As a consequence, Green Chemistry has emerged in the last decades[41-50] enabling the synthetic chemists to produce greener and more sustainable processes using the principles of Green Chemistry. The most important aspect of Green Chemistry is the concept of design. The object of Green Chemistry is to reshape the way of the synthesis in which the chemists visualize; necessarily focus on the development of alternative sustainable variants for the existing ones; and the development of various synthetic strategies including the environmental considerations at the design stage of any chemical process.[51]

Green Chemistry definitions change based upon the focus. Green Chemistry or Sustainable Chemistry[52,53] can be defined as "the utilisation of a set of principles that reduce or eliminate the use or generation of hazardous substances in the design, manufacture and application of chemical products." The term Green Chemistry, coined by staff at the US EPA in the 1990s,[54] helped to bring focus to an increasing interest in developing more environmentally friendly chemical processes and products. A variety of terms[55] have been postulated for Green Chemistry to lay emphasis on the possibility of the use of chemical processes. The most common ones can be stated as follows:

- Sustainable Chemistry
- Environmentally Benign Chemistry
- Clean Chemistry
- Ecological Chemistry

- Atom Economy
- Benign by Design Chemistry
- Environmentally Friendly Chemistry
- Ecochemistry

Green Chemistry is an emerging field which endeavours to work at the molecular level for achieving sustainability. The field has attained a global interest as it goes beyond the research laboratory to the industry, education and the environment due to its ability to control chemical methods to meet the economic and environmental goals simultaneously.[56] It has simply become a new environmental priority which provides a design for chemical evolution and can direct the synthetic chemists to accomplish sustainable practices during chemical research, development and manufacturing. Green Chemistry can be portrayed by the twelve principles (Figure **1.4**) and works as a cohesive system of these principles which provided the framework of sustainable design.[57] Green Chemistry recognizes the relevant chemical priorities and directs the research efforts towards significant and timely endeavours. These principles[51,58] can deliver higher efficiency, reduced environmental burden during synthetic chemical process and can provide a new landscape for exploration of scientific processes based upon the new measures of Chemistry.

Figure 1.4: Principles of Green Chemistry

Following section is intended to provide a brief overview about the principles of Green Chemistry.

1. Prevention of waste

This is the fundamental principle of Green Chemistry which states that chemical processes should be designed as to minimise the waste levels during the process design rather than to treat or clean up the afterwards generated waste. The development of preventive strategies at the design stage of synthesis is of utmost requirement for sustainability.

2. Atom economy

The term ‘atom economy’as one of the principles of Green Chemistry was introduced by Barry Trost in 1990.[59,60] Atom economy is a simple yet useful tool which refers to the concept of the maximum use of initial materials so that the final product incorporates the maximum number of atoms of the reactants. Thus, it represents an efficient strategy towards the sustainable synthesis.

3. Less hazardous chemical syntheses

This Green Chemistry principle strives towards the aspect of the hazards associated with the chemicals and the chemistry developed. As phrased by Anastas and Warner,[52] "wherever practicable, synthetic methods should be designed to use and generate substances which possess little or no toxicity to human health and environment". Less toxic materials imply lower hazards to the workers in industry and research laboratories and less pollution to the environment.

4. Designing safer chemicals

Designing should become a fundamental aim of Green Chemists to achieve the desired function and properties of the products but the sustainable view also pays attention to its environmental profile minimising their toxicity to human being and environment.

5. Safer solvents and auxiliary substances

Since solvents are the part and parcel of the research of a synthetic organic chemist. The ideal situation would be not to use any solvent as including any auxiliary in the synthetic process always entails the efforts and energy to remove it from the system. And if used, they should be replaced or reduced with the less toxic chemical products.

6. Design for energy efficiency

Rising concerns over the depletion of sources of energy and increase in the consumption of energy together have pushed for the search of renewable energy sources and the development of more energy efficient processes.

Reducing the energy barrier of a chemical reaction or choosing appropriate reactants so that the synthetic transformations can be conducted at ambient temperatures and pressures.

7. Use of renewable feedstocks and raw materials

It has been calculated approximately that most of our manufacturing products are derived from petrochemical feedstocks.[61-67] The depletion of these resources can affect our consumer life and economy very badly. Raw materials used in the chemical process should be renewable or have very low toxicity.

8. Reduced use of derivatives

This principle reminds chemists to modify their old ways of production of chemicals with more chemical steps and additional materials. Nowadays, chemists should aim towards reducing the unnecessary derivatisation in the synthetic routes, i.e., use of any blocking groups, protection/deprotection strategies, etc.

9. Use of catalysis and catalytic reagents

This principle is at the heart of the Green Chemistry principles. Catalysis or the use of catalytic reagents can improve the efficiency of a reaction and yield of the products by lowering the required energy input. It generates less waste when compared to the stoichiometric activating reagents with greater product selectivity.

10. Design for degradation

Persistence of the chemical products in the environment has been known since the early stages of the industrial development.[68-73] Most chemical products and consumer items do not degrade easily causing the environmental problems. This principle aims at designing of the materials and chemical products in such a way that they break down into biodegradable products so that they become eco-friendly.

11. Real-time analysis for pollution prevention

This principle is primarily process-oriented and finds its most relevance in the industrial applications. Green Chemists must take into account the monitoring in a reactor which is crucial in pollution control and accident prevention.

12. Inherently safer chemistry for accident prevention

Our working environment is plentiful of dangerous substances and processes. All types of hazards whether its toxicity, or physical hazards such as explosivity or flammability, allergic to humans, etc. and global hazards should be addressed in the design of chemicals and processes.

Thus, Green Chemistry aims to stop or minimise the use of materials which are dangerous for the health and safety of workers and consumers and should be replaced by safer alternatives to prevent accidents wherever possible.

1.3 Alternative methods used in Green Chemistry

The emerging areas of Green Chemistry visualize the minimum hazard as the performance criterion while designing new chemical processes. One of the thrust areas for achieving this target is to explore alternative reaction conditions and eco-friendly reaction media to achieve the desired chemical transformations with minimum by-products wherever possible. Some of the non-conventional methods of reaction activation used in organic synthesis are discussed as follows:

1.3.1 Microwave irradiation

A microwave is a part of electromagnetic spectrum, having the wavelength between 0.01 and 1 meter which operates in a frequency range between 0.3 and 30 GHz.[74] The region of the electromagnetic spectrum which lies in between the infrared and radio wave is denoted as microwave region.[75,76] The microwave radiations are non-ionising, unlike the other forms of radiation such as X-rays and γ-rays and for this reason, do not modify the molecular structure of the compound being irradiated. Microwave ovens are used in organic synthesis since old times and various instruments used can be shown (Figure **1.5**) as:

Figure 1.5: Instruments using microwave irradiation for synthesis

However, for the use at laboratory scale, a frequency of 2.45 GHz is preferred as this frequency is found to have the right depth of penetration for laboratory reaction conditions. Due to this, microwave irradiation has become an accepted way of heating samples. Microwave chemistry is the science of applying microwave irradiation to chemical reactions.[77-79] The use of microwave irradiation in chemistry led to the introduction of new concepts because the absorption and transmission of energy is completely different from the conventional modes of heating. Revolution in organic synthesis has been greatly endorsed by microwave-

assisted method by which the small molecules can be easily built up to larger ones within a very small fraction of time as compared to conventional methods of heating.[80,81]

The pioneering work regarding the application of microwave energy in organic synthesis dates back to the 1980s by Gedge *et al.*[82] and Giguere *et al.*[83] Since then, the production of microwave-assisted reactions in organic chemistry has experienced exponential increase due to the several benefits associated with the process.[84] Microwave-assisted organic synthesis has significantly improved the speed, reduced the cost and energy making it a sustainable process and is now far and wide accepted as one of the 'green chemistry' measures.[85]

In the past, microwave chemistry was often used whenever all other techniques to perform a reaction had failed, or when extremely longer reaction times or higher temperatures were required to complete a reaction. But this practice is now changing gradually, and routine synthetic transformations are being carried out using microwave heating. Microwave heating has several advantages as compared to the conventional methods of heating which are mentioned as:

- Uniform heating occurs throughout the material.
- High efficiency of heating.
- Process speed is increased.
- Reduction in formation of by-products.
- Environmental heat loss can be avoided.
- Reduce the energy loss due to heating of reaction vessel.
- Low operation cost.

1.3.1.1 Principles involved in the microwave heating

The basic mechanism behind the microwave heating involves the interaction of the polar molecules or ions with the oscillating electromagnetic waves of particular frequency. The phenomenon of generation of heat with the help of electromagnetic irradiation can be either by dipolar polarisation method or conduction mechanism.[74,84]

It is often assumed that microwave heating employs the ability of some compounds (whether they are liquids or solids) to convert electromagnetic energy

into heat. Similar to all other electromagnetic radiations, microwave irradiation can be divided into an electric field component and a magnetic field component. The advantage of the use of microwave irradiation is that it is rapid and volumetric, with the whole material being heated simultaneously. Two chief mechanisms are proposed which are responsible for the microwave heating which can be schematically shown (Figure **1.6**) as:

(i) Dipolar polarisation (ii) Conduction mechanism

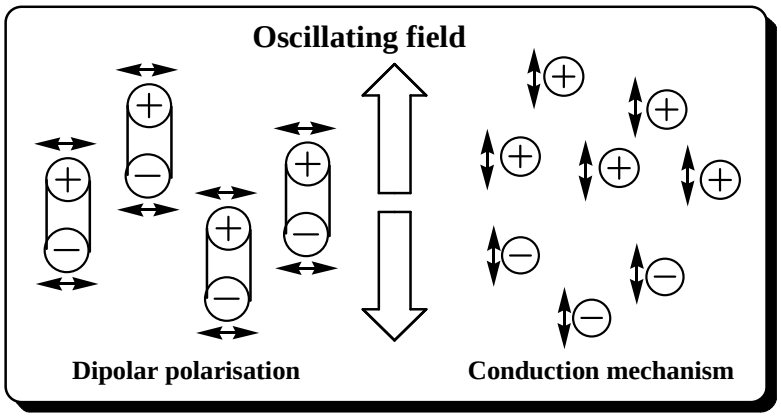


Figure 1.6: Methods of heating by microwave irradiation

Dipolar polarisation

For a liquid mixture to interact with microwave irradiation, certain requirements are there to be fulfilled. Polar molecules or dipolar species are the material for dipolar polarisation method. When the liquid mixture is exposed to an oscillating field of appropriate frequency, dipolar species try to re-orient themselves with respect to the oscillating field. In this process, they collide with each other due to their random motion and energy is released as heat because of molecular friction and dielectric loss. The key requirement for the process of dipolar polarisation is that the frequency of the oscillating field should be sufficient enough to cause the ample inter-particle interaction. The amount of heat generation in the process is directly related to the nature of the dipolar species and the frequency of the applied field.

If the oscillating field oscillates too quickly for the molecules to respond, the dipole does not have enough time to align itself with the field before the field changes the direction again. On the other hand, if the frequency of the oscillating field is too low, the molecules follow the field so well that there is no random motion generated between the dipolar molecules. However, if the applied field is in the

intermediate frequency region, the dipole has enough time to respond and undergo the random motion due to rapid collisions resulting in the generation of heat.

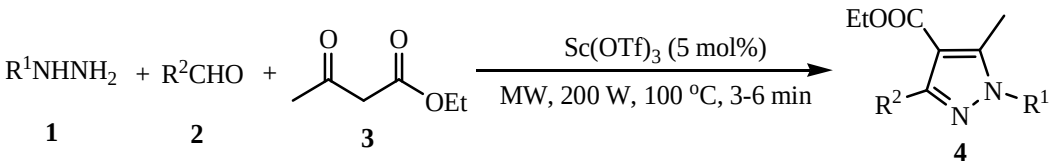
Conduction mechanism

In this mechanism, any mobile charge carriers (e.g. electrons or ions, etc.) will move through the liquid medium under the influence of microwave's electric field. This results in the energy loss due to the increased collision rate of the charged ions on conversion of kinetic energy to heat. The movement of ions in the liquid medium by the interaction with the microwave irradiation is a much stronger interaction as compared to the corresponding motion of dipoles. Thus, when the ionic species were exposed to the microwave irradiation, they heat up extremely quickly. And, this property can be utilised to improve the heating ability of non-polar species upon exposure to microwave irradiation.

There has been some debate over the use of microwave frequency, which can cause brain tumour. Microwave irradiation can leak out and damage human cells and tissues by weakened immune system and so many other illnesses and infection.

1.3.1.2 Examples of the microwave heating

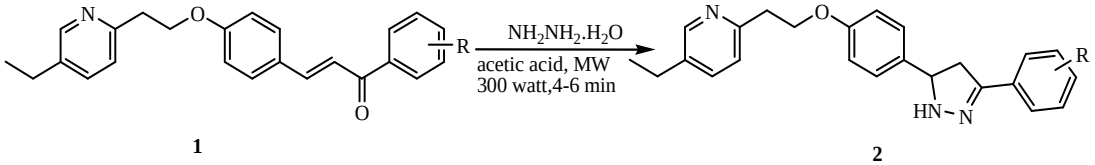
Singh *et al.*[86] reported an efficient and green synthesis of the functionalized pyrazole derivatives **4** using microwave irradiation under solvent-free conditions by the reaction of phenyl hydrazine **1**, aldehydes **2** and ethyl acetoacetate **3** (Scheme **1**).



1 **2** **3** **4**

Scheme 1

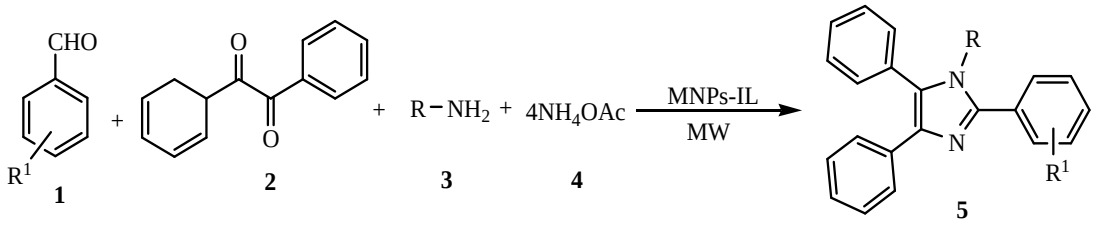
Patel *et al.*[87] accomplished the synthesis of 2-pyrazolines **2** from pyridine based chalcone **1** using microwave irradiation (Scheme **2**).



1 **2**

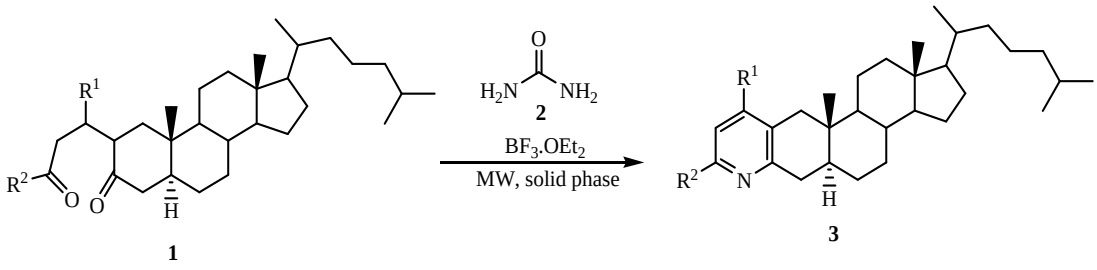
Scheme 2

Safari *et al.*[88] also developed a simple and one-pot synthesis of substituted imidazoles **5** using aldehydes **1**, benzil **2**, amines **3** and ammonium acetate **4** under solvent-free conditions and microwave irradiation catalysed by immobilized ionic liquid on super paramagnetic nanoparticles as an effective catalyst (Scheme **3**).



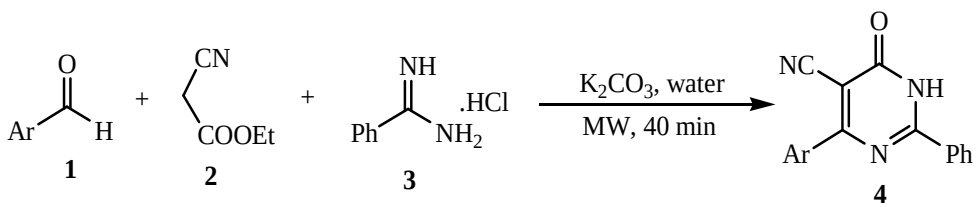
Scheme 3

Boruah *et al.*[89] synthesized novel steroidal heterocycles containing 4,6-diaryl substituted pyridine structural moiety **3** from the Michael reaction of steroidal ketones **1** with urea **2** in presence of $BF_3.Et_2O$ as the catalyst under microwave irradiation (Scheme **4**).



Scheme 4

Xavier *et al.*[90] described an aqueous mediated protocol for the synthesis of pyrimidine derivatives **4** by the multicomponent reaction of aldehyde **1**, ethylcyanoacetate **2** and benzamidine hydrochloride **3** under microwave irradiation (Scheme **5**).



Scheme 5

1.3.2 Ultrasound irradiation

Ultrasound irradiation is the part of the electromagnetic spectrum which lies in the range of about 20 kHz to 10 MHz[91-93] which can be sub-divided into three main regions:

(a) Low frequency high power ultrasound (20-100 kHz)

(b) High frequency medium power ultrasound (100 kHz-1 MHz)

(c) High frequency low power ultrasound (1 MHz-10 MHz).

Ultrasound irradiation has been an alternative source of energy for organic reactions which are generally proficient when compared with the conventional methods of heating. Sonochemistry is the study of the use of ultrasound irradiation to uphold the chemical reactions.[94-96] The study of sonochemistry is related to the understanding of the effect of sonic waves and its effects on chemical reactions. Since the last three decades, ultrasound-assisted organic synthesis has been developed as a green synthetic approach by the researchers at the global level which is being used to enhance the organic reactions.[97-100]

The notable features of the ultrasound-assisted organic approach works by the process of 'acoustic cavitation' which involves the formation, growth and collapse of micro-bubbles under the effect of an ultrasonic field.[101] Ultrasonic bath and ultrasound probe system are used in organic synthesis since old times and they can be shown (Figure **1.7**) as:

Figure 1.7: Instruments using ultrasonic irradiation for synthesis

1.3.2.1 Principles involved in the ultrasonication

Ultrasound irradiation, when made to pass through a liquid medium, causes the liquid to vibrate mechanically. As a result, it generates the sound waves within

the liquid. And, under normal conditions, the dissolved gas molecules in the liquid medium tend to grow and get collapsed under the action of ultrasound waves.[102] The whole phenomenon is known as "acoustic cavitation". There are three theories proposed for the whole process of cavitation: the hot-spot, the electrical and the plasma theory. The most accepted and popular theory is the hot-spot theory. Localised hot spots are generated from the vigorous collapse of the bubbles which create drastically high temperatures and pressures inside the collapsing cavity in the liquid medium for an extremely shorter span of time. Enormous amounts of energy is produced by the bubble collapse in liquid medium from the conversion of kinetic energy of liquid medium for heating the contents of the bubble.[103] (Figure **1.8**)

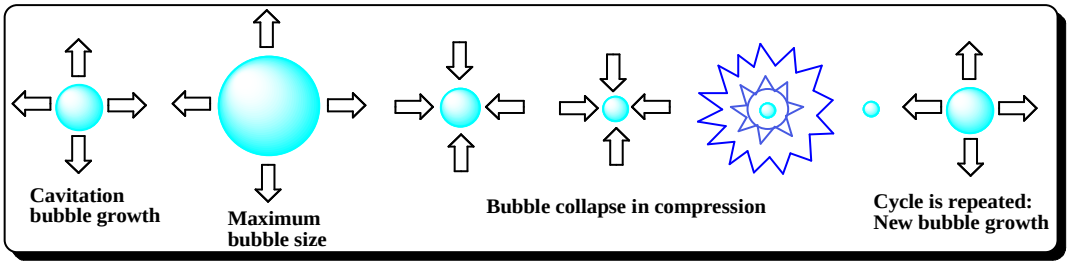


Figure 1.8: Phenomenon of acoustic cavitation

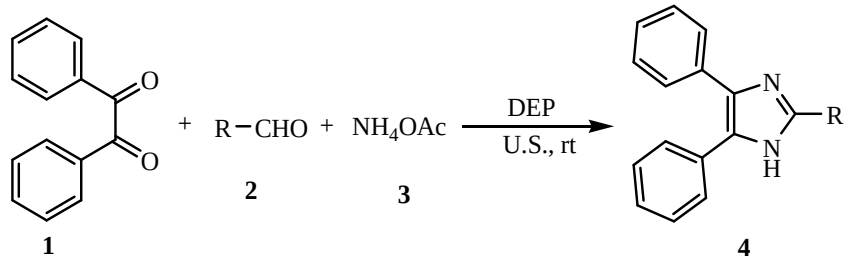
As a consequence, various strong physical effects outside the bubble such as shear-forces, jets and shock waves develop which induces the molecular fragmentation and highly reactive species are produced. As the formation of these implosive bubbles concentrate the energy into very small volumes to produce very high temperature and pressure and causes the formation of high-speed jets from the liquid to the liquid surface which leads to the initiation and acceleration of chemical transformations.[104,105]

The diverse effects of the ultrasound include significant improvements in various fields such as material science, life science, medicine and chemistry. Consequently, sonication can efficiently conduct many homogeneous and heterogeneous as well as transition metal catalysed organic reactions at ambient reaction conditions. Assistance of ultrasound irradiation enhances the yields, selectivities and shortens the reaction times. So, the use of ultrasound irradiation has proved to be the way towards the green synthesis.

But at high levels of exposure, ultrasound is capable to cause permanent damage to biological tissues, including teratogenic effects, pain and eventual rupture of the ear-drum through heating, acoustic cavitation and radiation force. And, for diagnostic purposes, which are at lower levels of exposure, ultrasound does not generally cause heating beyond physiological range.

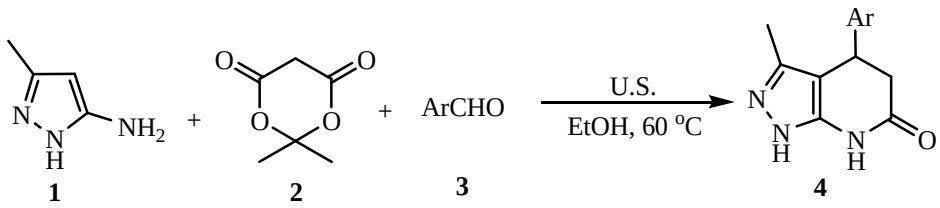
1.3.2.2 Examples of the ultrasonication

Nagargoje *et al.*[106] described a one pot, three-component reaction of benzoin/benzil **1**, an aldehyde **2**, and ammonium acetate **3** using diethyl bromophosphate as a mild oxidant for the synthesis of imidazole derivatives **4** under ultrasound irradiation (Scheme **6**).



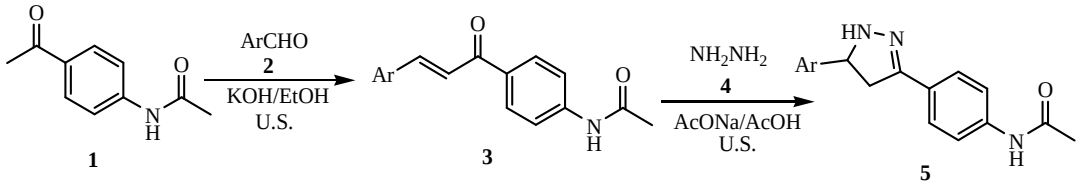
Scheme 6

Mamaghani *et al.*[107] reported a simple and efficient ultrasound-assisted synthesis of novel 4-aryl-3-methyl-4,5-dihydro-1*H*-pyrazolopyridin-6-ones **4** by the one-pot three-component reaction of 5-amino-3-methyl-1*H*-pyrazole **1**, meldrum's acid **2** and various aryl aldehydes **3** (Scheme **7**).



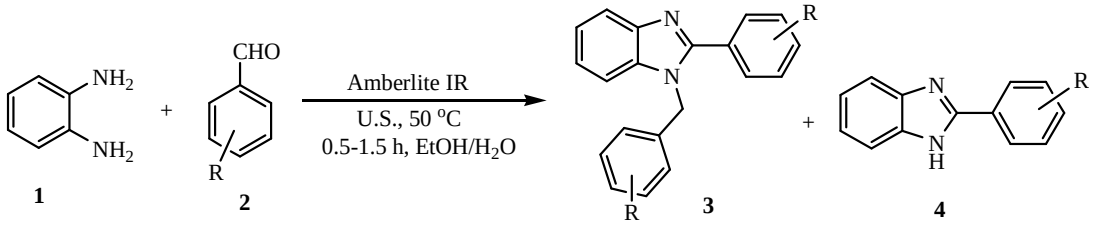
Scheme 7

Pacheco *et al.*[108] reported Claisen-Schmidt condensation reaction of 4-acetamidoaceto phenone **1** and aromatic aldehydes **2** under ultrasonic irradiation for the synthesis of acetylaminochalcones **3** which undergo cyclocondensation with hydrazine **4** to afford pyrazoline derivatives **5** in the presence of sodium acetate and aqueous acetic acid under ultrasonic irradiation (Scheme **8**).



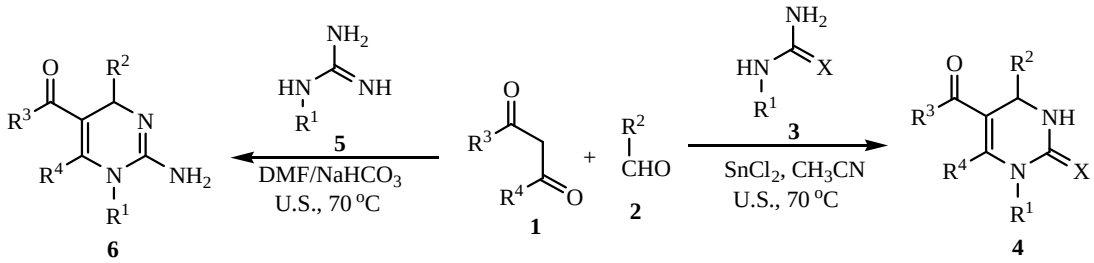
Scheme 8

Park *et al.*[109] reported a simple and eco-friendly route for the one pot synthesis of biologically significant 2-aryl-1-arylmethyl-1*H*-benzimidazoles **3/4** by the reaction of phenylene diamine **1** and various aldehydes **2** in EtOH/H_2O solvent mixture using Amberlite IR-120 under ultrasound irradiation (Scheme **9**).



Scheme 9

Ansari *et al.*[110] described the ultrasound-assisted synthesis of 3,4-dihydropyrimidin-2-thiones **4** by the reaction of 1,3-dicarbonyl compound **1**, an aldehyde **2**, diamino compound **3**, using $SnCl_2 \cdot 2H_2O$ as a catalyst and acetonitrile as a solvent. The authors also reported the synthesis of 2-amino-1,4-dihydropyrimidines **6** *via* the reaction of an aldehyde, guanidine hydrochloride **5**, 1,3-dicarbonyl compound and sodium bicarbonate as a catalyst in DMF (Scheme **10**).



Scheme 10

1.3.3 Infrared irradiation

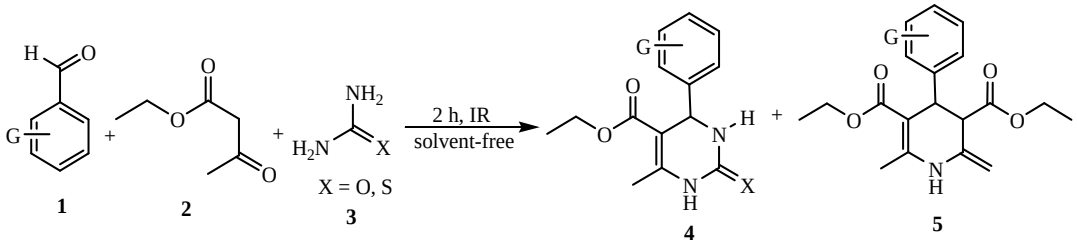
Infrared irradiation in organic synthesis has been the focus of considerable attention in recent years. Infrared irradiation is also an alternative energy source, and is an economic process working under solvent-less conditions, contributing towards the development of environmentally benign methods.[111-113] Infrared radiation could be more extensively used, based on its ability to promote fast heating of solution.

Infrared lamps are electrical device which emit infrared irradiation. They have many uses, and are most commonly used in heating process in the laboratory.[113-116]

However, exposure to infrared irradiation induces dermal angiogenesis, inflammatory cellular infiltration and cumulative oxidative DNA damage in humans, interrupts the dermal extracellular matrix by inducing matrix metalloproteinases, and modifies dermal structural proteins, thereby adding to premature skin aging.[112b]

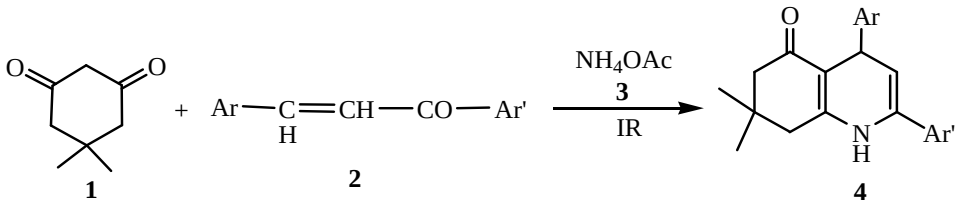
1.3.3.1 Examples of the infrared irradiation

Miranda *et al.*[113] studied the condensation reaction of various benzaldehydes **1** with ethyl acetoacetate **2** and urea/thiourea **3** to synthesize Biginelli and Hantzsch esters **4/5**. The reaction was performed in solvent-less conditions under infrared radiation (Scheme **11**).



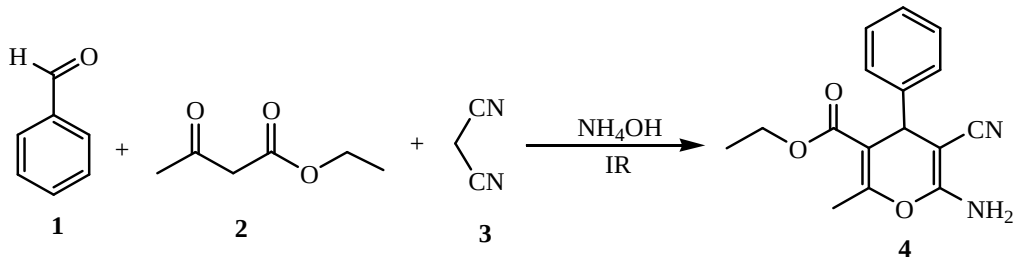
Scheme 11

Wang *et al.*[114] reported the synthesis of substituted 5-oxo-octahydroquinoline derivatives **4** by the condensation reaction of 5,5-dimethyl-1,3-cyclohexane-dione **1**, 1,3-diaryl-2-propen-1-one **2** and ammonium acetate **3** under infrared irradiation in the absence of solvent (Scheme **12**).



Scheme 12

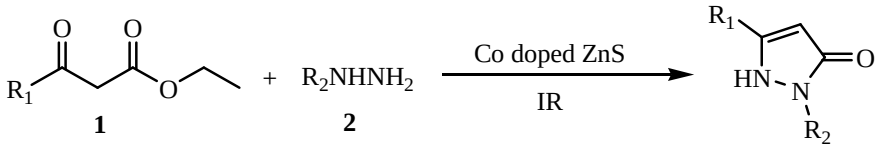
Vázquez *et al.*[115] explained a simple and versatile synthesis of 4*H*-pyran derivatives **4** *via* a three-component cyclocondensation of aldehydes **1**, ethyl acetoacetate **2**, and malononitrile **3**, using ammonium hydroxide as the catalyst, promoted by infrared irradiation (Scheme **13**).



1 **2** **3** **4**

Scheme 13

Dandia *et al.*[116] explored a simple and efficient methodology for the synthesis of pyrazolone derivatives **3** from the reaction of ethyl acetoacetate **1** and substituted phenyl hydrazine **2** using cobalt doped ZnS nanoparticles as a recyclable catalyst under infrared irradiation (Scheme **14**).



1 **2** **3**

Scheme 14

1.4 Alternative reaction media

Nowadays, the main goal of 'sustainable chemistry' is to increase the process selectivity, to maximize the use of starting materials, to develop environmentally benign conditions, and to replace the hazardous, expensive reagents and solvents with eco-friendly materials. On the other hand, science and technology is shifting emphasis on economically and environmentally benign and sustainable processes. Keeping this objective in mind, there is a need to search for alternative reaction media which can replace volatile organic solvents and hazardous chemicals in the field of organic synthesis.

1.4.1 Water

With the increasing emphasis on the concerns over the environmental impact, the list of organic processes performed efficiently in water has been expanded with the continuous increase of number of various organic transformations.

Water, which is nature's solvent and also known as universal solvent possesses unique structural form, which is the consequence of its distinguished physical and chemical properties. Water exhibits powerful hydrogen bonding, large

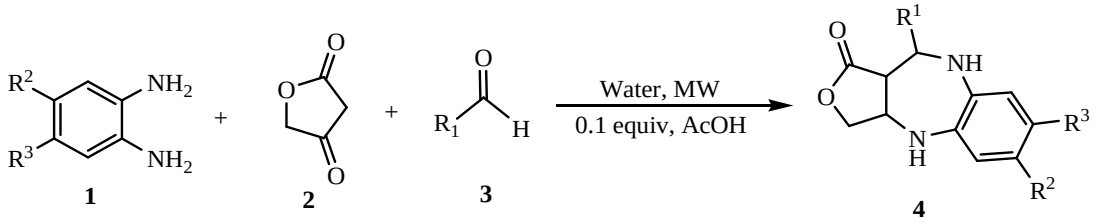
dielectric constant, high heat capacity and a wide temperature range in which it remains in liquid state. These advantageous characteristics have contributed to many factors, such as the hydrophobic effect, enhanced hydrogen bonding in the transition state, and high cohesive energy of water.[117,118]

From both environmental and economic points of view, water has emerged as a relevant reaction medium to perform organic reactions since water addresses several aspects of green chemistry such as easy availability, environmental acceptability, safe handling, easy workup and cost effectiveness for small and bulk scale process industries. In addition, reactions performed in water are found to be faster and more selective than in conventional organic solvents and also exhibit unique reactivity and selectivity[119,120] leading to the process development of synthetic protocols.

Several types of terms have been used to describe the reactions occurring in water, viz., '*in-water*', '*on-water*', etc. It is not possible to define markedly if the reaction is occurring '*in-water*' or '*on-water*'. However, certain attributes are there to define these reactions: the extent of solubility of the reactant(s) in water. If the organic reactants are soluble giving clear solutions in water, the effects operating simultaneously are (i) the hydrophobic effect, (ii) hydrogen bonding effects, (iii) solvent polarity effects; which may or may not accelerate the reaction rates. If the organic reactants are insoluble in water, the reactions are described as being *on-water* involving the two-phase system which proceeds by the interaction of phases with transition states and reactants.[117,118]

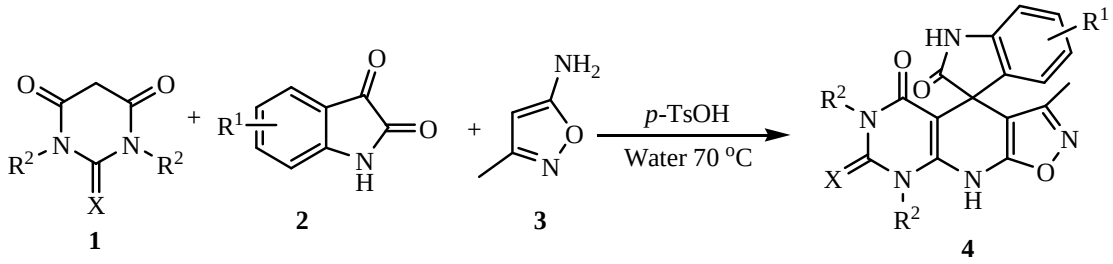
After the pioneering contribution based on studies done by Breslow[121-124] and Grieco,[125,126] showing that water can be used to enhance the Diels-Alder reactions,[127] one of the first cases of which was originally reported using water as a reaction medium.

Microwave-assisted three-component reaction of substituted benzene-1,2-diamine **1**, tetronic acid **2** and aldehydes **3** in the presence of acetic acid proceeded rapidly in water under microwave irradiation to afford benzo[*f*]azulen-1-ones **4** in good yields as reported by Wang *et al.*[128] (Scheme **15**)



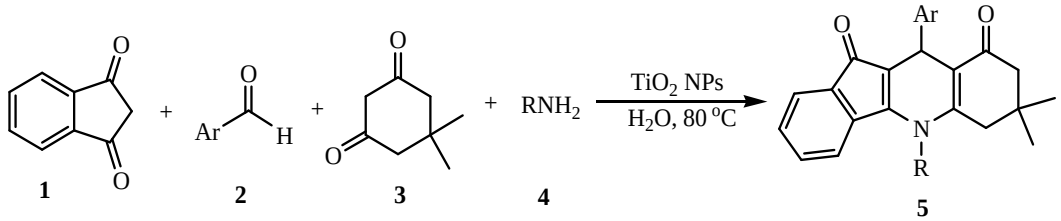
Scheme 15

An approach for the synthesis of spiro[indoline-isoxazolopyrido[2,3-*d*] pyrimidine]triones **4** *via* the multi-component reaction of an barbituric acid **1**, isatin **2**, and isoxazole **3** in water using *p*-toluene sulfonic acid as a catalyst had been reported by Rahmati *et al*.[129] (Scheme **16**)



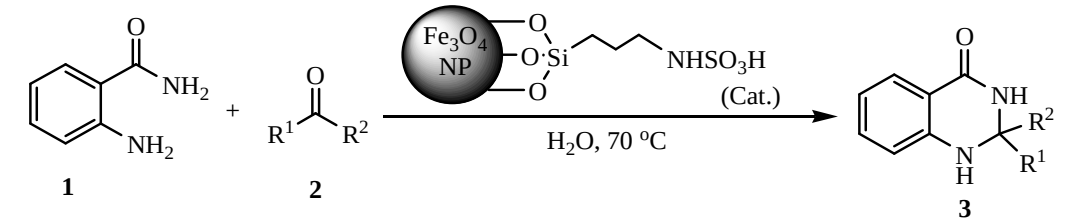
Scheme 16

Abdolmohammadi *et al*.[130] developed a simple, efficient approach for the synthesis of indeno[1,2-*b*]quinolinediones **5** through one-pot, four-component, coupling reaction of indane-1,3-dione **1**, aldehyde **2**, dimedone **3** and primary amine **4** using TiO_2 nanoparticles as a heterogeneous catalyst in aqueous medium (Scheme **17**).



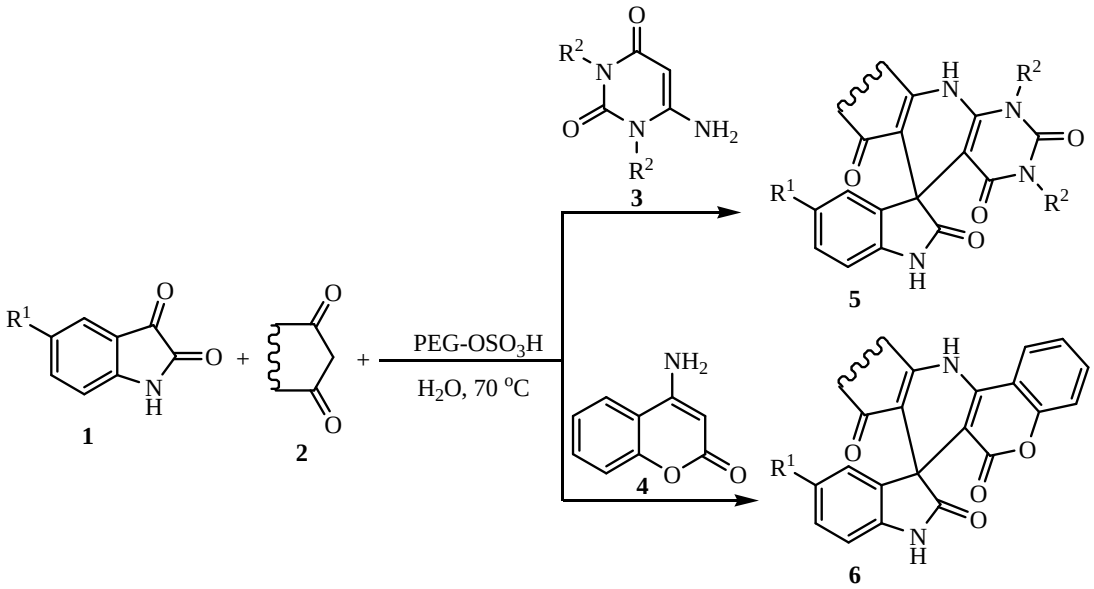
Scheme 17

A simple and eco-friendly procedure for the synthesis of 2-substituted-2,3-dihydroquinazolin-4(1*H*)-ones **3** from the reaction of anthranilamide **1** with aldehydes/ketones **2** using N-propylsulfamic acid supported onto magnetic Fe_3O_4 nanoparticles (MNPs-PSA) as a catalyst had been described by Rostami *et al*.[131] (Scheme **18**)



Scheme 18

Three-component domino reaction of 1,3-diketo compound (cyclohexane-1,3-dione, indane-1,3-dione, dimedone, and 1,3-dimethylbarbituric acid) **2**, 6-aminouracil/4-aminocoumarin **3/4** and isatin **1** in the presence of PEG-OSO_3H (20 mol%) proceeded rapidly in water at 70 ^oC and were completed within 1-2 h to afford a library of uracil and coumarin fused spirooxindole derivatives **5/6** in good yields as reported by Das *et al.* [132] (Scheme **19**)



Scheme 19

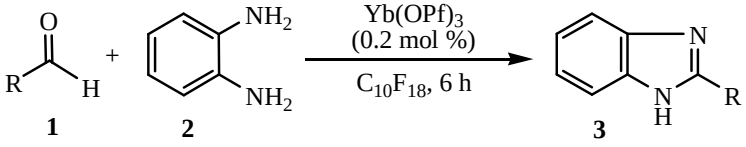
1.4.2 Fluorous solvents

Environmentally benign methodologies have attained major significance in the field of organic synthesis, especially at the industrial level. One of the interesting advances in this field is provided by the extraction of fluorinated compounds. The last two decades witnessed a rapid development of the fluorous chemistry since the birth and definition of fluorous biphasic concept by Horvath and Rabai.[133,134] Horvath introduced the term 'fluorous' analogous to the 'aqueous' for highly fluorinated alkane, ether and tertiary amine solvents.

Fluorous solvents have the advantage of thermally-controlled miscibility with the organic solvents.[135,136] That is, a fluorous/organic solvent system is observed to be monophasic at elevated temperatures and reverts back to the biphasic system at lower temperatures. Thus, it maximises the reaction efficiency by the elimination of problems related to the mass transfer across the two phases. Thus, the key to smooth reaction is the attainment of homogeneous conditions.

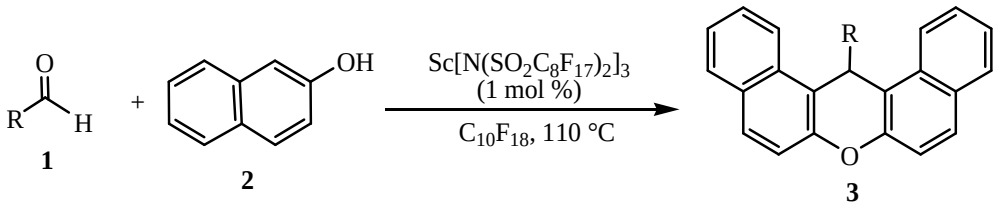
Fluorous solvents are characterised by high density, high stability,[137,138] low surface tension, good biocompatibility, sensitive temperature-dependent miscibility with organic solvents, and hydrophobic property.[139-142]

Cai *et al.*[143] accomplished the catalytic condensation reaction of aldehydes **1** and *o*-phenylenediamine **2** for the synthesis of benzimidazole derivatives **3** using Ytterbium perfluoro octanesulfonates ($Yb(OPf)_3$) as catalysts in fluorous solvent perfluorodecalin (Scheme **20**).



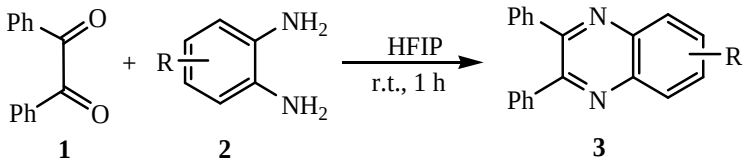
Scheme 20

Cai *et al.*[144] also proposed an efficient synthesis of substituted-14*H*-dibenzo[*a,j*]xanthenes **3** by the one-pot condensation of aryl or alkyl aldehydes **1** with β-naphthol **2** by using Scandium bis(perfluorooctanesulfonyl)imide complex as catalyst and perfluorodecalin as solvent (Scheme **21**).



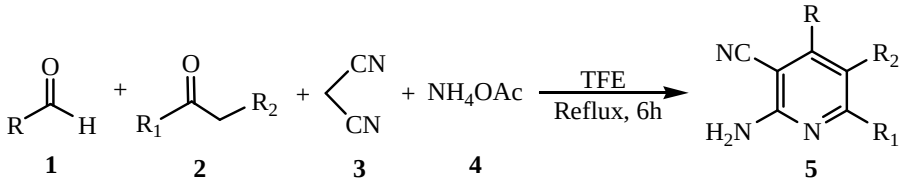
Scheme 21

Khaksar *et al.*[145] explored the use of hexafluoroisopropanol (HFIP) as an effective reaction medium for the synthesis of quinoxaline derivatives **3** through the reaction of 1,2-dicarbonyl compounds **1** with aryl 1,2-diamines **2** (Scheme **22**).



Scheme 22

Khaksar *et al.*[146] described the four-component reaction of aldehydes **1**, ketones **2**, malononitrile **3**, and ammonium acetate **4** in trifluoroethanol for the synthesis of 2-amino-3-cyanopyridine derivatives **5**. This reaction proceeds *via* one-pot four-component reaction using trifluoroethanol as a reusable reaction medium (Scheme **23**).



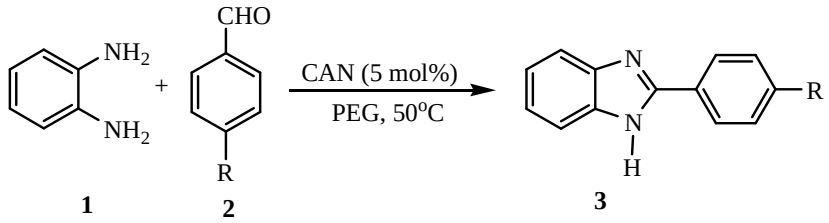
Scheme 23

1.4.3 Poly Ethylene Glycols

Polyethylene glycols (PEG)s is a family of solvents, which has emerged as an inexpensive substitute for volatile organic solvents.[147-150] PEG is a hydrophilic polymer, easily soluble in water and other organic solvents including toluene and acetone, but is insoluble in less polar solvents such as hexane, cyclohexane or diethyl ether, etc.[151-153]

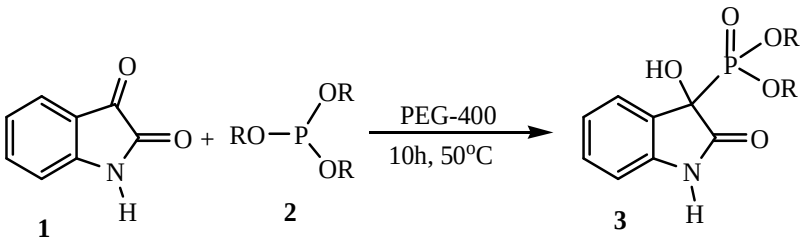
Polyethylene glycol (PEG) and its derivatives are known to be relatively nontoxic, thermally stable, easily recoverable, negligible vapor pressure, easily degradable and environmentally benign media for chemical reactions.[154-157]

A novel route had been reported by Kidwai *et al.*[158] for the synthesis of benzimidazole derivatives **3** by the reaction of *o*-phenylenediamine **1** and aldehydes **2** in good yields with small amount of CAN in PEG (polyethylene glycol) (Scheme **24**).



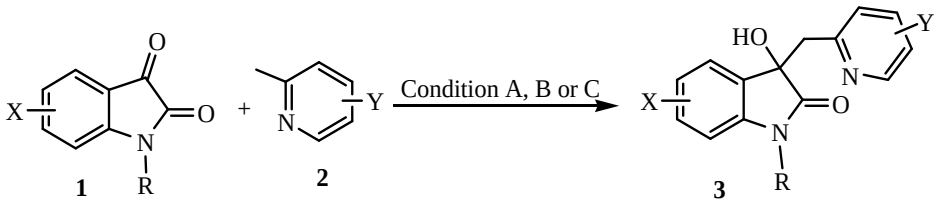
Scheme 24

Polyethylene glycol (PEG-400)[159] had been used as non-toxic solvent for the synthesis of α-oxindole-α-hydroxyphosphonate derivatives **3** by a one-pot reaction of isatin **1** with trialkyl phosphites **2** under catalyst-free conditions in excellent yields (Scheme **25**).



Scheme 25

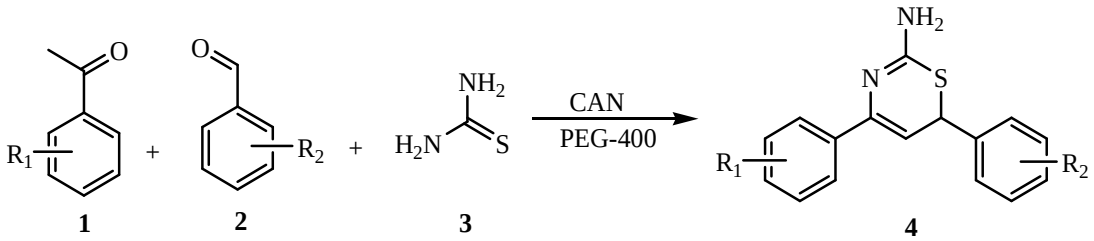
An environmentally benign protocol had also been used by Raghu *et al.*[160] for the synthesis of 3-(Pyridylmethyl)-3-hydroxy-2-oxindole derivatives **3** by the reaction of isatin **1** and substituted pyridine **2** under mild and catalyst-free conditions using polyethylene glycol (PEG-400) as a solvent (Scheme **26**).



Conditions: (A) H_2O/MW; (B) I_2/Dioxane; (C) PEG- 400/60°C

Scheme 26

Singh *et al.*[161] developed a one-pot ceric ammonium nitrate (CAN) catalysed multi-component synthesis of 1,3-thiazine derivatives **4** using ketones **1**, aldehydes **2** and thiourea **3** in PEG-400 (Scheme **27**).



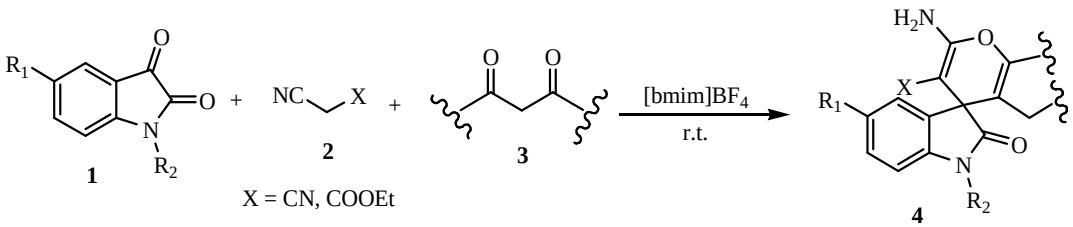
Scheme 27

1.4.4 Ionic liquids

Ionic liquids (ILs), a special class of molten salts composed of organic cations and organic or inorganic anions, have drawn the attention of numerous of chemists' in the last two decades.[162-164] ILs are widely recognized as 'green' alternatives to classical molecular solvents in organic synthesis because of their distinctive physicochemical properties, such as low vapour pressure, high chemical and thermal stability, solvating ability, behaviour as both acidic or basic catalysts and ease of recovery and reuse.[165-167]

Interestingly, they are also known as 'designer solvents'[168] as their solubility, viscosity, density, acidic or basic character, refractive index and associated catalyzing ability can be easily tuned by modification of the structure of their anion/cation for different applications. Due to these attractive features, ionic liquids have emerged as potent solvents, exhibiting nearly all kinds of interactions with reacting species, where upon they give rise to improved yields and rate enhancements.[162,167]

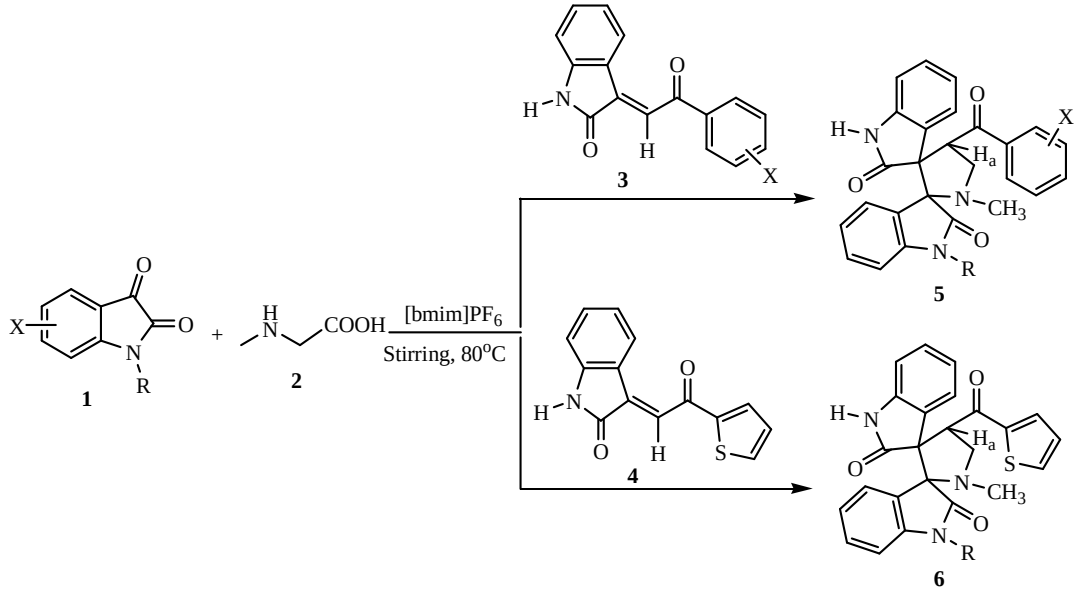
A simple approach for the synthesis of fused spiro[4*H*-pyran-oxindole] derivatives **4** by the one-pot three-component reaction between isatin **1**, malononitrile/ethyl cyano-acetate **2** and various 1,3-dicarbonyl compounds **3** was reported by Moghadam *et al.*[169] under ionic liquid [bmim]BF_4 at room temperature (Scheme **28**).



Scheme 28

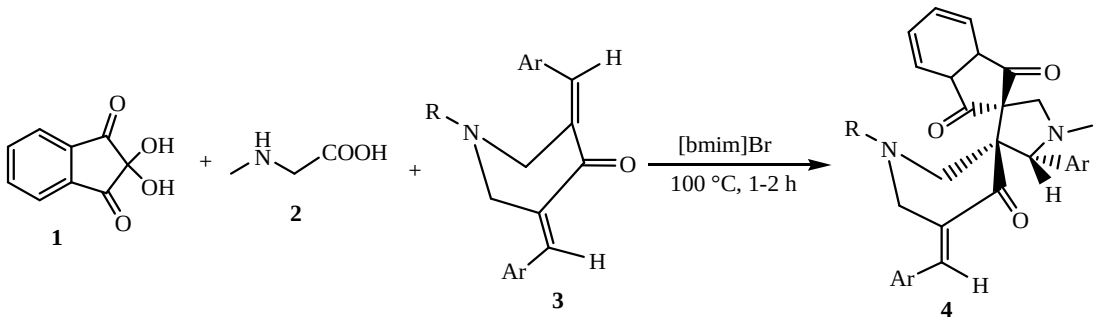
A one-pot synthetic strategy for the synthesis of novel dispiropyrrolidine-bisoxindole derivatives **5/6** had been reported by Jain *et al.*[170] *via* a three component

1,3-dipolar cycloaddition reaction of isatin **1**, sarcosine **2** and Knoevenagel adduct 3-aroylmethyleneindol-2-one **3** or 3-(2-oxo-2-(thiophen-2-yl)ethylidene)indol-2-one **4** in [bmim]PF_6 (Scheme **29**).



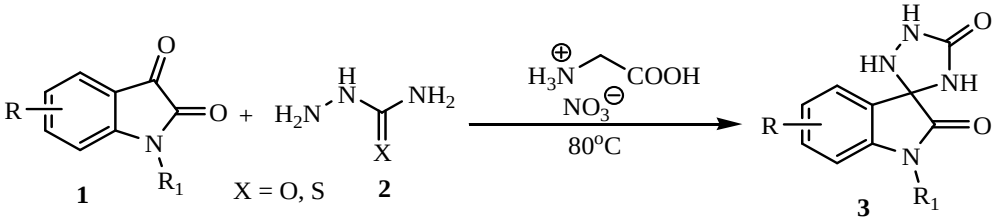
Scheme 29

Perumal *et al.*[171] described a general and efficient process for the selective synthesis of novel 1-methyl-4-arylpyrrolo-(spiroindan-1′,3′-dione)-spiro-1″-methyl/ benzyl-5″-(arylmethylidene)pipe- ridin-4″-one derivatives **4** *via* one pot three-component 1,3-dipolar cycloaddition reaction of ninhydrin **1**, sarcosine **2** and Knoevenagel adduct **3** in [bmim]Br ionic liquid (Scheme **30**).



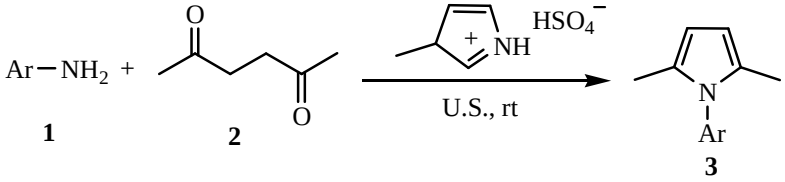
Scheme 30

Pore *et al.*[172] reported glycine nitrate mediated protocol for proficient synthesis of novel spiro-1,2,4-triazolidinone derivatives **3** by the one pot reaction of isatin **1**, semicarbazide/ thiosemicarbazide **2** in water with excellent yield (Scheme **31**).



Scheme 31

Zang *et al.*[173] described an efficient synthesis of N-substituted pyrroles **3** through the reaction of amines **1** with 2,5-hexanedione **2** under ultrasonic irradiation using ionic liquid [hmim]HSO_4 as catalyst (Scheme **32**).



Scheme 32

1.5 Alternative Catalysts

1.5.1 Nanocatalysts

Historically, catalysis has been understood as a way to accelerate chemical reactions. Initially in the field of catalysis, where the requirement was to make simple reactions kinetically accessible, entire focus was on the rate of reaction.[174,175] However, in recent applications, more and more complex reaction networks are at play, and there is a vital requisite not only to promote a given desired conversion but also to avoid any undesirable side reactions.

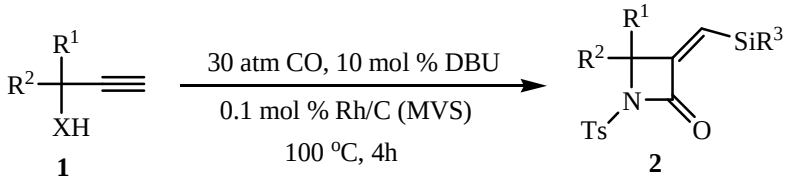
In the present scenario, various economical and effective alternative catalysts have been developed as the core of various green technologies to meet the demands of renewable energy in our society.[176] So far, to achieve better reaction activity, selectivity, and stability, a lot of research efforts have been devoted for the development of nanocatalysts[177] and nano-catalysed reactions[179] which have attracted considerable attention with the aim of finding significant applications in the pharmaceutical and fine chemical industries.[177-179]

The combination of catalysis and nanotechnology has opened new possibilities to create controlled structures and geometries to investigate and optimize a broad range of catalytic processes. Easy isolation and purification in

organic synthesis is an essential requirement and the simple separation of the catalyst from the reaction mixture is an advantage in organic synthesis, particularly for the pharmaceutical industry.[180,181]

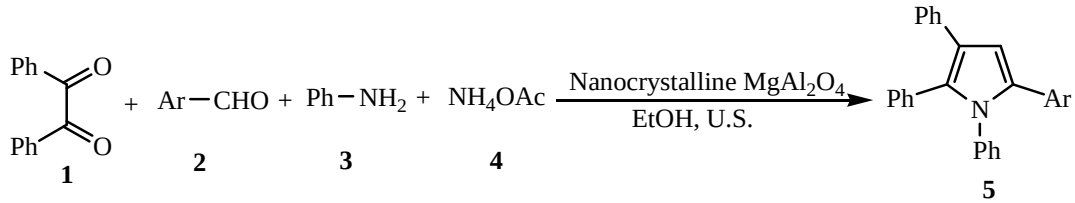
Magnetic nanoparticles have been known for several years, but research on their potential use in medicinal and pharmaceutical industry,[182-184] in particular, for biomedical applications,[185,186] is extensively research topic because of their unique physicochemical properties.

Aronica *et al.*[187] described the synthesis of β-lactams **2** from propargyl tosyl amides **1** catalysed by rhodium nanoparticles derived from mesitylene-solvated Rh atoms (Scheme **33**).



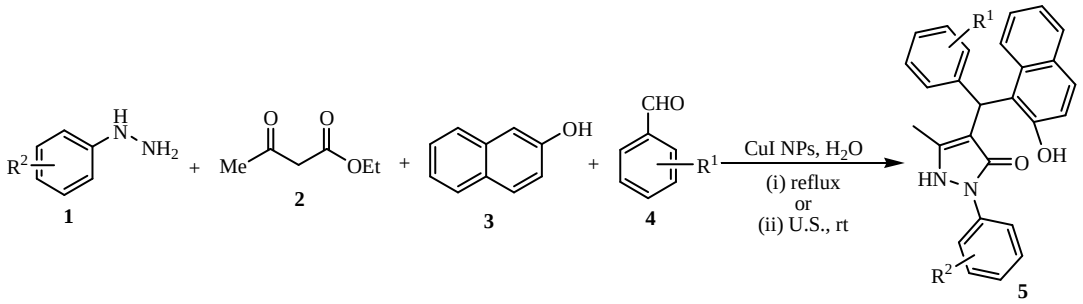
Scheme 33

An efficient four-component sonochemical synthesis of 1,2,4,5-tetrasubstituted imidazoles **5** from the reaction of benzil **1**, an aldehyde **2**, primary aromatic amine **3** and ammonium acetate **4** using nanocrystalline $MgAl_2O_4$ as an effective catalyst was described by Safari *et al.*[188] (Scheme **34**)



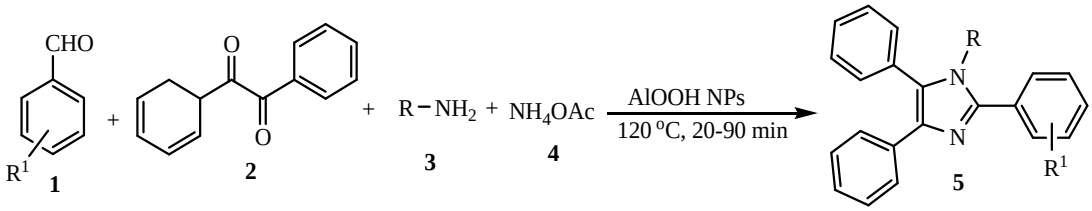
Scheme 34

Ziarati *et al.*[189] explored a simple and green process for the synthesis of pyrazolones **5** from the multicomponent reaction of substituted phenyl hydrazine **1**, methyl acetoacetate **2**, β-naphthol **3** and aldehydes **4** using CuI nanoparticles as reusable catalyst under reflux and sonication conditions (Scheme **35**).



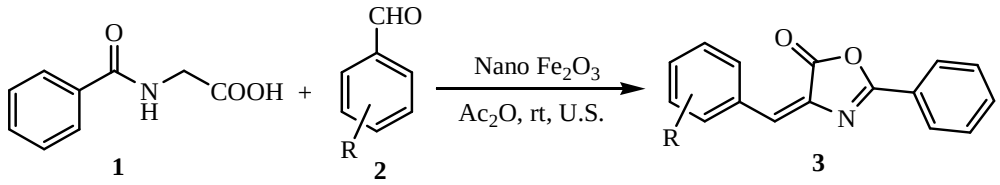
Scheme 35

Keivanloo *et al*.[190] described an efficient green procedure for the synthesis of highly substituted imidazoles **5** from the reaction of an aldehyde **1**, benzil **2**, primary aromatic amine **3** and ammonium acetate **4** using boehmite nanoparticles as catalyst (Scheme **36**).



Scheme 36

Ahmadi *et al*.[191] proposed a green procedure to synthesize azlactones **3** from the reaction of hippuric acid **1** and aromatic aldehydes **2** using Fe_2O_3 nanoparticles under ultrasonic irradiation. This reaction is an example of the Erlenmeyer–Plöchl reaction. Reactions proceed *via* S_N1 pathway (Scheme **37**).



Scheme 37

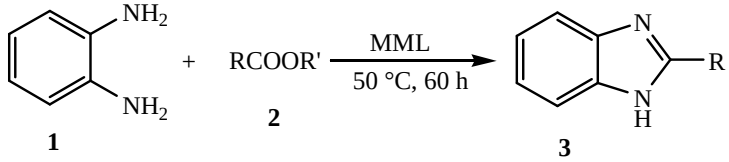
1.5.2 Biocatalysts

Over the last two decades, biocatalysis has emerged as an important scientific technology to meet the demand for the green and sustainable synthesis of pharmaceuticals and other chemical products.[192,193] Biocatalytic processes are attaining increasing importance in organic synthesis from their unique selectivity advantages over traditional methods.[193-196]

Biocatalysis has many interesting features from Green Chemistry point of view as the reactions are generally performed under mild conditions of temperature and pressure using eco-compatible biodegradable catalysts, like enzymes, fruit juices, etc.[197-199] Moreover, a high degree of selectivity is also observed during the chemical transformations without the need for the functional group activation and protection which is often required in traditional organic syntheses.[200-202]

Enzymes are well known as practical biocatalysts passessing vast potential and, in particular, are being increasingly used on an industrial scale for bio-transformation,[203] preparation of fine chemicals, and synthesis of enantiopure pharmaceuticals.[204–206] Thus, more and more natural resources will be transformed into organic products catalysed by biocatalysts in the near future. And, the conventional chemical syntheses can be replaced by these biocatalysed syntheses, which often employ mild reagents and require low energy input.

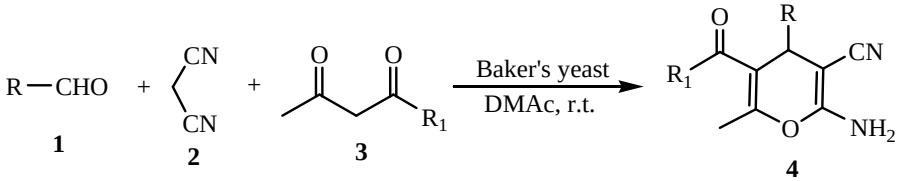
Wang *et al.*[207] found immobilized lipase from *Mucor miehei* (MML) to be an efficient biocatalyst for the synthesis of 2-alkylbenzimidazole derivatives **3** under solvent-free domino acylation/cyclization reactions of *o*-phenylenediamine **1** and fatty acid esters **2** (Scheme **38**).



1 **2** **3**

Scheme 38

Baker's yeast is reported to be an efficient catalyst by Pratap *et al.*[208] for the synthesis of polyfunctionalized 4*H*-pyrans **4** *via* one-pot three-component cyclocondensation of arylaldehydes **1**, malononitrile **2** and β-dicarbonyls **3** in organic medium (Scheme **39**).



1 **2** **3** **4**

Scheme 39

1.6 Objectives of the present work

Heterocyclic structural motifs occur in many bioactive natural products and synthetic drugs,[21] agrochemicals[22] and so many other industrially important compounds of general use like dyes,[15] paints,[15] etc. and these structural units serve as important reactants as well as intermediates in organic synthesis.[9,10]

Heterocyclic compounds possess an array of biological activities. Most of the clinically relevant drugs accessible in the market are heterocyclic compounds. In view of the enormous medicinal importance of heterocyclic compounds as potential antiviral, antibacterial, antifungal, anti-inflammatory, antihypertensive, antitumor, antidepressants, agents etc[23-29] synthesis of novel heterocyclic systems has always been the primary objective of organic chemists in the continuous search for newer bioactive molecules.

The present work is an effort in the direction of the development of environmentally benign procedures using non-conventional methods and has been carried out with the aim to explore newer green synthetic approaches under the title "**Synthesis and Characterization of Some Medicinally Important Spiro and Condensed Heterocyclic Compounds through Green Approach**" by using more sustainable approaches for devising alternative, clean, efficient, economic and nature friendly methodology for diversity-oriented combinatorial synthesis of potential bioactive compounds.

The versatility of these techniques has been explored in terms of their synthetic utility for bioactive heterocycles using green methodologies.

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CHAPTER 2

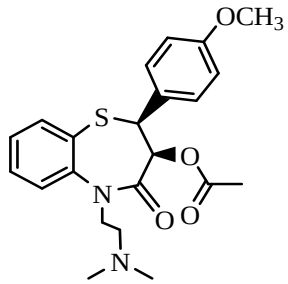
Selective Synthesis and Characterization of Pyrazolo [3,4-e][1,4]thiazepinone Derivatives in Aqueous Media under Catalyst-free Condition using Ultrasound Irradiation

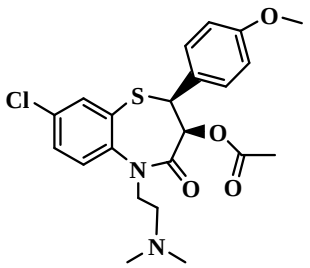
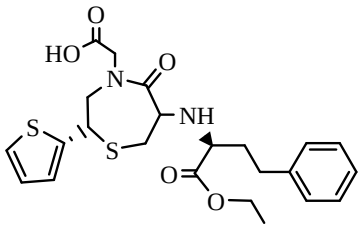
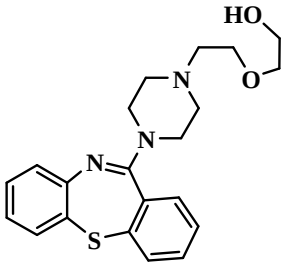
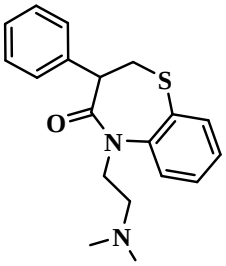
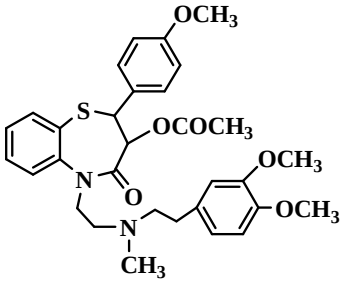
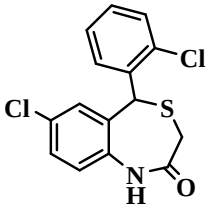
2.1 INTRODUCTION

Compounds containing heterocyclic ring systems are of great importance and receive special attention as they belong to the class of compounds with proven utility in medicinal chemistry.[1] Thia-aza heterocycles have attracted considerable prominence because of their wide biological and pharmacological activities.[2] Among these heterocycles, 1,4-thiazepine derivatives have attracted much attention as an important structural motif in medicinal chemistry owing to their significant therapeutic and biological activities.[3]

In recent years, some thiazepinones have also been described as calcium channel antagonists, HIV-1 enzyme integrase, tyrosine kinase enzyme inhibitor,[4] reverse transcriptase inhibitors, and antitumor agents.[5] Numerous heteroannulated bioisosteric analogs of this core fragment were reported as potent inhibitors of Herpes simplex virus type 1 (HSV-1) replication, compounds possessing H1 antihistamine activity, dopamine D2 receptors, and selective antagonists of 5-HT1A and vasoconstrictor agents.[6] Various thiazepine derivatives exhibits angiotensin-converting enzyme inhibition,[7] which is important for development of Temocapril drug,[8] used for the treatment of hypertension. Compounds from different 1,4-benzothiazepine type structural classes, such as CGP37157 and Diltiazem have been reported to inhibit mNCE activity.[9] (**Table 2.1**)

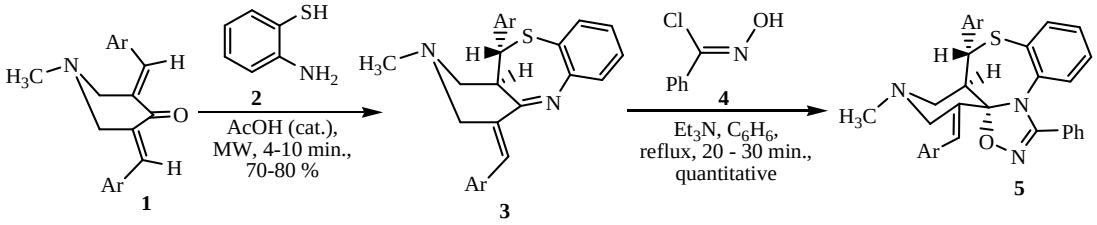
Table 2.1: Medicinally relevant thiazepine derivatives

S. No.	Name of the compound	Structure of the compound	Use of the compound [8,10-16]
1	Diltiazem		The calcium channel blocker Diltiazem lowers the heart rate in man which probably contributes to its clinical effectiveness in heart disease and hypertension.

S. No.	Name of the compound	Structure of the compound	Use of the compound [8,10-16]
2	Clentiazem		Clentiazem has been shown to have cerebrovascular protective properties. It has both vasorelaxing and negative inotropic actions.
3	Temocapril		Temocapril is an ACE (angiotensin converting enzyme) inhibitor primarily used in the treatment of hypertension and congestive heart failure, diabetic nephropathy.
4	Quetipine		Quetiapine is a dopamine, serotonin, and adrenergic antagonist, and a potent antihistamine with clinically tested anticholinergic properties.
5	Tiazesim		Triazesim has been found to posses coronary vasodilating and anti-depressant activity.
6	DTZ323		DTZ323 is the one of the most potent Ca^{2+} channel antagonists. It has been reported to block voltage-dependent L-type calcium channel currents selectively.
7	CGP37157		CGP37157 is widely used to explore the role of mitochondria in cell Ca^{2+} handling, by its blocking effect of the mitochondrial Na^{+}/Ca^{2+} exchanger.

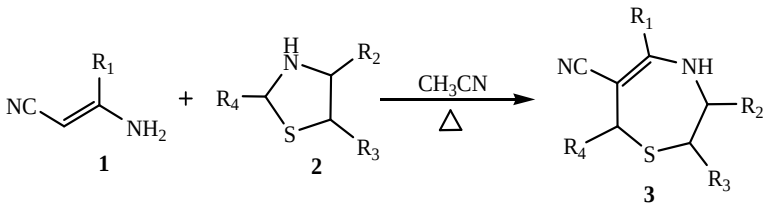
Detailed literature survey shows that different protocols have been developed for the synthesis of medicinally important thiazepine derivatives. They involve:

A series of new substituted [1,2,4]oxadiazolo[5,4-*d*]pyrido[3,4-*c*][1,5] benzothiazepines **5** were developed by Perumal *et al.*[17] by the two-step reaction of 1-methyl-3,5-bis(arylidene)-4-piperidones **1** and *o*-aminothiophenol **2**, 2-methyl-11-aryl-hexahydropyrido-benzothiazepines **3** and (Z)-benzoyl chloride oxime **4** in the presence of a catalytic amount of triethylamine in reflux conditions (Scheme **1**).



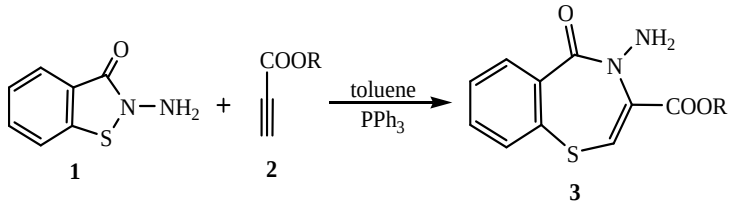
Scheme 1

Calvo *et al.*[18] synthesized a wide library of substituted tetrahydro [1,4]thiazepines **3** by simple heating of β-enaminonitrile **1** and thiazolidine derivatives **2** in acetonitrile (Scheme **2**).



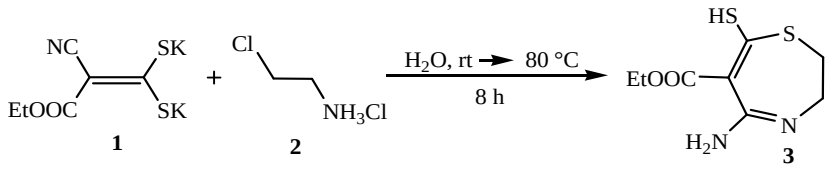
Scheme 2

Vicini *et al.*[19] discussed a one-pot reaction between 2-aminobenzo[*d*] isothiazolone **1** and alkyl propiolates **2** in the presence of triphenylphosphine leading to the corresponding alkyl 4-amino-5-oxobenzo [*f*][1,4]thiazepine-3-carboxylate derivatives **3** (Scheme **3**).



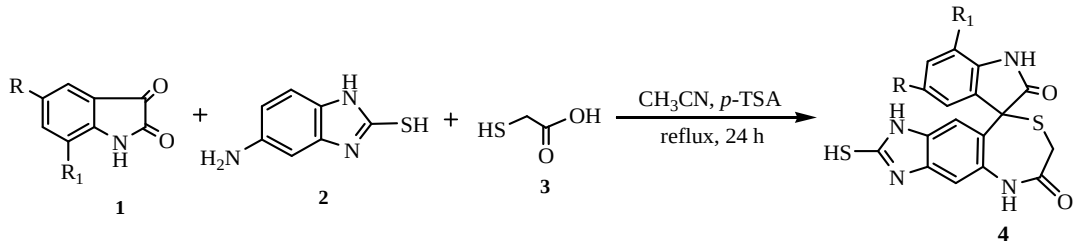
Scheme 3

Synthesis of (4*E*,6*E*)-ethyl-5-amino-2,3-dihydro-7-mercapto-1,4-thiazepine-6-carboxylate derivatives **3** by the reaction of ethyl-2-cyano-3,3-dimercaptoacrylate dipotassium salt **1** with 2-chloroethylamine hydrochloride **2** in water as a solvent had been described by Bakavoli *et al.*[20] (Scheme **4**)



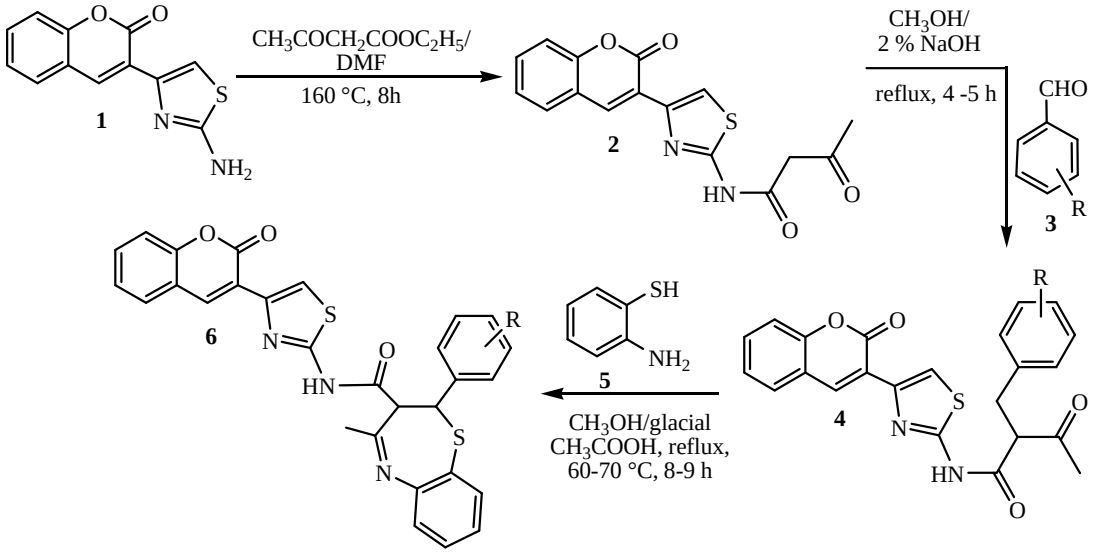
Scheme 4

An efficient synthesis of novel spiro(imidazo-benzo[1,2-*e*][1,4]thiazepine)-indoline derivatives **4** has been accomplished by Anisetti *et al.*[21] from isatins **1**, 5-amino-2-mercapto benzimidazole **2** and mercapto acetic acid **3** in acetonitrile (Scheme **5**).



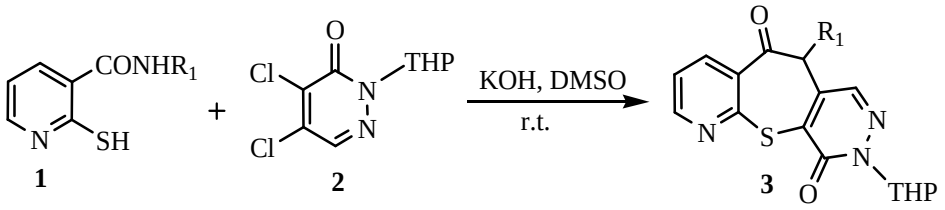
Scheme 5

Raval *et al.*[22] synthesized a variety of thiazolyl-2-substituted phenyl-2,3-dihydrobenzo[*b*][1,4]thiazepine-3-carboxamide compounds **6** from the multistep reaction of substituted amino-thiazolyl-2*H*-chromenone **1**, substituted chromenyl thiazolyl butanamide **2**, aldehyde **3**, substituted arylidene-chromenyl thiazol-2-yl butanamides **4**, and 2-aminothiophenol **5** under reflux conditions (Scheme **6**).



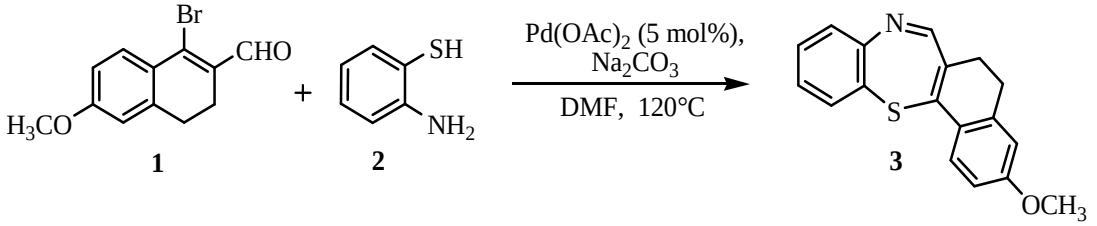
Scheme 6

Ma *et al.*[23] studied a transition metal-free route to synthesize different pyridazinopyrido[3,2-*f*][1,4]thiazepine dione derivatives **3** by the reaction of N-substituted 2-mercaptonicotinamides **1** and 2-tetrahydropyranyl-4,5-dichloropyridazin-3-ones **2** (Scheme **7**).



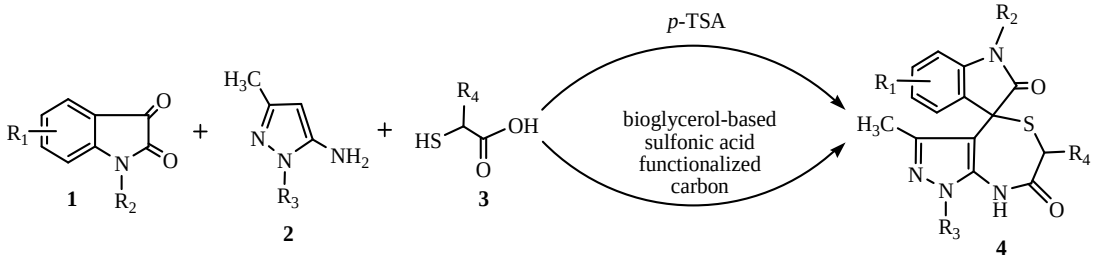
Scheme 7

Gogoi *et al.*[24] described the preparation of steroid/nonsteroid 2-arylsubstituted fused benzo[*b*][1,4]thiazepines **3** from $Pd(OAc)_2$ catalyzed reaction of steroidal/ nonsteroidal halovinyl aldehydes **1** and 2-aminothiophenols **2** in DMF as solvent under reflux condition (Scheme **8**).



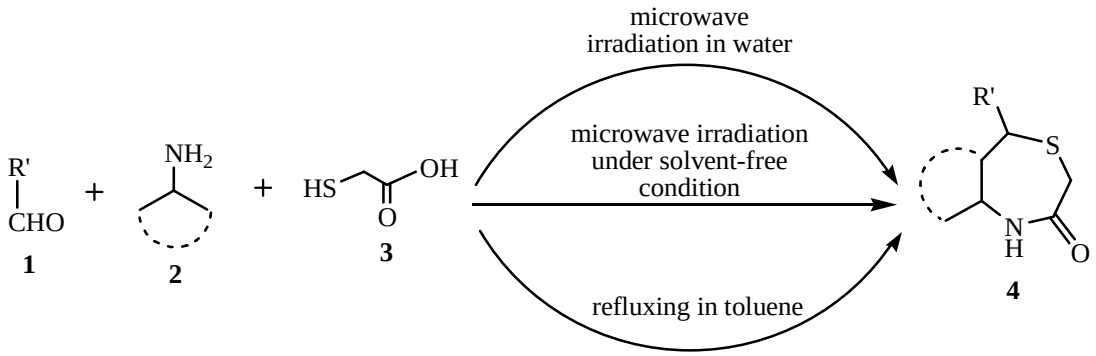
Scheme 8

Use of substituted isatin **1**, 5-amino-3-methylpyrazole **2**, and thioacid **3** as the starting materials to construct spiro[indoline-pyrazolo[3,4-*e*][1,4] thiazepine] dione derivatives have attracted considerable attention. The reported procedures for this protocol involve a wide spectrum of reagents including *p*-TSA in acetonitrile,[25] bioglycerol-based sulfonic acid functionalized carbon in acetonitrile.[26] (Scheme **9**)



Scheme 9

Further, the use of substituted aldehydes **1**, anilines **2**, and thioacid **3** as the starting materials to construct pyrazolo[3,4-*e*][1,4]thiazepine derivatives have been also involved using microwave irradiation in water,[27] microwave irradiation under solvent-free conditions,[28] under refluxing in toluene.[29] (Scheme **10**)



Scheme 10

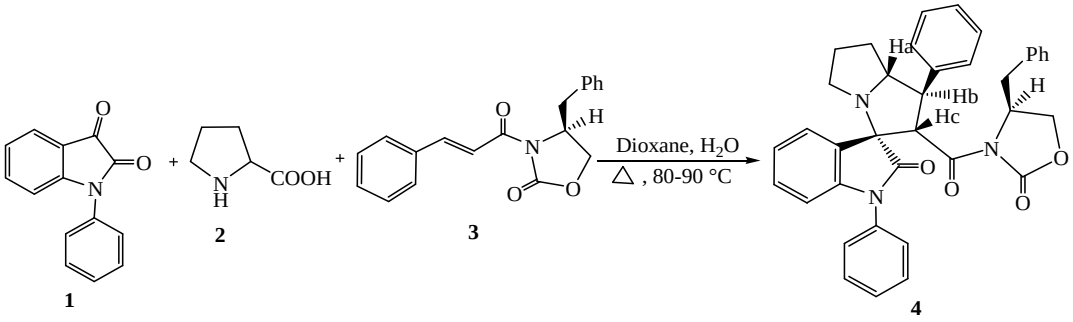
Despite the novelty of these methods, low yields, long reaction times, difficult product isolation procedures and the use of volatile solvents are some of the drawbacks of these procedures. However, straightforward and efficient one-pot reactions are still limited. In summary due to the importance of these compounds and lack of efficient methods for the synthesis of pyrazolo-thiazepines, development of new methods for their synthesis is of great interest.

2.1.1 Use of water as solvent

With increasing environmental awareness in chemical research and industry, the challenge of creating a sustainable environment calls for development of clean synthetic procedures.[30] In order to eliminate organic solvents, they are replaced by water as a solvent for converting the conventional chemical reactions into the greener ones.[31] It is presumed that water facilitates the organic reaction in non-polar media by significant factors[32] like a hydrophobic effect, enhanced hydrogen bonding in the transition state and cohesive energy density. Water is one of the most environmentally benign, cheap and non-flammable medium for carrying out organic synthesis.[33] Besides, catalyst-free synthetic methods have attracted immense interest not only for laboratory synthesis but also in chemical industry, because of reduced pollution, lower cost, mild conditions, and ease of purification.[34]

Detailed literature survey shows that different protocols have been developed using water as a reaction medium. Some of them are discussed are as follows:

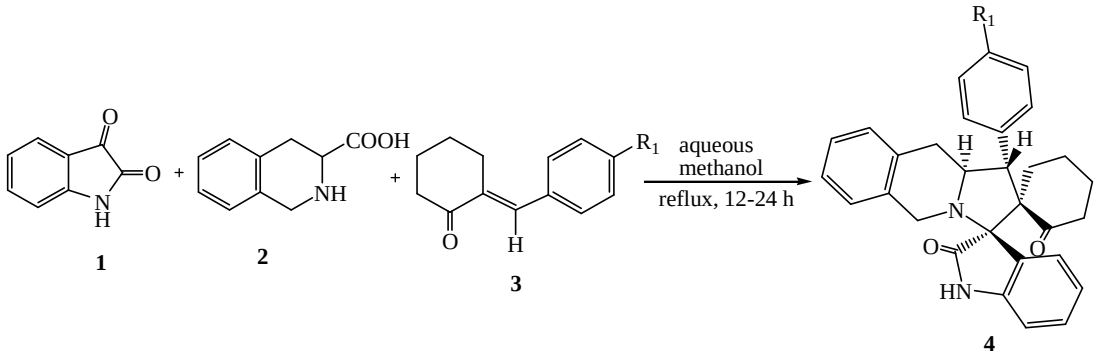
Ganguly *et al*.[35] devised a convenient way to synthesize the enantiomerically pure spirooxindolopyrrolizidine derivatives **4** involving a three-component 1,3-dipolar cycloaddition reaction of N-phenyl isatin **1** with proline **2** and (S)-4-benzyl-3-cinnamoyloxazolidin-2-one **3** in aqueous dioxane at 80-90 °C (Scheme **11**).



1 **2** **3** **4**

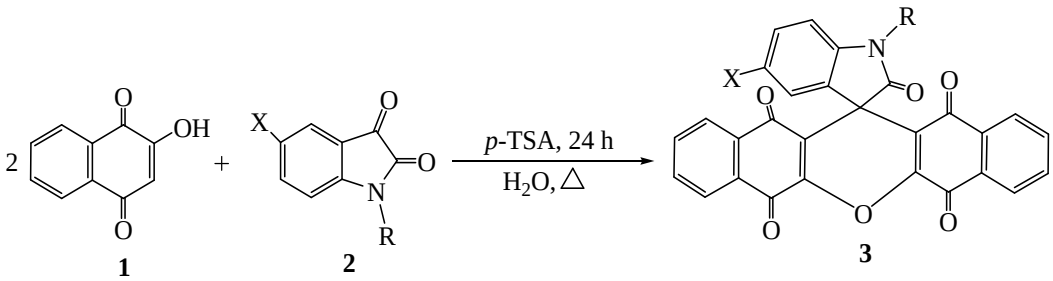
Scheme 11

Raghunathan *et al*.[36] explained an efficient synthesis of dispiro [oxindolecyclohexanone]pyrroloisoquinoline ring system **4** by the 1,3-dipolar cycloaddition reaction of isatin **1**, tetrahydroisoquinoline-3-carboxylic acid **2** with the *(E)*-2-arylidene-1-cyclohexanones **3** (Scheme **12**).



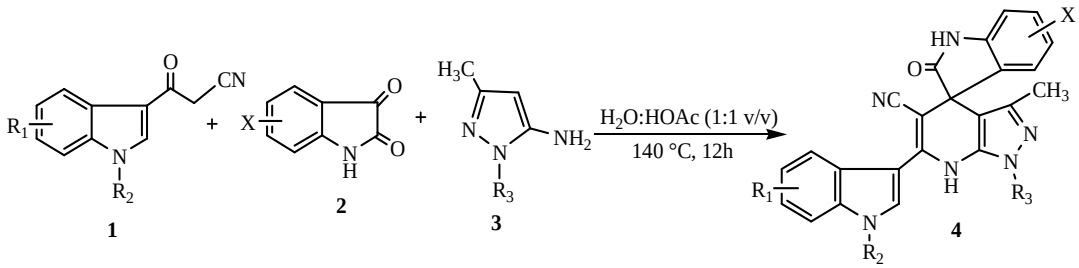
Scheme 12

Bazgir *et al.*[37] undertook the synthesis of spiro[dibenzo[*b,i*]xanthene-13,3′-indoline]-pentaone derivatives **3** through a cyclo-condensation reaction of 2-hydroxynaphthalene-1,4-dione **1** with isatins **2** using water as the reaction medium (Scheme **13**).



Scheme 13

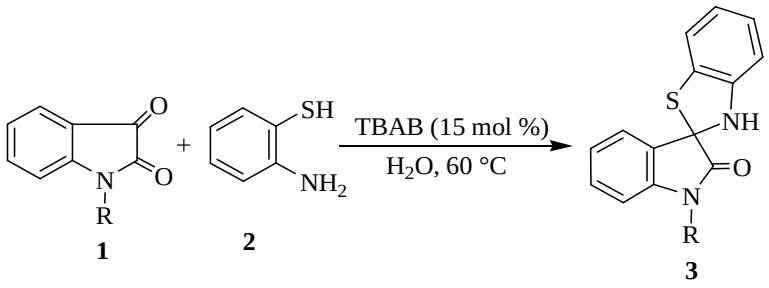
Ji *et al.*[38] demonstrated a simple and atom economical approach for the synthesis of highly functionalized 6′-(1*H*-indol-3-yl)-1′,7′-dihydrospiro[indoline-3,4′-pyrazolo[3,4-*b*]pyridine]-2-one derivatives **4** from the one-pot, three-component reaction of 3-cyanoacetyl indoles **1**, isatins **2**, and 1*H*-pyrazol-5-amines **3** in H_2O/HOAc in high yields (Scheme **14**).



Scheme 14

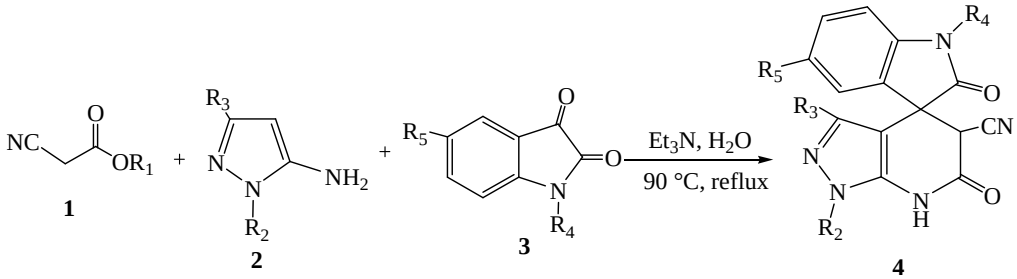
Jain *et al.*[39] described an efficient and green protocol for the synthesis of 3′*H*-spiro[indole-3,2′-[1,3]benzothiazole]-2(1*H*)-ones **3** by the reaction of indole-

2,3-diones **1** with 2-aminothiophenol **2** using tetrabutylammonium bromide (TBAB), as a surfactant in aqueous medium (Scheme **15**).



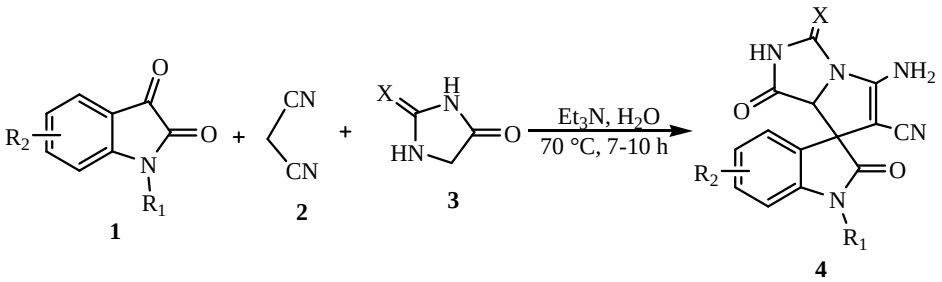
Scheme 15

Rahmati *et al.*[40] described a green and efficient one-pot, three-component condensation reaction of an alkyl cyanoacetate **1**, aminopyrazole **2**, and isatin **3** in water to give 2,6′-dioxo-1′,5′,6′,7′-tetrahydrospiro[indoline-3,4′-pyrazolo[3,4-*b*] pyridine]-5′-carbonitrile derivatives **4** at 90 °C, using Et_3N as a catalyst with good yields (Scheme **16**).



Scheme 16

Choudhury *et al.*[41] synthesized pharmacologically proficient spiro[indoline-3,7′-pyrrolo[1,2-*c*]imidazole]-6′-carbonitrile derivatives **4** by a sequential three-component one-pot reaction of the readily available starting materials such as isatin **1**, malononitrile **2** and substituted hydantoin **3** catalyzed by Et_3N in water, an environmentally friendly reaction medium (Scheme **17**).



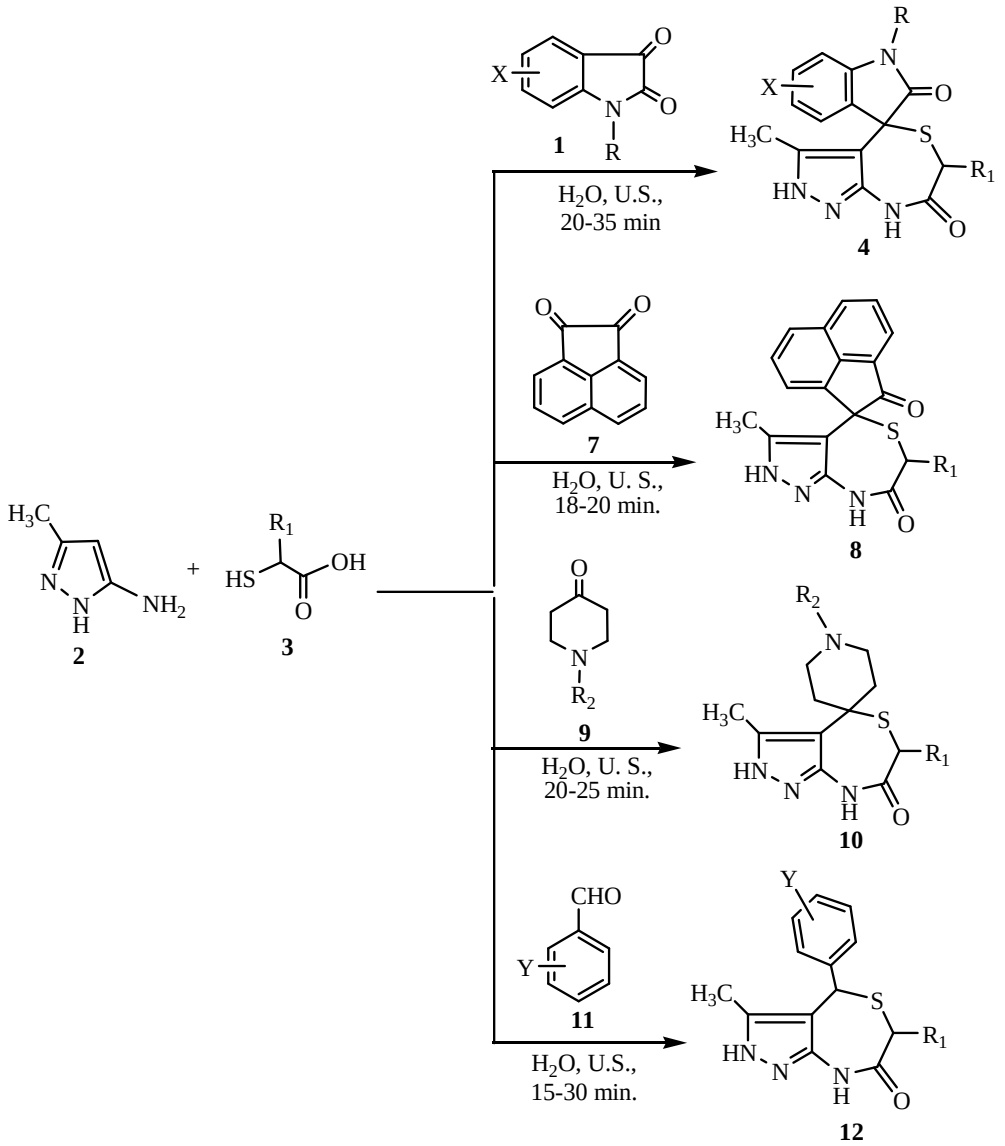
Scheme 17

2.2 EXPERIMENTAL SECTION

The application of ultrasonic irradiation in organic reactions is a promising technique. Ultrasonic irradiation has also found to be used to influence selectivity and yields of reactions.[42]

In this context, clean and rapid multicomponent reactions (MCRs) have played an important role in this process.[43] According to the principles of green chemistry; synthetic methods should be designed to use substances that exhibit little or no toxicity to human health and environment. Thus, the possibility of performing multicomponent reactions in aqueous conditions under ultrasonic irradiation to enhance their efficiency is attractive from an economic as well as a green point of view.

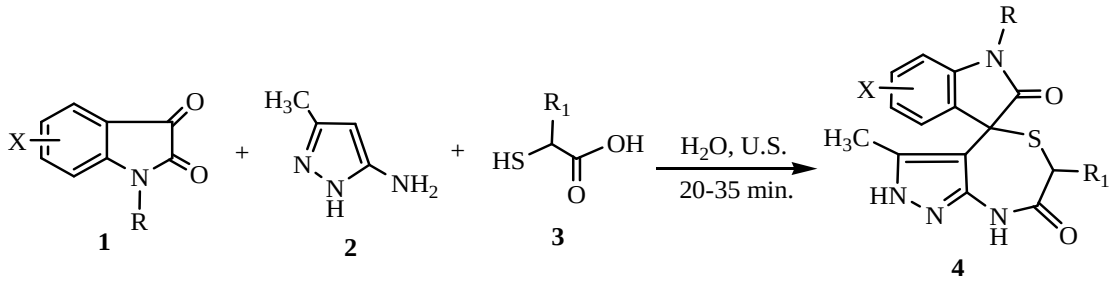
Hence, in continuation of our work to develop new green chemical routes to synthesize spiro heterocyclic derivatives using water as solvent,[44] and guided by the following two objectives: (1) to develop a new procedure without utilizing basic/acidic catalysts and volatile organic solvents, (2) to evaluate the versatility and selectivity of these procedures. Therefore, we have investigated a three component reaction of isatin (**1**), 5-amino-3-methylpyrazole (**2**), and α-mercaptocarboxylic acid (**3**) under simple but effective catalyst-free conditions in water under sonication for the first time (**Scheme 2.1**).



Scheme 2.1: Synthesis of pyrazolo[3,4-*e*][1,4]thiazepinone derivatives

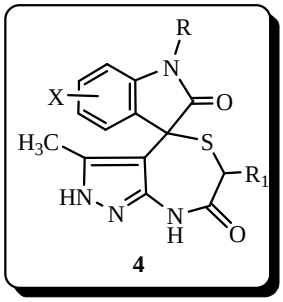
2.2.1 Synthesis of spiro[indoline-3,4'-pyrazolo[3,4-*e*][1,4]thiazepine diones (4a-i)

An equimolar mixture of indole-2,3-dione **1** (0.294 g, 2 mmol), 5-amino-3-methylpyrazole **2** (0.194 g, 2 mmol) and α-mercaptocarboxylic acids **3** (0.184 g, 2 mmol) in water (10 ml) were taken in a flask. The flask was attached to a 12 mm tip diameter probe and the reaction mixture was sonicated for the specified period at 50% power of the processor at 4 s pulse mode. At the end of the reaction period, TLC was checked and the flask was detached from the probe and the content was transferred into a beaker. The formed product was filtered and washed well with water to afford the pure crystalline product (**Scheme 2.2**). All the synthesized compounds are summarized in **Table 2.2**.



Scheme 2.2: Synthesis of spiro[indoline-3,4'-pyrazolo[3,4-*e*][1,4]thiazepine diones

Table 2.2: Synthesis of spiro[indoline-3,4'-pyrazolo[3,4-*e*][1,4]thiazepine]dione (**4a-i**) derivatives under sonication



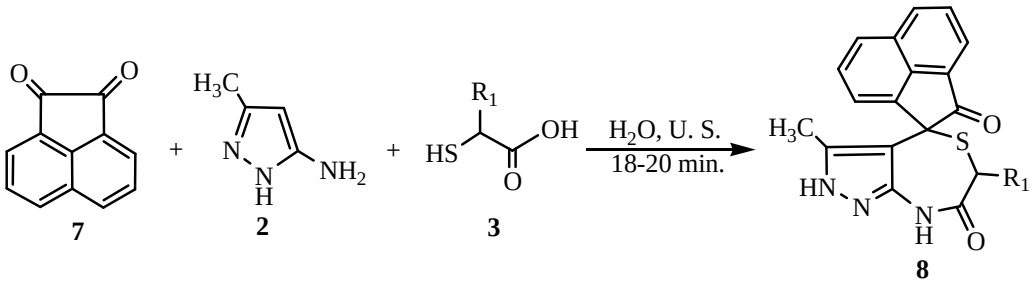
Entry	Product	X	R	R_1	Time (min.)	Yield* (%)	Mp (^oC)	Color
1	**4a**	H	H	H	20	91	182-184	White
2	**4b**	H	H	CH_3	20	92	172-174	White
3	**4c**	5-CH_3	H	H	25	90	279-281	White
4	**4d**	5-CH_3	H	CH_3	30	89	317-319	Off-white
5	**4e**	5-Cl	H	H	25	90	194-196	Yellow
6	**4f**	5-Br	H	H	35	88	329-331	Yellow
7	**4g**	5,7-diCH_3	H	H	30	90	324-326	White
8	**4h**	H	CH_2-Ph	H	35	91	234-236	Off-white
9	**4i**	H	CH_2-Ph	CH_3	30	90	255-257	Off-white

***** = Isolated Yield

2.2.2 Synthesis of spiro[acenaphthylene-1,4'-pyrazolo[3,4-*e*][1,4]thiazepine] ones (**8a-b**)

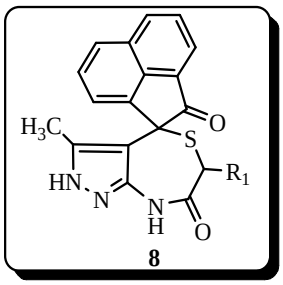
An equimolar mixture of acenaphthylene-1,2-dione **7** (0.364 g, 2 mmol), 5-amino-3-methylpyrazole **2** (0.194 g, 2 mmol) and α-mercaptocarboxylic acids **3** (0.184 g, 2 mmol) in water (10 ml) were taken in a flask. The flask was attached to a 12 mm tip diameter probe and the reaction mixture was sonicated for the specified period at 50% power of the processor at 4 s pulse mode. At the end of the reaction

period, TLC was checked and the flask was detached from the probe and the content was transferred into a beaker. The formed product was filtered and washed well with water to afford the pure crystalline product (**Scheme 2.3**). All the synthesized compounds are summarized in **Table 2.3**.



Scheme 2.3: Synthesis of spiro[acenaphthylene-1,4'-pyrazolo[3,4-*e*] [1,4]thiazepine]one

Table 2.3: Synthesis of spiro[acenaphthylene-1,4'-pyrazolo[3,4-*e*][1,4] thiazepine]one **(8a-b)** derivatives under sonication



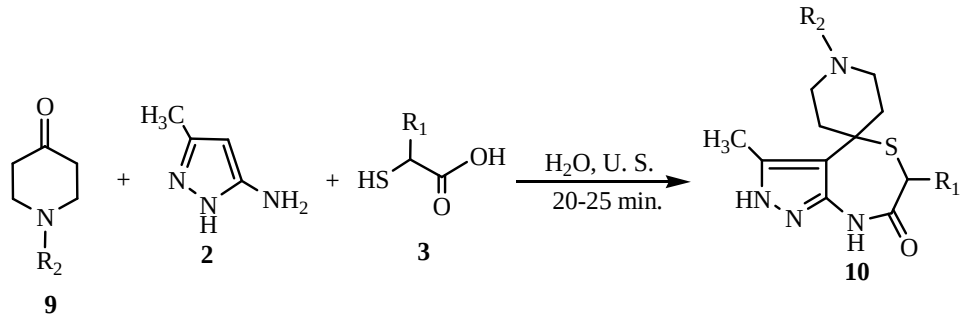
Entry	Product	R_1	Time	Yield*	Mp (°C)	Color
1	**8a**	H	20	91	275-277	Yellow
2	**8b**	CH_3	18	90	272-274	Yellow

***** = Isolated Yield

2.2.3 Synthesis of spiro[piperidine-4,4'-pyrazolo[3,4-*e*][1,4]thiazepine]ones (10a-b)

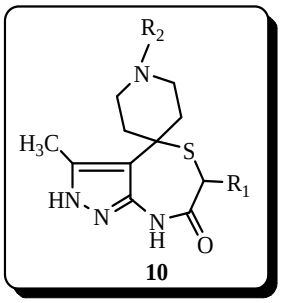
An equimolar mixture of piperidinone **9** (0.378 g, 2 mmol), 5-amino-3-methylpyrazole **2** (0.194 g, 2 mmol) and α-mercaptocarboxylic acids **3** (0.184 g, 2 mmol) in water (10 ml) were taken in a flask. The flask was attached to a 12 mm tip diameter probe and the reaction mixture was sonicated for the specified period at 50% power of the processor at 4 s pulse mode. At the end of the reaction period, TLC was checked and the flask was detached from the probe and the content was

transferred into a beaker. The formed product was filtered and washed well with water to afford the pure crystalline product (**Scheme 2.4**). All the synthesized compounds are summarized in **Table 2.4**.



Scheme 2.4: Synthesis of spiro[piperidine-4,4'-pyrazolo[3,4-*e*][1,4]thiazepine]ones

Table 2.4: Synthesis of spiro[piperidine-4,4'-pyrazolo[3,4-*e*][1,4]thiazepine]one **(10a-b)** derivatives under sonication



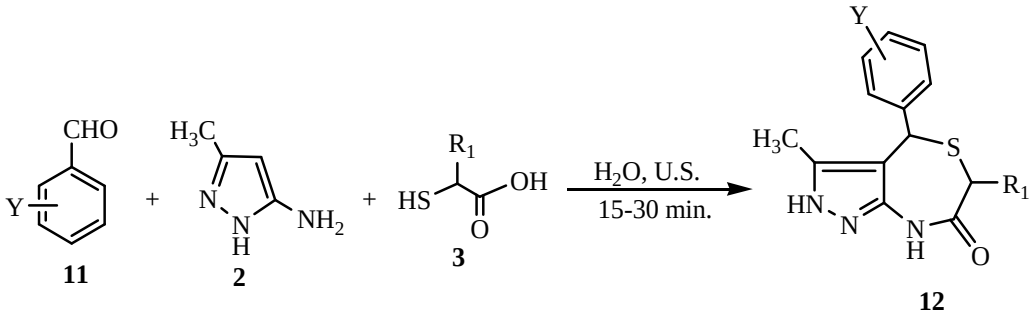
Entry	Product	R_1	R_2	Time (min.)	Yield* (%)	Mp (°C)	Color
1	**10a**	H	CH_2Ph	20	86	156-158	Off-white
2	**10b**	H	BOC	25	88	152-154	White

***** = Isolated Yield

2.2.4 Synthesis of substituted-aryl pyrazolo[3,4-*e*][1,4]thiazepines (12a-i)

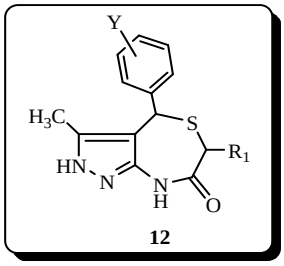
An equimolar mixture of aldehydes **11** (0.240 g, 2 mmol), 5-amino-3-methylpyrazole **2** (0.194 g, 2 mmol) and α-mercaptocarboxylic acids **3** (0.184 g, 2 mmol) in water (10 ml) were taken in a flask. The flask was attached to a 12 mm tip diameter probe and the reaction mixture was sonicated for the specified period at 50% power of the processor at 4 s pulse mode. At the end of the reaction period, TLC was checked and the flask was detached from the probe and the content was transferred into a beaker. The formed product was filtered and washed well with

water to afford the pure crystalline product (**Scheme 2.5**). All the compounds synthesized are summarized in **Table 2.5**.



Scheme 2.5: Synthesis of substituted-aryl pyrazolo[3,4-*e*][1,4]thiazepinones

Table 2.5: Synthesis of substituted-aryl pyrazolo[3,4-*e*][1,4]thiazepinone **(12a-i)** derivatives under sonication



Entry	Product	Y	R_1	Time (min.)	Yield* (%)	Mp (°C)	Color
1	**12a**	4-CH_3	H	15	90	299-301	White
2	**12b**	4-CH_3	CH_3	15	88	153-155	White
3	**12c**	4-OCH_3	H	15	89	267-269	White
4	**12d**	4-OCH_3	CH_3	18	90	165-167	White
5	**12e**	4-F	H	20	93	182-184	White
6	**12f**	4-F	CH_3	20	91	154-156	Light-yellow
7	**12g**	4-NO_2	H	20	89	193-195	Yellow
8	**12h**	2-Cl	H	30	83	126-128	White
9	**12i**	4-Br	H	25	87	283-285	Yellow

***** = Isolated Yield

2.3 CHARACTERIZATION OF THE COMPOUNDS SYNTHESIZED

To confirm the structure of the compounds synthesized and to know the position of various functional groups and presence of heteroatoms in the compounds, characterized by their melting points, various spectral studies including IR, Mass, ^{1}H NMR, ^{13}C NMR and single crystal X-ray analysis.

2.3.1 IR and Mass spectral studies

IR spectra of the compounds synthesized was recorded on Shimadzu FT-IR 8400S spectrophotometer using KBr pellets. Mass spectra of the representative compound were obtained using Waters UPLC-TQD Mass spectrometer at 70 eV. (**Table 2.6**)

Compound **4b** was isolated as white crystalline solid with melting point as 172-174 °C. The IR spectrum showed absorptions at 1660, 1710 cm^{-1} which clearly indicated two carbonyl groups. Further, the presence of molecular ion peak at m/z 315.0 (M+1) in the mass spectrum confirmed the formation of the **4b**.

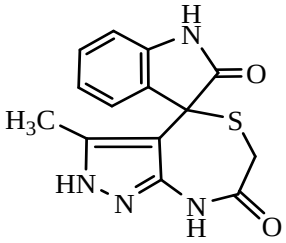
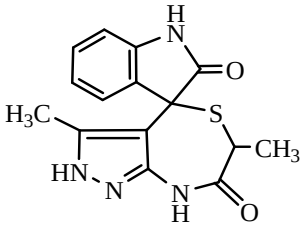
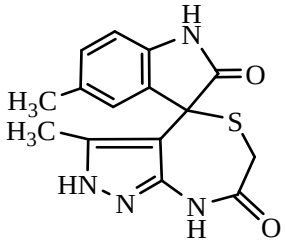
2.3.2 ^{1}H NMR and ^{13}C NMR spectral studies

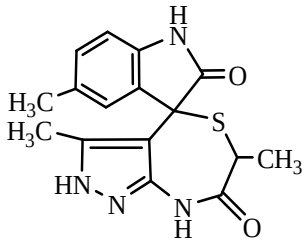
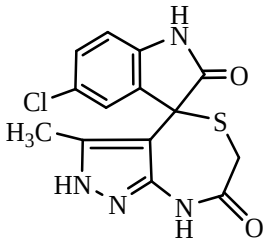
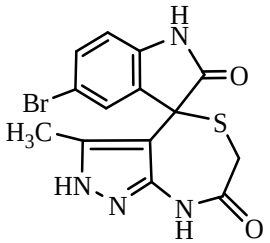
^{1}H NMR and ^{13}C NMR spectra of the compounds synthesized were recorded in DMSO-d_6 using TMS as an internal standard on a Bruker 400 Avance III Spectrophotometer at 400, 100 MHz and Bruker Spectrophotometer at 300, 75 MHz. (**Table 2.6**)

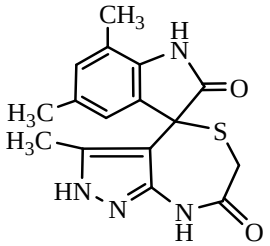
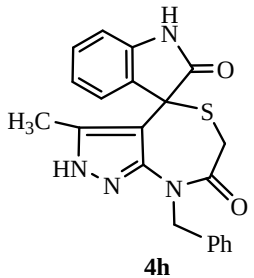
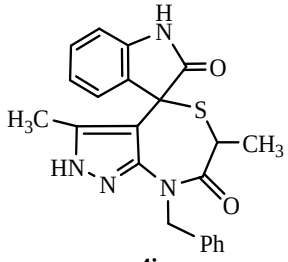
The ^{1}H NMR spectrum of **4b** revealed a sharp singlet at δ 1.44 due to the methyl protons present in the pyrazole ring. The methyl protons of the thiazepine ring are observed to be appeared as a multiplet at δ 2.50 and a quartet at δ 4.52 (J = 6.6 Hz) was assigned as the CH proton at same carbon atom. The –NH protons in the compound showed three singlet peaks at δ 10.04, 10.79 and 12.49 and the four aromatic protons appeared in the region δ 6.93-7.02 ppm. In ^{13}C NMR spectrum of **4b**, the two carbonyl carbon atoms resonated at δ 172.9, 178.0. Signal at δ 48.9 can be attributed to the spiro carbon and the signals at δ 110.0-146.0 confirmed the presence of aromatic carbons while methyl carbon atoms appeared at δ 9.2 and 15.5 ppm. The relative configuration was also unambiguously confirmed by X-ray crystallography of the compound **4b**.

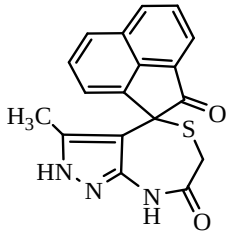
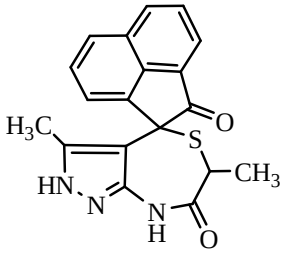
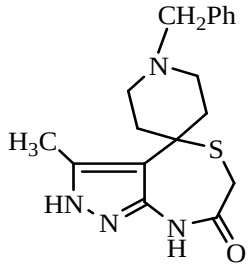
In the single crystal X-ray structure of the compound **4b**, the pyrazole ring is fused with the thiazepine ring. Four atoms of the ring, i.e., C7, C9, C12 and N3 are co-planar with the fused pyrazole ring whereas the atoms S1, C14 and C13 are not in the plane and are present above and below the plane of the fused ring system.[45] Further, the isatin ring, which is attached with the C7 atom, is perpendicular with respect to the plane of the seven-membered thiazepine ring.

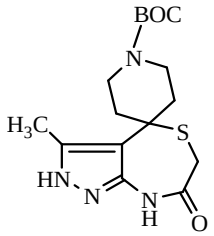
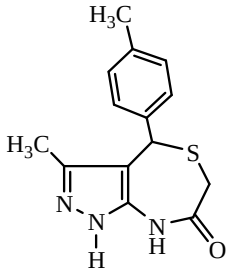
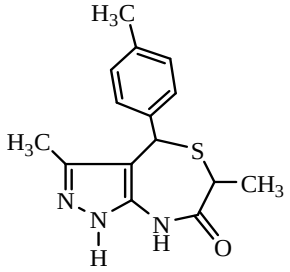
Table 2.6: IR, Mass, ^{1}H NMR and ^{13}C NMR spectral data of the compounds synthesized **(4a-i), (8a-b), (10a-b) and (12a-i)**

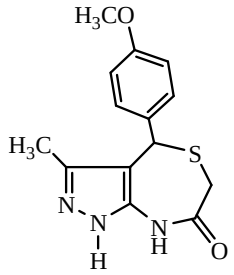
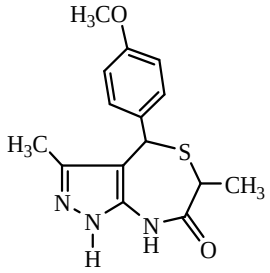
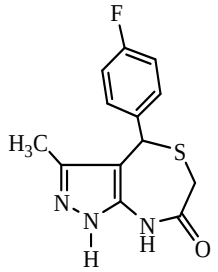
Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
1	 **4a**	3369 (NH), 3134 (NH), 2918 (NH), 1708 (CO), 1650 (CO), 1468, 1409, 771	301 m/z as $[M+H]^+$ for $C_{14}H_{12}N_4O_2S$	1.43 (s, 3H, CH_3), 3.04 (d, *J* = 15.2 Hz, 1H, CH), 4.42 (d, *J* = 15.2 Hz, 1H, CH), 6.96-7.32 (m, 4H, Ar-H), 10.09 (s, 1H, NH), 10.83 (s, 1H, NH), 12.48 (s, 1H, NH)	9.8 (CH_3), 29.5 (CH_2), 56.1 (spiro C), 106.1, 110.1, 122.6, 124.5, 129.0, 129.8, 137.4, 141.1, 146.1, 171.3 (C=O), 177.9 (C=O)
2	 **4b**	3392 (NH), 3244 (NH), 3169 (NH), 2915, 1710 (CO), 1660 (CO), 1360, 1414, 769	315 m/z as $[M+H]^+$ for $C_{15}H_{14}N_4O_2S$	1.44 (s, 3H, CH_3), 2.50 (m, 3H, CH_3), 4.52 (q, *J* = 6.6 Hz, 1H, CH), 6.93-7.02 (m, 4H, Ar-H), 10.04 (s, 1H, NH), 10.79 (s, 1H, NH), 12.49 (s, 1H, NH)	9.2 (CH_3), 15.5 (CH_3), 34.0 (CH), 48.9 (spiro C), 104.1, 110.0, 122.5, 124.4, 128.6, 129.6, 141.0, 146.0, 162.2, 172.9 (CO), 178.0 (CO)
3	 **4c**	3365 (NH), 3197 (NH), 2981 (NH), 2904, 1712 (CO), 1669 (CO), 1474, 1403, 677	315 m/z as $[M+H]^+$ for $C_{15}H_{14}N_4O_2S$	1.44 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), 3.02 (d, *J* = 15.2 Hz, 1H, CH), 4.43 (d, *J* = 15.2 Hz, 1H, CH), 6.84-7.10 (m, 3H, Ar), 10.06 (s, 1H, NH), 10.69 (s, 1H, NH), 12.45 (s, 1H, NH)	9.9 (CH_3), 20.4 (CH_3), 29.4 (CH_2), 48.2 (spiro C), 105.5, 109.8, 124.8, 128.8, 129.9, 131.2, 138.5, 142.4, 147.0, 171.2 (C=O), 177.8 (C=O)

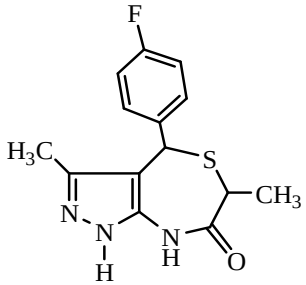
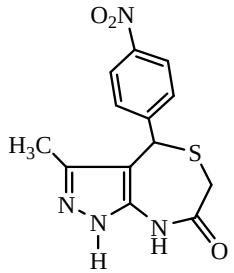
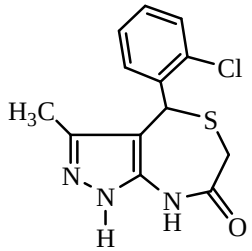
Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
4	 **4d**	3316 (NH), 3126 (NH), 2988 (NH), 2921, 1713 (CO), 1665 (CO), 1492, 1410, 709	329 m/z as $[M+H]^+$ for $C_{16}H_{16}N_4O_2S$	1.28 (d, *J* = 7.2 Hz, 3H, CH_3), 1.45 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.82 (q, *J* = 7.2 Hz, 1H, CH), 6.91-7.23 (m, 3H), 9.64 (s, 1H, NH), 10.72 (s, 1H, NH), 11.23 (s, 1H, NH)	10.5 (CH_3), 15.6 (CH_3), 19.8 (CH_3), 32.6 (CH), 47.9 (spiro C), 110.3, 122.5, 124.0, 127.3, 130.7, 132.4, 134.1, 140.6, 145.8, 169.8 (C=O), 174.5 (C=O)
5	 **4e**	3378 (NH), 3164 (NH), 2956 (NH), 1708 (CO), 1665 (CO), 1476, 1404, 724	335 m/z as $[M+H]^+$ for $C_{14}H_{11}ClN_4O_2S$	1.70 (s, 3H, CH_3), 3.41 (d, *J* = 14.8 Hz, 1H, CH), 4.67 (d, *J* = 14.8 Hz, 1H, CH), 6.85-7.23 (m, 3H, Ar), 10.02 (s, 1H, NH), 10.59 (s, 1H, NH), 12.11 (s, 1H, NH)	10.2 (CH_3), 25.9 (CH_2), 45.9 (spiro C), 100.2, 110.5, 124.1, 125.4, 127.3, 132.4, 141.1, 152.2, 158.4, 170.4 (C=O), 177.3 (C=O)
6	 **4f**	3336 (NH), 3145 (NH), 2964 (NH), 1709 (CO), 1670 (CO), 1438, 1415, 834	380 m/z as $[M+H]^+$ for $C_{14}H_{11}BrN_4O_2S$	1.52 (s, 3H, CH_3), 3.39 (d, *J* = 15.2 Hz, 1H, CH), 4.61 (d, *J* = 15.2 Hz, 1H, CH), 6.83-7.20 (m, 3H, Ar-H), 10.04 (s, 1H, NH), 10.50 (s, 1H, NH), 12.05 (s, 1H, NH)	9.9 (CH_3), 23.9 (CH_2), 46.8 (spiro C), 105.6, 110.5, 123.1, 124.4, 126.3, 131.4, 145.1, 150.2, 154.4, 171.4 (C=O), 176.3 (C=O)

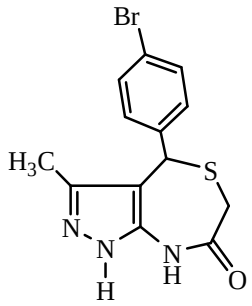
Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
7	 **4g**	3324 (NH), 3186 (NH), 2912 (NH), 1707 (CO), 1665 (CO), 1472, 1429, 827	329 m/z as $[M+H]^+$ for $C_{16}H_{16}N_4O_2S$	1.44 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 3.05 (d, *J* = 15.2 Hz, 1H, CH), 4.41 (d, *J* = 15.2 Hz, 1H, CH), 6.84-7.10 (m, 2H, Ar-H), 10.10 (s, 1H, NH), 10.59 (s, 1H, NH), 12.42 (s, 1H, NH)	10.5 (CH_3), 19.7 (CH_3), 20.4 (CH_3), 29.4 (CH_2), 48.2 (spiro C), 109.8, 124.8, 128.8, 129.9, 131.2, 134.7, 136.7, 138.5, 145.7, 171.2 (C=O), 177.8 (C=O)
8	 **4h**	3368 (NH), 3183 (NH), 2991, 1702 (CO), 1680 (CO), 1434, 1402, 737	391 m/z as $[M+H]^+$ for $C_{21}H_{18}N_4O_2S$	1.43 (s, 3H, CH_3), 3.03 (d, *J* = 15.2 Hz, 1H, CH), 4.18 (d, *J* = 14.8 Hz, 1H, CH), 4.85 (d, J =15.2 Hz, 1H, CH), 5.08 (d, *J* = 15.6 Hz, 1H, CH), 6.96-7.35 (m, 9H, Ar-H), 10.15 (s, 1H, NH), 10.80 (s, 1H, NH)	10.4 (CH_3), 29.5 (CH_2), 48.2 (spiro C), 56.1 (CH_2), 110.1, 115.4, 122.6, 123.6, 124.5, 128.6, 129.0, 129.8, 135.5, 137.4, 139.56, 141.1, 146.7, 171.3 (C=O), 177.9 (C=O)
9	 **4i**	3326 (NH), 2982 (NH), 2914, 1710 (CO), 1680 (CO), 1473, 1421, 785	405 m/z as $[M+H]^+$ for $C_{22}H_{20}N_4O_2S$	1.01 (s, 3H, CH_3), 1.42 (d, *J* = 7.6 Hz, 3H, CH_3), 3.98 (q, *J* = 7.2 Hz, 1H, CH), 4.79 (d, *J* = 15.2 Hz, 1H, CH), 5.10 (d, *J* = 15.6 Hz, 1H, CH), 6.42-6.75 (m, 9H, Ar-H), 9.91 (-NH, s), 11.96 (-NH, s)	9.5 (CH_3), 15.1 (CH_3), 34.0 (CH), 48.8 (spiro C), 49.0, 109.7, 122.0, 124.2, 128.2, 128.9, 140.7, 173.5 (CO), 178.1 (C=O)

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
10	 **8a**	3346 (NH), 3173 (NH), 2916, 1720 (CO), 1695 (CO), 1481, 1415, 757	336 m/z as $[M+H]^+$ for $C_{18}H_{13}N_3O_2S$	1.37 (s, 3H, CH_3), 3.13 (d, *J* = 14.8 Hz, 1H, CH), 4.19 (d, *J* = 14.8 Hz, 1H, CH), 6.28 7.10 (m, 6H, Ar-H), 10.55 (s, 1H, NH), 12.23 (s, 1H, NH)	10.2 (CH_3), 28.9 (CH_2), 51.2 (spiro C), 102.3, 120.2, 122.1, 124.0, 127.3, 127.8, 128.3, 130.5, 136.2, 137.3, 169.4 (C=O), 196.8 (C=O)
11	 **8b**	3317 (NH), 2980 (NH), 2929, 1720 (CO), 1677 (CO), 1476, 1412, 692	350 m/z as $[M+H]^+$ for $C_{19}H_{15}N_3O_2S$	1.14 (s, 3H, CH_3), 1.21 (d, *J* = 7.2 Hz, 3H, CH_3), 4.43 (q, *J* = 7.6 Hz, 1H, CH), 7.43-8.38 (m, 6H, Ar-H), 10.13 (s, 1H, NH), 12.52 (s, 1H, NH)	9.8 (CH_3), 15.3 (CH_3), 35.4 (CH), 53.7 (spiro C), 106.7, 111.4, 122.0, 124.2, 125.8, 129.2, 129.3, 129.3, 129.8, 132.5, 138.9, 146.3, 172.9 (C=O), 198.7 (C=O)
12	 **10a**	3321 (NH), 2974 (NH), 2937, 1722 (CO), 1475, 1411, 906, 824, 703	343 m/z as $[M+H]^+$ for $C_{18}H_{22}N_4OS$	1.46 (s, 3H, CH_3), 2.34 (m, 4H, $2CH_2$), 2.53 (m, 4H, $2CH_2$), 3.20 (d, *J* = 15.2 Hz, 1H, CH), 4.16 (d, *J* = 15.2 Hz, 1H, CH), 4.85 (d, 1H, CH, *J* = 14.8 Hz,), 5.06 (d, 1H, CH, *J* = 14.8 Hz,), 6.21-7.20 (m, 5H, Ar-H), 9.55 (s, 1H, NH), 12.23 (s, 1H, NH)	8.9 (CH_3), 30.4 (CH_2), 34.2 (CH_2), 48.2 (spiro C), 50.2, 61.8, 107.1, 118.1, 129.1, 137.4, 140.5, 142.1, 151.2, 170.9 (C=O)

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
13	 **10b**	3280 (NH), 2963 (NH), 2825, 1723 (CO), 1697, 1479, 1378, 860, 765, 693	353 m/z as $[M+H]^+$ for $C_{12}H_{15}BN_4O_2S$	1.23 (s, 9H, $3CH_3$), 1.58 (s, 3H, CH_3), 2.54 (m, 4H, $2CH_2$), 3.14 (m, 4H, $2CH_2$), 3.34 (d, *J* = 15.2 Hz, 1H, CH), 4.32 (d, *J* = 15.2 Hz, 1H, CH), 9.52 (s, 1H, NH), 12.26 (s, 1H, NH)	9.9 (CH_3), 28.0 (CH_2), 31.5, 35.1, 49.1 (spiro C), 50.7, 77.6, 108.0, 151.9, 153.9, 160.7, 171.7 (C=O)
14	 **12a**	3197 (NH), 2953 (NH), 2906, 1704 (CO), 1475, 1402, 687	274 m/z as $[M+H]^+$ for $C_{14}H_{15}N_3OS$	1.43 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 3.03 (d, *J* = 14.8 Hz, 1H, CH), 4.23 (d, *J* = 14.8 Hz, 1H, CH), 5.24 (s, 1H, CH), 6.82-7.11 (m, 4H, Ar-H), 9.86 (s, 1H, NH), 12.56 (s, 1H, NH)	10.5 (CH_3), 21.2 (CH_3), 33.4 (CH_2), 42.8 (CH), 104.8, 116.2, 125.3, 127.5, 128.6, 141.7, 142.6, 148.4, 170.4 (C=O)
15	 **12b**	3283 (NH), 2976 (NH), 2920, 1710 (CO), 1488, 1418, 717	288 m/z as $[M+H]^+$ for $C_{15}H_{17}N_3OS$	1.11 (d, *J* = 7.2 Hz, 3H, CH_3), 1.34 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 4.28 (q, *J* = 7.2 Hz, 1H, CH), 5.28 (s, 1H, CH), 6.74-7.05 (m, 4H, Ar-H), 9.98 (s, 1H, NH), 12.64 (s, 1H, NH)	10.9 (CH_3), 18.9 (CH_3), 20.1 (CH_3), 30.4 (CH), 42.5 (CH), 108.9, 118.2, 129.3, 137.5, 141.7, 142.6, 146.8, 148.4, 173.4 (C=O)

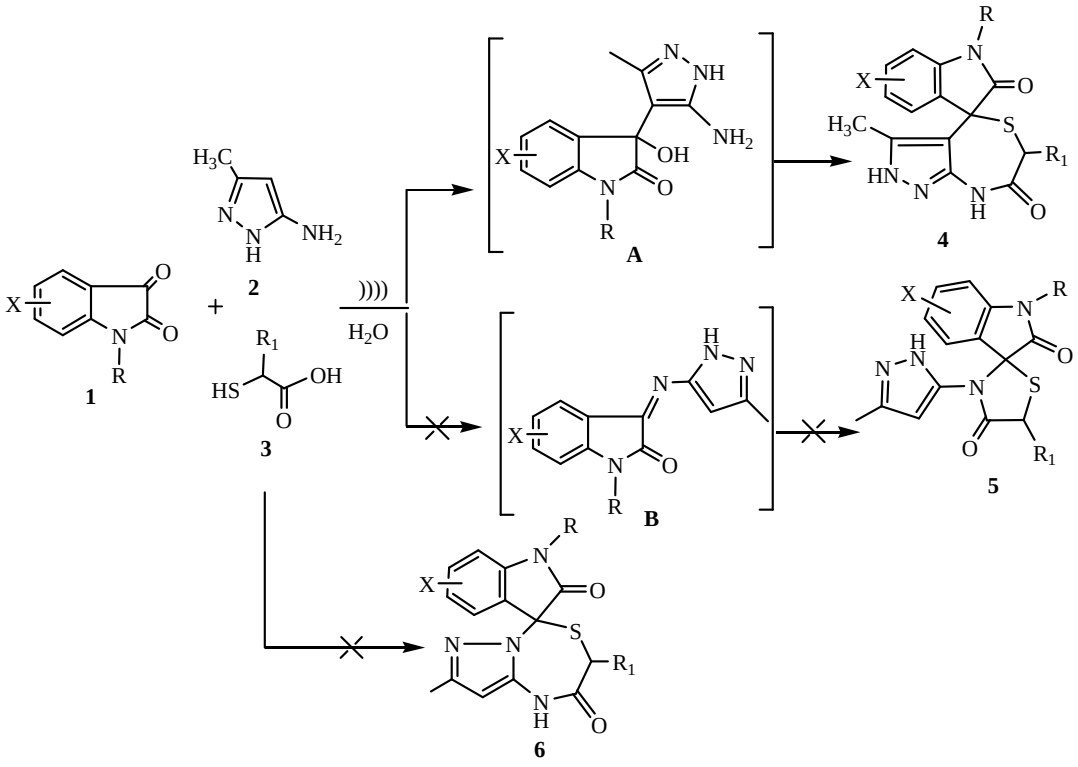
Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
16	 **12c**	3321 (NH), 2989 (NH), 2902, 1712 (CO), 1463, 1429, 770	290 m/z as $[M+H]^+$ for $C_{14}H_{15}N_3O_2S$	1.98 (s, 3H, CH_3), 3.07 (d, *J* = 15.2 Hz, 1H, CH), 3.72 (s, 3H, OCH_3), 4.36 (d, *J* = 15.2 Hz, 1H, CH), 5.24 (s, 1H, CH), 6.86-7.35 (m, 4H, Ar-H), 10.01 (s, 1H, NH), 12.69 (s, 1H, NH)	10.5 (CH_3), 33.5 (CH_2), 42.8 (CH), 55.0 (OCH_3), 99.8, 104.8, 113.6, 128.9, 132.4, 138.6, 151.6, 158.1, 171.2 (C=O)
17	 **12d**	3281 (NH), 2976 (NH), 2925, 1703 (CO), 1480, 1414, 714	304 m/z as $[M+H]^+$ for $C_{15}H_{17}N_3O_2S$	1.18 (d, *J* = 7.2 Hz, 3H, CH_3), 1.45 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 4.23 (q, *J* = 7.2 Hz, 1H, CH), 5.29 (s, 1H, CH), 6.99-7.25 (m, 4H, Ar-H), 9.95 (s, 1H, NH), 12.66 (s, 1H, NH)	10.9 (CH_3), 18.9 (CH_3), 30.4 (CH), 43.2 (CH), 52.8 (OCH_3), 110.7, 119.2, 127.3, 138.5, 144.7, 145.6, 148.8, 154.9, 172.4 (C=O)
18	 **12e**	3271 (NH), 3015 (NH), 2934, 1701 (CO), 1465, 1397, 637	278 m/z as $[M+H]^+$ for $C_{13}H_{12}FN_3OS$	1.34 (s, 3H, CH_3), 3.08 (d, *J* = 14.8 Hz, 1H, CH), 4.22 (d, *J* = 14.8 Hz, 1H, CH), 5.25 (s, 1H, CH), 6.83-7.09 (m, 4H, Ar-H), 10.18 (s, 1H, NH), 12.41 (s, 1H, NH)	10.4 (CH_3), 30.7 (CH_2), 41.8 (CH), 108.8, 126.3, 128.5, 130.6, 142.7, 143.6, 148.4, 154.7, 172.4 (C=O)

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
19	 **12f**	3304 (NH), 2988 (NH), 2941, 1708 (CO), 1444, 1409, 817	292 m/z as $[M+H]^+$ for $C_{14}H_{14}FN_3OS$	1.16 (d, *J* = 7.2 Hz, 3H, CH_3,), 1.47 (s, 3H, CH_3), 4.28 (q, *J* = 7.6 Hz, 1H, CH), 5.31 (s, 1H, CH), 6.67-7.24 (m, 4H, Ar-H), 10.12 (s, 1H, NH), 12.12 (s, 1H, NH)	10.1 (CH_3), 19.4 (CH_3), 33.5 (CH), 44.1 (CH), 52.9, 111.7, 119.8, 126.8, 139.7, 145.8, 148.9, 150.9, 153.5, 172.9 (C=O)
20	 **12g**	3295 (NH), 2973 (NH), 2919, 1684 (CO), 1538, 815, 764	305 m/z as $[M+H]^+$ for $C_{13}H_{12}N_4O_3S$	1.32 (s, 3H, CH_3), 3.15 (d, *J* = 15.2 Hz, 1H, CH), 4.22 (d, *J* = 14.8 Hz, 1H, CH), 5.29 (s, 1H, CH), 6.29-7.05 (m, 4H, Ar-H), 10.21 (s, 1H, NH), 12.73 (s, 1H, NH)	10.9 (CH_3), 31.4 (CH_2), 43.9 (CH), 111.8, 122.3, 129.5, 131.6, 143.7, 145.6, 149.4, 156.7, 173.8 (C=O)
21	 **12h**	3328 (NH), 3018 (NH), 2951, 1693 (CO), 1427, 1409, 757	294 m/z as $[M+H]^+$ for $C_{13}H_{12}ClN_3OS$	1.58 (s, 3H, CH_3), 3.04 (d, *J* = 14.8 Hz, 1H, CH), 4.04 (d, *J* = 15.2 Hz, 1H, CH), 5.19 (s, 1H, CH), 6.88-7.19 (m, 4H, Ar-H), 10.82 (s, 1H, NH), 12.44 (s, 1H, NH)	10.4 (CH_3), 30.7 (CH_2), 42.5 (CH), 108.8, 126.3, 128.5, 130.6, 142.7, 143.6, 148.4, 154.7, 172.4 (C=O)

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
22	 **12i**	3301 (NH), 2968 (NH), 2911, 1709 (CO), 1516, 1458, 639	337 m/z as $[M+H]^+$ for $C_{13}H_{12}BrN_3OS$	1.79 (s, 3H, CH_3), 3.11 (d, *J* = 15.2 Hz, 1H, CH), 4.29 (d, *J* = 15.2 Hz, 1H, CH), 5.22 (s, 1H, CH), 6.93-7.27 (m, 4H, Ar-H), 9.78 (s, 1H, NH), 12.19 (s, 1H, NH)	11.2 (CH_3), 34.5 (CH_2), 40.3 (CH), 105.0, 122.5, 124.6, 129.2, 139.5, 141.6, 147.3, 152.8, 173.8 (C=O)

2.4 RESULTS AND DISCUSSION

The present reaction is chemo as well as regioselective and three products spiro[indoline-3,4'-pyrazolo[3,4-*e*][1,4]thiazepine]dione **(4)**, spiro[indole[3,2'] thiazolidinone]-dione **(5)** and spiro[indoline-3,5'-pyrazolo[1,5-*c*][1,3,5] thiadiazepine]-dione **(6)** can be formed because in addition to -NH_2 group, two nucleophilic centers are present in 5-aminopyrazole **(3)** (**Scheme 2.6**). The formation of pentacyclic ring system **5** occurs if the reaction go through the intermediate 3-indolylimine **B** as observed in our previous study. While the formation of heptacyclic ring system **4** involves the first formation of Baylis-Hillman type adduct **A**. The X-ray crystal structure of the representative compound **4b** confirmed the chemoselective formation of **4**. Further, the formation of regioisomer **6** is ruled out due to the higher nucleophilicity of C-4 over N-1 in the 5-aminopyrazole (**2**), which is also proved by other workers.[46]



Scheme 2.6: Selective synthesis of spiro[indole-3,4'-pyrazolo[3,4-*e*][1,4]thiazepine**]** derivatives

A mixture of isatin **1**, 5-amino-3-methylpyrazole **2**, and α-mercaptoacetic acid **3** was irradiated under sonication at room temperature for 20 minutes in water to furnish a white solid, characterised as 3'-methyl-6',8'-dihydrospiro[indoline-3,4'-pyrazolo[3,4-*e*][1,4]thiazepine]dione **4a**. Initially, a series of experiments were

carried out for the one-pot, three-component reaction to optimize the reaction conditions (**Table 2.7**). Reaction mixture was irradiated under sonication in presence of various catalysts such as acids *p*-TSA, AcOH, $InCl_3$ and surfactant CTABr. No satisfactory results were obtained under a variety of catalytic conditions and tedious work-up was also required for separation.

In order to evaluate the effect of solvent, various solvents such as CH_2Cl_2, DMF and ethanol were used for the model reaction under sonic condition. As shown in **Table 2.7**, low yield of the target product **4a** was obtained in hexane and DCM. Reaction in DMF and ethanol resulted in 65 % and 69 % yields, respectively. From the economical and environmental point of view, H_2O was chosen as the reaction medium for all further reactions. As I_{max} (maximum cavitation intensity) and T_{Imax} (the temperature at which Imax is reached) of any solvent has a profound effect in sonochemical reactivity (for water I_{max} is 100), I_{max} of water is responsible for the increase in the reaction rate compared to the other solvents for which I_{max} is less.[47]

Table 2.7: Optimization of conditions for synthesis of **4a**[a]

Entry	Solvent	Condition	Catalyst	Time	Yield[b] (%)
1	H_2O	US	AcOH	30 min	72
2	H_2O	US	*p*-TSA	30 min	75
3	H_2O	US	$InCl_3$	30 min	74
4	H_2O	US	CTABr	30 min	78
5	H_2O	US	-	20 min	92
6	Hexane	US	-	60 min	57
7	DCM	US	-	60 min	48
8	DMF	US	-	60 min	65
9	Ethanol	US	-	60 min	69
10	H_2O	Stirring	-	20 min	No reaction
11	H_2O	Stirring	-	1 h	Mixture of products
12	H_2O	Reflux	-	20 min	Traces

[a] Reaction conditions: indole-2,3-dione **1a** (2 mmol), 5-amino-3-methylpyrazole **2** (2 mmol) α-mercaptoacetic acid **3a** (2 mmol) in 10 ml solvent.

[b] isolated yield.

In order to verify the effect of ultrasound irradiation on this reaction, the model reaction was also carried out in the absence of ultrasound under conventional manners in both stirring and refluxing conditions using water as a solvent (entries 10-12). As shown in **Table 2.7**, under high speed stirring at magnetic stirrer, no reaction was observed at same time but a mixture of products was obtained after prolonged reaction time. While under at reflux, traces of compound was detected by TLC. Thus, ultrasonic irradiation was found to have beneficial effect on the synthesis of pyrazolo[3,4-*e*][1,4]thiazepinone derivatives which was superior to the traditional method with respect to yields, reaction time, and particularly while considering the basic green chemistry concept.

Encouraged by this success, the optimized reaction conditions were extended to other substituted isatins and thioacid, Table **2.2** showed that all the substrates reacted smoothly and efficiently affording **4a-i** in good to excellent yields. A single product was isolated in all the cases and no trace of the other isomers was formed even after prolonged reaction time.

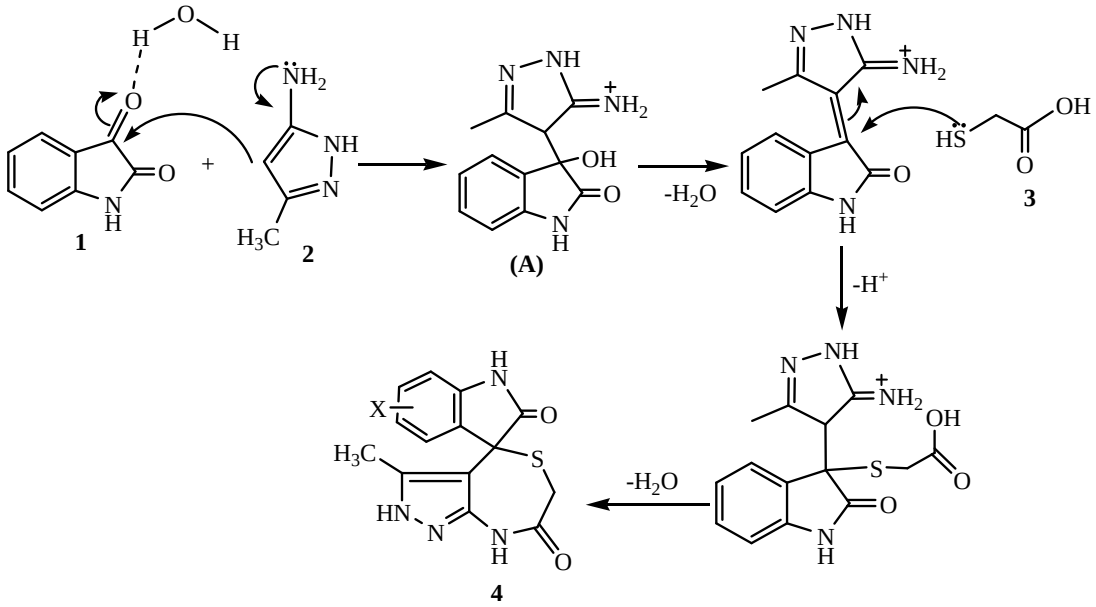
To further explore the potential of this protocol for synthesis of other pyrazolo[3,4-*e*][1,4]thiazepine compounds and for assessing the generality of optimized reaction conditions, we have also investigated the present multicomponent reaction using acenaphthylene-1,2-dione **7**, piperidinone **9** and substituted aromatic aldehydes **11** to obtain spiro[acenaphthylene-1,4'-pyrazolo[3,4-*e*][1,4]thiazepine] dione derivatives **8**, spiro[piperidinone-1,4'-pyrazolo[3,4-*e*] [1,4]thiazepine]dione **10** and substituted aryl-pyrazolo[3,4-*e*][1,4]thiazepine **12** respectively (Table **2.3**, **2.4** and **2.5**).

2.4.1 Mechanism

Ultrasound waves passes through liquid medium to give rise to sinusoidal variation in the bulk pressure, which induces cavitaion which creates, enlarges, and implodes gaseous and vaporous cavities in an irradiated liquid, involving enhancement of mass transfer to start a chemical reactions.[48] Possible nuclei for occurrence of cavitation events are gas pockets trapped in the walls and crevices of the solid and reactor wall, or they could be small bubbles already present in the medium. Thus, ultrasound irradiation activates the reaction mixture by inducing high local temperature and pressure generated inside the cavitation bubble and its

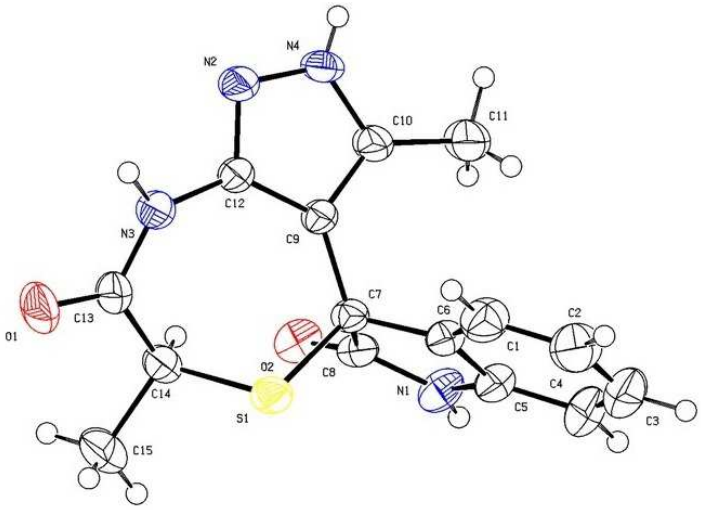
interfaces when it collapses which accelerates the reaction rate and shorten the reaction time.

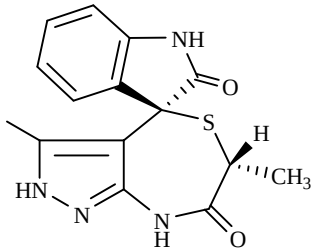
The plausible role of water in promoting the reaction can be rationalized by the hydrogen bond formation between water and the 3-carbonyl of isatin which increases the electrophilic character of the 3-carbon of the isatin. The hydrogen atom of water works as a Brønsted acid and activate the formation of Baylis-Hillman type adduct **(A)**. Also, the oxygen of water may coordinate with the hydrogen of thiol, and as a result, functions as a Brønsted base. This dual activation mode[49] by water may be important in promoting Michael addition reaction of intermediate **(A)** with thioacid more efficiently under neutral conditions followed by dehydration forming the final product **4.** (**Scheme 2.7**)



Scheme 2.7: Possible mechanism for the synthesis of pyrazolo[3,4-*e*][1,4] thiazepinone derivatives

In addition to chemo and regioselectivity, interesting results were obtained with respect to diastereoselectivity. The present multicomponent reaction led to the formation of compounds with two asymmetric centers (R = CH_3). Hence, the final products **4b, 4d** and **4i** may be obtained either as diastereomeric pairs with (3R,6'S)- or (3R,6'R)- relative configuration of these centers or as their mixture. The ^{1}H NMR spectrum of compound **4b (Entry 2, Table 2.6)** showed the formation of only one diastereomer and the relative configuration of diastereomer (3R,6'S) was unambiguously confirmed by X-ray crystallography (**Figure 2.1**).[50]

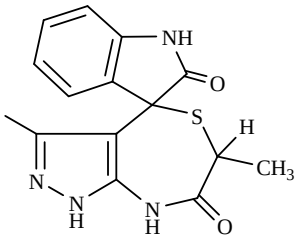




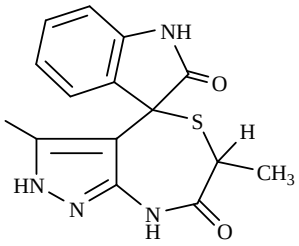
(3R,6'S)

Figure 2.1: The crystal structure of compound **4b**

In addition, the X-ray structure also indicates that among the two tautomeric forms (I) and (II) in **Figure 2.2**, only form II was generated.



(I)



(II)

Figure 2.2: Two tautomeric forms (I) and (II) of **4b**

2.5 CONCLUSION

In summary, we describe herein an efficient protocol for the highly selective multi-component reaction in water under ultrasound irradiation. It is noteworthy that the present reaction proceeded without the addition of any acid or base catalyst. Compared with the conventional methods, the procedure offers several advantages including excellent yields, shorter reaction time, clean reactions, and minimal environmental effects.

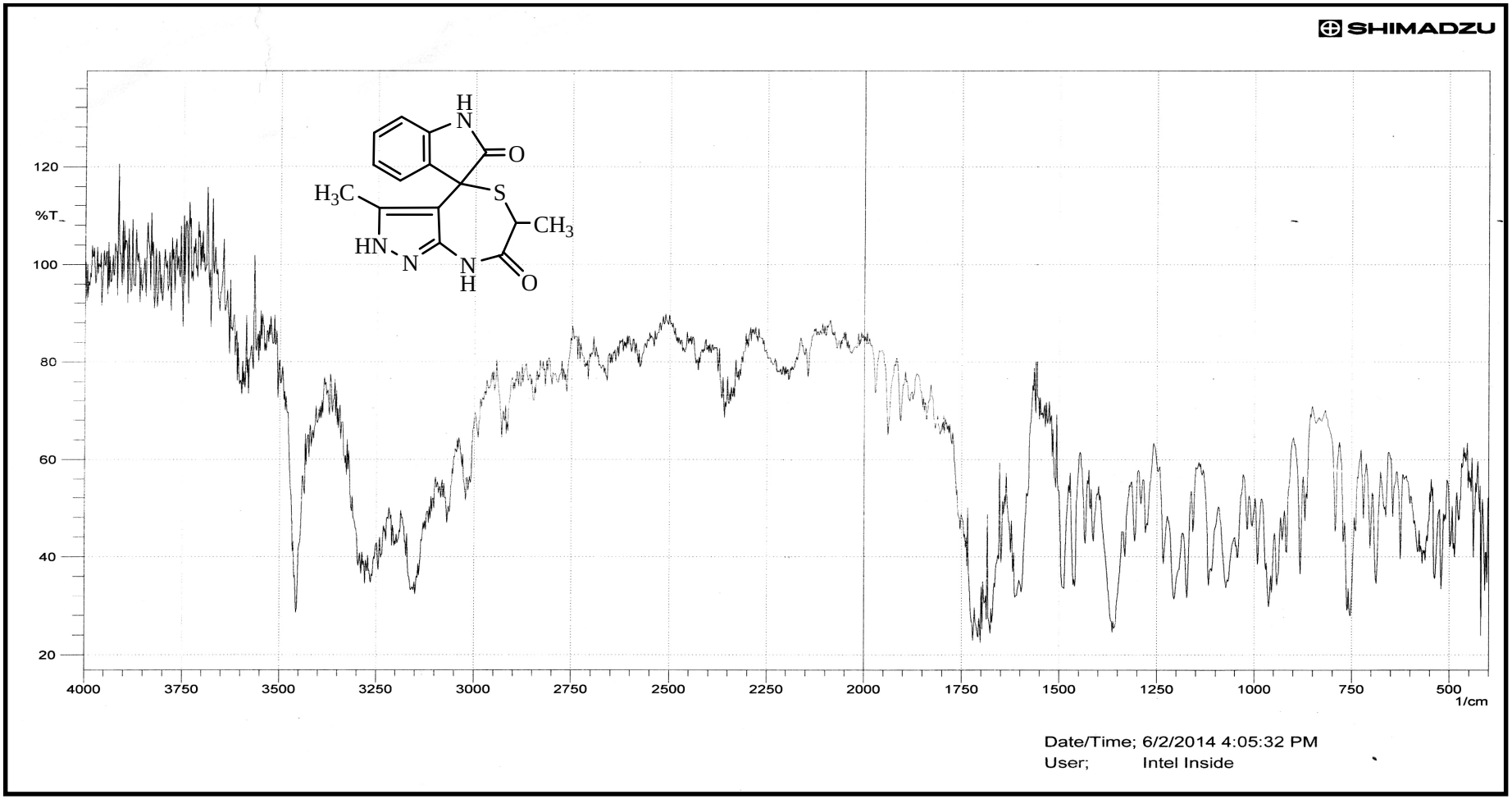


Figure 2.3: FT-IR spectrum of **3',6'-dimethyl-6',8'-dihydrospiro[indoline-3,4'-pyrazolo[3,4-*e*][1,4]thiazepine]-2, 7'(2'*H*)-dione (4b)**

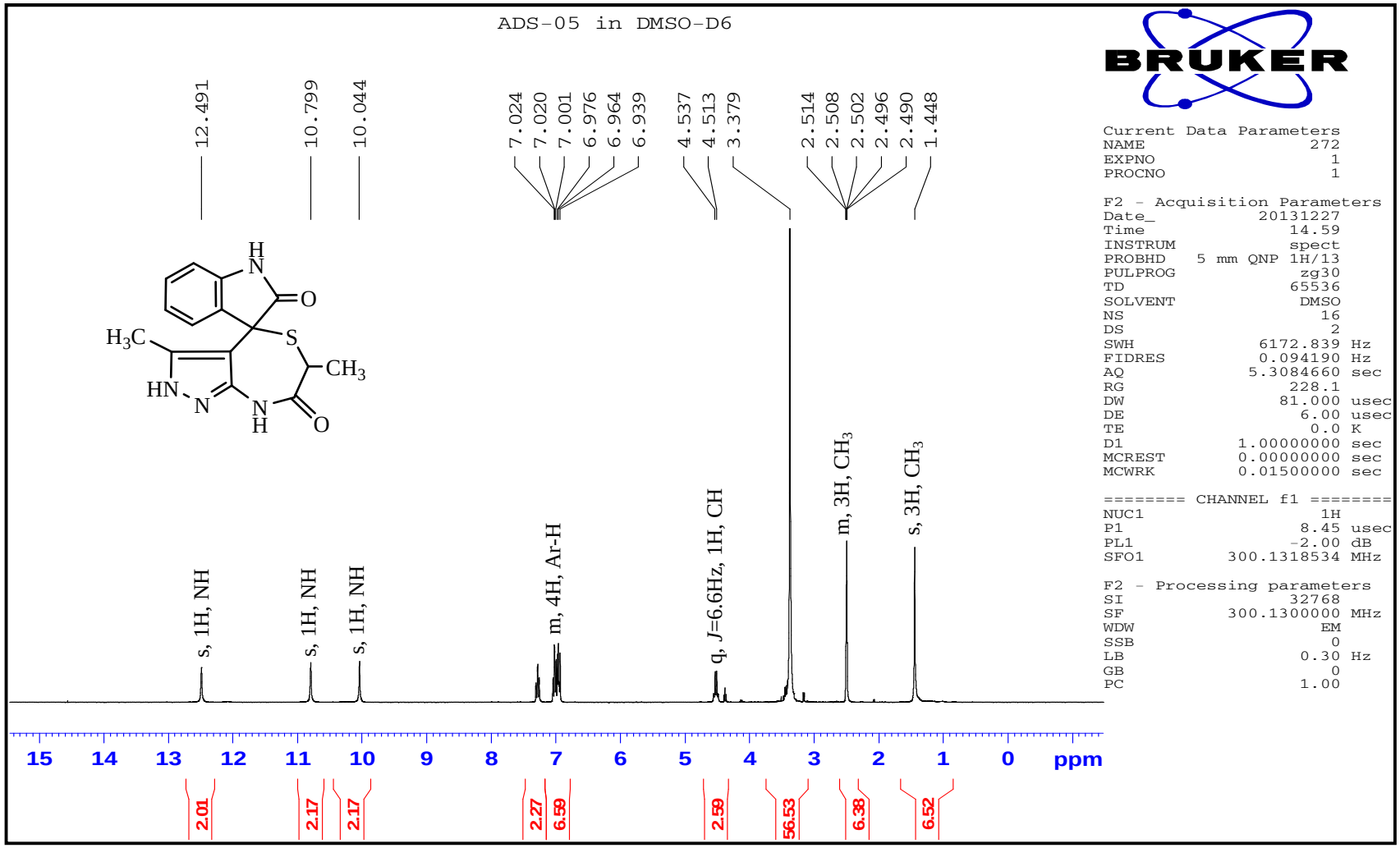


Figure 2.4: ^{1}H NMR spectrum of **3',6'-dimethyl-6',8'-dihydrospiro[indoline-3,4'-pyrazolo[3,4-*e*][1,4] thiazepine]-2,7'(2'*H*)-dione (4b)**

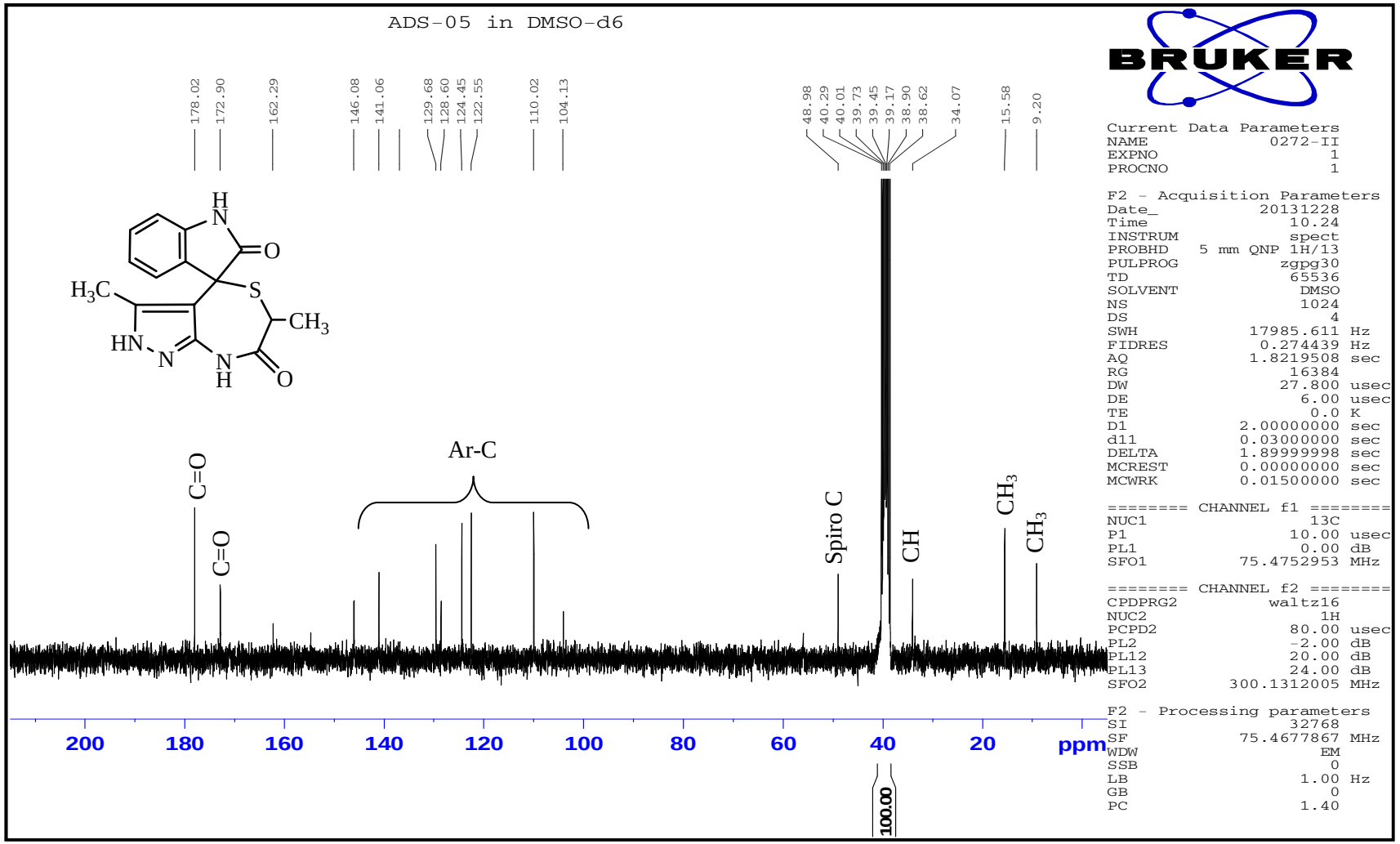


Figure 2.5: ^{13}C NMR spectrum of **3',6'-dimethyl-6',8'-dihydrospiro[indoline-3,4'-pyrazolo[3,4-*e*][1,4]thiazepine] -2,7'(2'*H*)-dione (4b)**

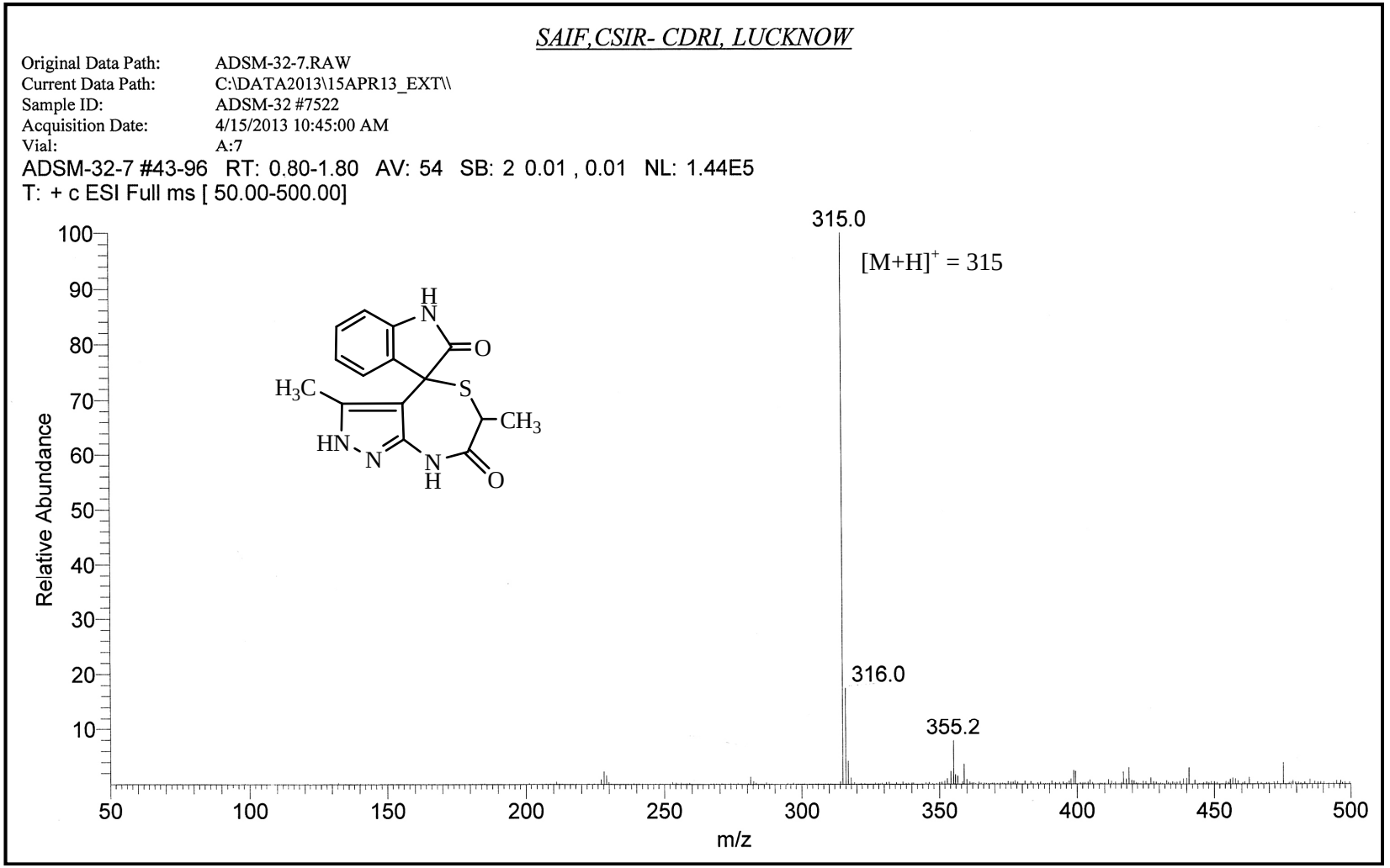


Figure 2.6: Mass spectrum of **3',6'-dimethyl-6',8'-dihydrospiro[indoline-3,4'-pyrazolo[3,4-*e*][1,4]thiazepine]-2,7'(2'*H*)-dione (4b)

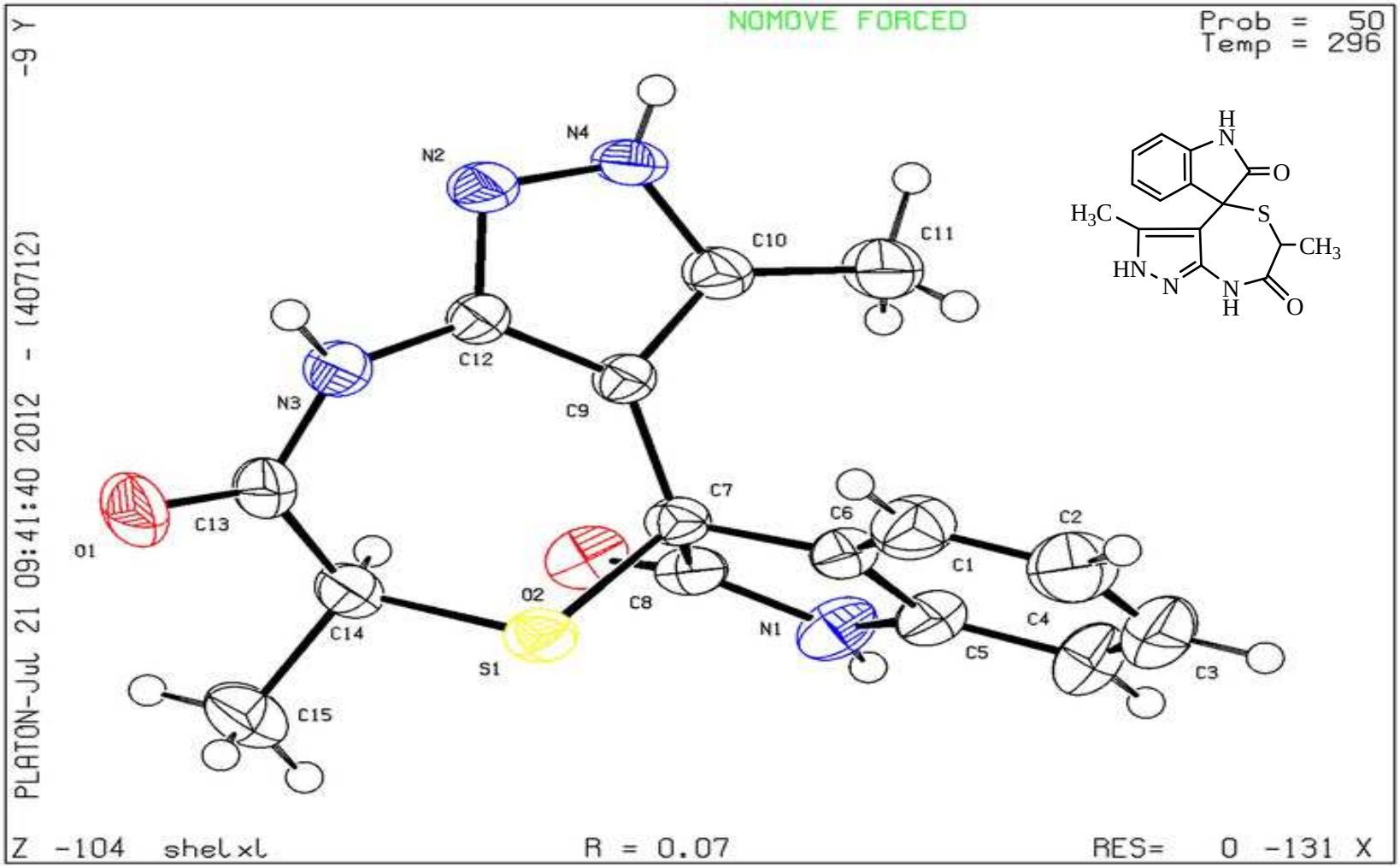


Figure 2.7: ORTEP diagram of **3',6'-dimethyl-6',8'-dihydrospiro[indoline-3,4'-pyrazolo[3,4-*e*][1,4]thiazepine]-2,7'(2'*H*)-dione (4b)**

Crystal data and structure refinement for 4b

Identification code	shelxl	
Empirical formula	C_{15} H_{14} N_4 O_2 S	
Formula weight	314.37	
Temperature	296(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21/c	
Unit cell dimensions	a = 11.9823(6) Å	α = 90°
	b = 8.3551(4) Å	β = 104.948(2)°
	c = 17.5510(9) Å	γ = 90°
Volume	1697.63(15) $Å^3$	
Z	4	
Density (calculated)	1.230 Mg/m^3	
Absorption coefficient	0.202 mm^{-1}	
F(000)	656	
Crystal size	0.50 x 0.40 x 0.30 mm	
Theta range for data collection	1.76 to 25.00°	
Index ranges	-14<=h<=14, -9<=k<=9, -16<=l<=20	
Reflections collected	24860	
Independent reflections	2982 [R(int) = 0.0211]	
Completeness to theta = 25.00	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9419 and 0.9058	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2982 / 0 / 206	
Goodness-of-fit on F^2	1.153	
Final R indices [I>2sigma(I)]	R1 = 0.0735, wR2 = 0.2496	
R indices (all data)	R1 = 0.0769, wR2 = 0.2592	
Extinction coefficient	0.042(8)	
Largest diff. peak and hole	2.037 and -0.269 $e.Å^{-3}$	

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50. Crystallographic data for **4b** have been deposited at the Cambridge Crystallographic Data Centre with the deposition number CCDC **912381**. Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/ deposit (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK; Fax: +44 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk).

CHAPTER 3

Chemoselective Synthesis and Characterization of Indolo[2,3-b] quinoxaline Derivatives catalyzed by Copper Doped Cadmium Sulphide Nanoparticles under Microwave Irradiation

3.1 INTRODUCTION

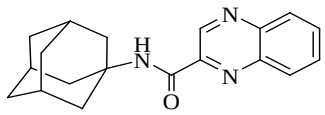
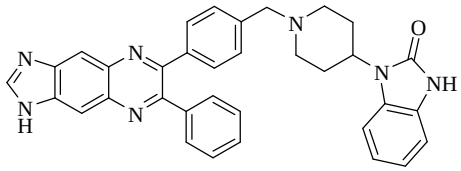
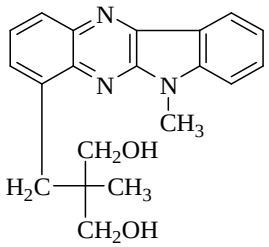
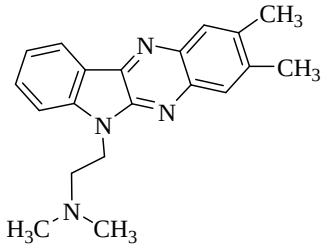
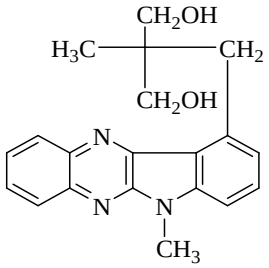
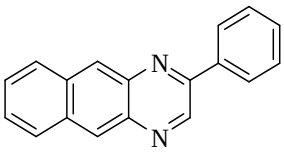
The study of heterocyclic compounds is of great interest from both a theoretical as well as a practical point of view. Heterocyclic compounds occur in a variety of naturally occurring compounds.[1a] A large number of heterocyclic compounds are essential in our day-to-day life.

The quinoxaline ring system is probably one of the common heterocyclic scaffolds used to build pharmacologically and biologically active molecules.[1b-d] In addition to their medicinal uses, quinoxaline derivatives have found various technical applications as semiconductors,[2] dyes,[3] and as ligands in coordination chemistry.[4]

Further, indole substituted diverse heterocyclic rings have been found in a fascinating array of bioactive natural products and pharmaceutical compounds.[5] An indolo[2,3-*b*]quinoxaline nucleus which accumulates quinoxaline as well as indole moieties integrates properties of both, and the synergism of both the heterocyclic moieties in a single nucleus results in the formation of molecules with the worthwhile biological activities.[6]

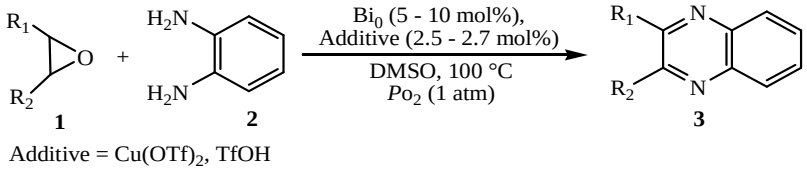
Derivatives of indolo[2,3-*b*]quinoxaline exhibit promising biological activities such as anticancer, anticonvulsant, antibacterial and antiviral activity.[7] Further, 6*H*-indolo[2,3-*b*]quinoxaline compounds, NCA0424 and NCA0465 represent an important series of DNA intercalating agents endowed with antiviral and cytotoxic activities[8a] and Allosteric dual Akt1 and Akt2 inhibitor afforded modest activity in cell-based IPKA Akt assays.[8b] Compound B-220 (2,3-dimethyl-6-(2-dimethylaminoethyl)-6*H*-indolo[2,3-*b*]quinoxaline) has shown remarkable activity against herpes virus,[9a,b] tricyclic benzo[*g*]quinoxaline acts as platelet derived growth factor receptor kinase inhibitor[9c] and NPS-2390 has been identified as an active mGluR1 fragment.[9d] (**Table 3.1**)

Table 3.1: Medicinally relevant quinoxaline derivatives

S. No.	Name of the compound	Structure of the compound	Use of the compound [8,9]
1	NPS-2390		NPS-2390 has been identified as an active mGluR1 fragment
2	Allosteric dual Akt1 and Akt2 inhibitor		Allosteric dual Akt1 and Akt2 inhibitor afforded modest activity in cell-based IPKA Akt assays
3	NCA0424		NCA0424 represent an important series of DNA intercalating agents endowed with antiviral and cytotoxic activities
4	B 220		Compound B-220 has shown remarkable activity against herpes virus
5	NCA0465		NCA0465 is a DNA intercalating agent with cytotoxic activities
6	Tricyclic benzo[*g*] quinoxaline		Tricyclic benzo[*g*]quinoxaline acts as platelet derived growth factor receptor kinase inhibitor

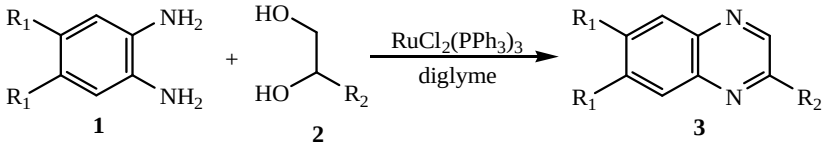
As a result of the above significant properties, extensive literature survey has been reported by the various workers to synthesize quinoxaline derivatives through a variety of approaches. Some of the conventional methods to synthesize quinoxaline moiety are given as under:

Antoniotti *et al.*[10] reported the Bi-catalyzed one-pot condensation of epoxides **1** and diamino derivatives **2** to access di-substituted quinoxalines **3**. The reaction proceeded in DMSO under molecular oxygen in the presence of copper triflate or triflic acid as additives (Scheme **1**).



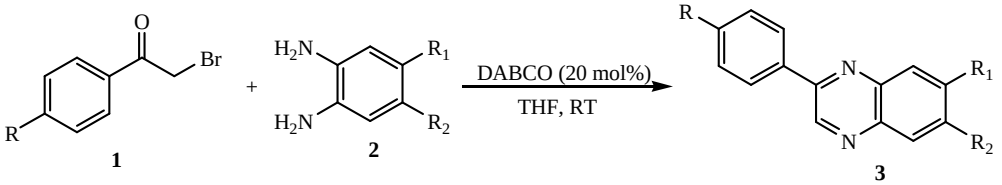
Scheme 1

An efficient synthesis of quinoxalines **3** was accomplished by Cho *et al.*[11] when *o*-phenylene diamines **1** were made to react with an array of 1,2-diols **2** in diglyme catalysed by ruthenium salt (Scheme **2**).



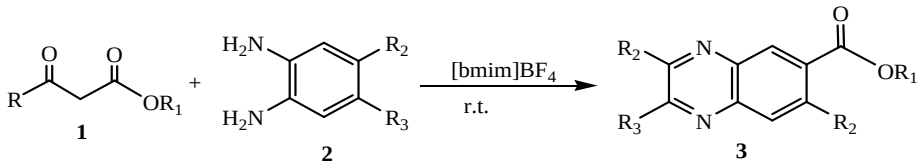
Scheme 2

Meshram *et al.*[12] utilized DABCO as a catalyst to afford functionalized quinoxalines **3** by the reaction of phenacyl bromides **1** and *o*-phenylene diamines **2** (Scheme **3**).



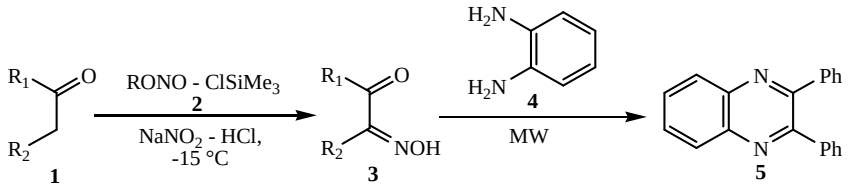
Scheme 3

In an alternate approach, Meshram *et al.*[13] also synthesized catalyst-free quinoxaline-2-carboxylate derivatives **3** by the reaction of α-halo-β-ketoesters **1** with 1,2-diamines **2** using ionic liquid as an environmentally benign solvent (Scheme **4**).



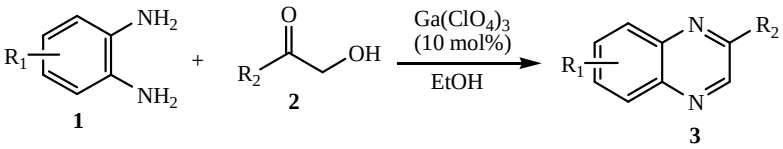
Scheme 4

Besides traditional heating methods discussed, microwave dielectric heating has also been employed to shorten the reaction times significantly by Nagendrappa *et al.*[14] for the synthesis of quinoxaline derivatives **5** by the two-step reactions of ketones **1** with substituted nitroso trimethyl chlorosilane **2** and substituted iminoketone **3** with 1,2-diamine **4** (Scheme **5**).



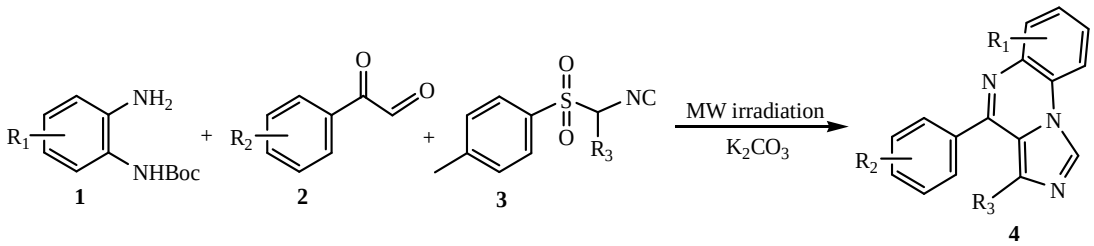
Scheme 5

Zhang *et al.*[15] demonstrated a new approach for the synthesis of 2-substituted quinoxalines **3** by $Ga(ClO_4)_3$-catalyzed cycloaddition of *o*-phenylenediamines **1** and α-hydroxyketones **2** (Scheme **6**).



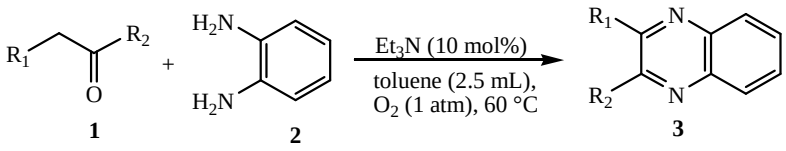
Scheme 6

Hulme *et al.*[16] described a facile one-pot multicomponent method for the construction of imidazo-[1,5-*a*]quinoxaline derivatives **4** in which two aromatic rings and four new chemical bonds are formed from various building blocks such as substituted carbamates **1**, substituted 2-oxoacetaldehydes **2** and isocyanosulfonylbenzenes **3** (Scheme **7**).



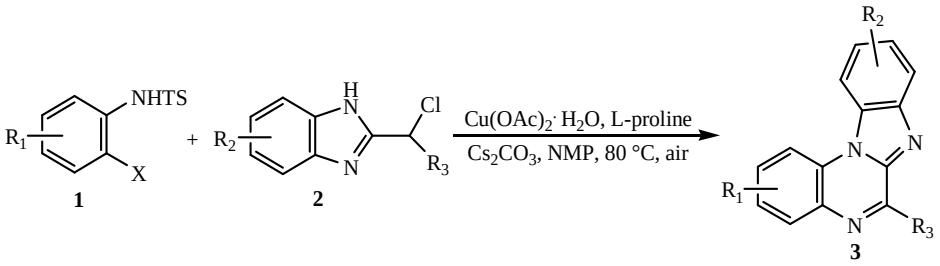
Scheme 7

Et_3N-catalyzed oxidative dehydrogenative coupling of α-unsubstituted carbonyl compounds **1** with aryl diamines **2** was invoked to give quinoxaline derivatives **3** using molecular oxygen as oxidant had been described by Zhang *et al.*[17] (Scheme **8**)



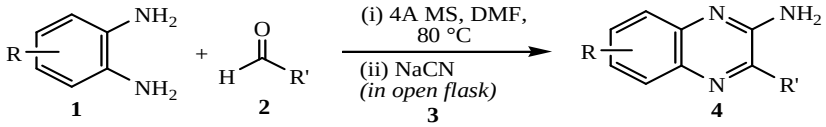
Scheme 8

Ma *et al.*[18] introduced a copper-catalyzed process for the construction of benzo[4,5]imidazo[1,2-*a*]quinoxaline derivatives **3** from N-tosyl-2-haloanilines **1** and 2-(chloromethyl)-1*H*-benzo[*d*]imidazoles **2** under mild conditions (Scheme **9**).



Scheme 9

An expedient and atom-economical method had been developed by Cheon *et al.*[19] for the assembly of 2-aminoquinoxalines **4** from readily available *o*-phenylenediamines **1**, aldehydes **2** and NaCN **3** under aerobic conditions (Scheme **10**).



Scheme 10

A general procedure for the synthesis of indolo[2,3-*b*]quinoxalines involves the condensation reaction of isatin analogs with 1,2-diamines.[20] A literature survey suggested that there are very few reports where quinoxaline moiety is assimilated with indole moiety.[20,21] The condensation reaction of 1,2-aryldiamines with 1,2-diketones is a primary route to construct the quinoxaline ring.

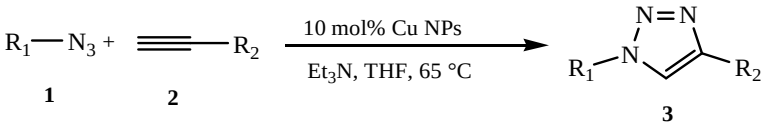
The reported procedures for this protocol involves a wide spectrum of reagents including clayzic,[22a] silicagel,[22b] Zr(IV) modified silica gel,[22c] alumina,[22d] DABCO,[22e] $Sm(OTf)_2$,[22f] glycerol,[22g] $CeCl_3.7H_2O$ in glycerine,[22h] $FeCl_3$ with morpholine,[22i] triethylamine/O_2,[22j] $Ga(ClO_4)_3$,[22k] and graphite.[22l]

However, the reported methods suffered from one or more of the following disadvantages like longer reaction times, low yields of the products, harsh reaction conditions, and use of excessive amounts of reagents, tedious workup procedures, and co-occurrence of several side reactions with less selectivity of the process. In addition, some of the catalysts and reagents are expensive, toxic, and air sensitive. Therefore, further development of the catalytic system will require advanced materials that can selectively catalyze chemical reactions with high reactivity and can be recycled through simple separation and regeneration processes.

Development in the field of catalysis is helping the scientists to face the challenges related to the economy of world, sustainability and energy. The societal pressure has been at the origin of the concept of nanoscience, which is an exponentially growing research field in modern science that involves the synthesis and application of nanoparticles of different sizes and shapes.[23] The exciting prospect of nanoscience is showing its potential in any conceivable domain. Every field from medicine and electronics to manufacturing and fashion stand to be benefitted from advances in nanotechnology.[24] Though nano-scale technology is multifaceted in its application, the use of nano-material as catalyst is perhaps the most intriguing. Further, the nano-material catalysed reactions provide the advantages of high atom efficiency, simplified isolation of product, and easy recovery and recyclability of the catalyst.[25]

CdS and Cu doped CdS NPs is an important semiconductor owing to its unique electronic and optical properties and its potential applications in solar energy conversion, nonlinear optical photoelectrochemical cells and heterogeneous photocatalysis.[26] Since the properties of the catalyst surfaces are closely correlated with the catalytic activities, the precise modification of the catalyst surface by introducing another component (dopant) or changing the morphology could facilitate the controlled tuning of the catalytic properties.[27] Cu has been extensively investigated as promoter elements to develop new or improved catalytic system in the past.[28] Some of the reactions using the Cu NPs are discussed below:

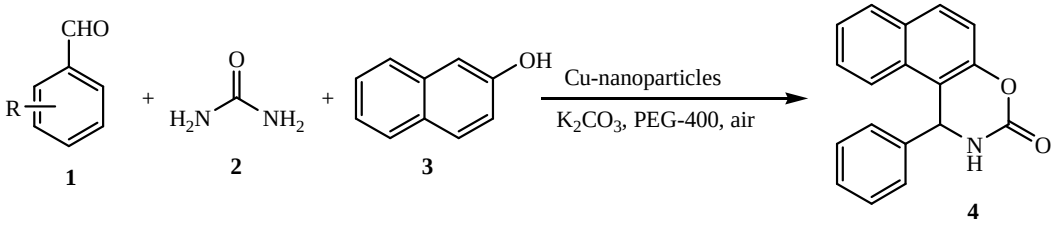
Alonso *et al.*[29] synthesized the copper nanoparticles to produce the substituted triazoles **3** using various azides **1** and alkynes **2** in shorter reaction period (Scheme **11**).



1 **2** **3**

Scheme 11

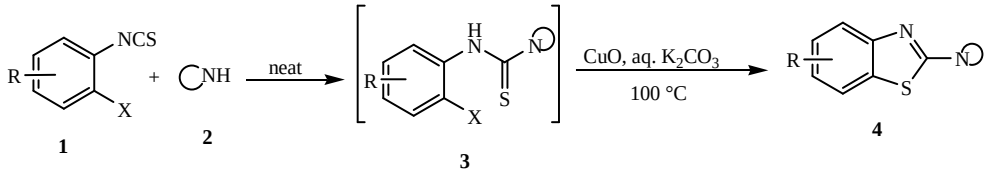
Kumar *et al.*[30] developed an efficient and green methodology for the synthesis of naphthalene condensed oxazinone derivatives **4** from aldehydes **1**, urea **2** and β-naphthol **3** in presence of copper nanoparticles as catalyst (Scheme **12**).



1 **2** **3** **4**

Scheme 12

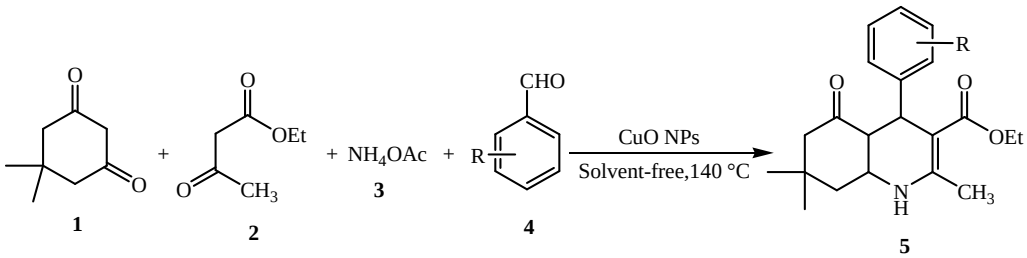
Patel *et al.*[31] studied a novel and efficient method for the synthesis of 2-aminobenzothiazole derivatives **4** from the *in situ* generated 2-halothioureas **3** by the reaction of substituted 2-haloisothiocyanatobenzenes **1** and a variety of secondary amines **2** in the presence of CuO nanoparticles (Scheme **13**).



1 **2** **3** **4**

Scheme 13

A simple, eco-friendly, green and efficient procedure for the synthesis of polyhydroquinoline derivatives **5** from dimedone **1**, ethyl acetoacetate **2**, ammonium acetate **3** and aldehydes **4** was reported by Ghomi *et al.*[32] This reaction proceeds *via* one pot multicomponent methodology using CuO nanoparticles under solvent-free conditions (Scheme **14**).

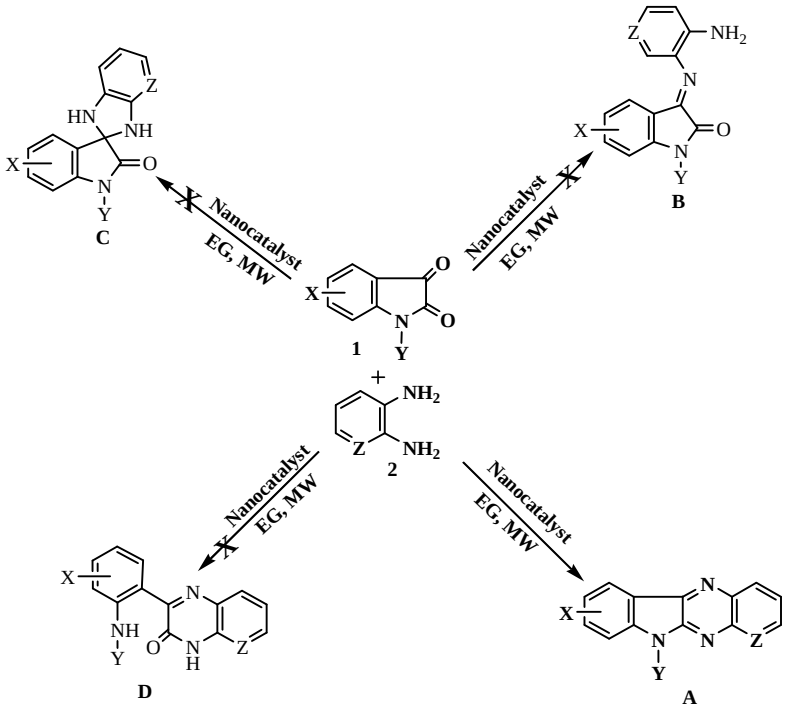


1 **2** **3** **4** **5**

Scheme 14

3.2 EXPERIMENTAL

As part of our efforts on the development of novel green protocols for heterocyclic frameworks and nanoparticle synthesis,[33] herein, we describe the synthesis, characterization and catalytic application of CdS and Cu doped CdS nanoparticles for the chemoselective synthesis of indolo[2,3-*b*]quinoxalines by the reaction of isatins with 1,2-diamine in ethylene glycol under microwave irradiation (**Scheme 3.1**).



Scheme 3.1 Chemoselective synthesis of indolo[2,3-*b*]quinoxaline derivatives

In the reaction of isatins with 1,2-diamines, the formation of the required indolo[2,3-*b*]quinoxalines is accompanied by the occurrence of 3-imino-isatin, spirobenzimidazole, and ring-opened quinoxalinone as side products and sometimes these products have been isolated as the main product. The present protocol gives indolo[2,3-*b*]quinoxalines selectively and exclusively.

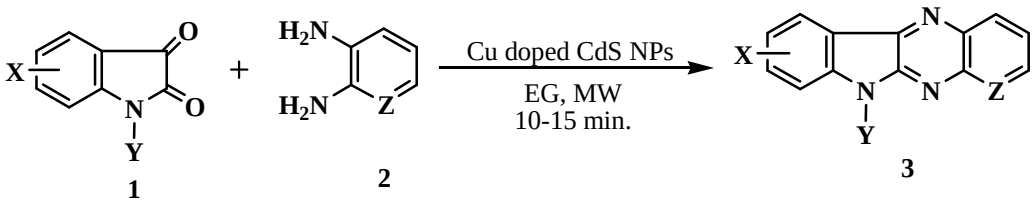
3.2.1 Synthesis of Catalyst

Simple aqueous chemical method[34] has been used to prepare Cu doped CdS nanoparticles. Nanoparticles of CdS were synthesized at room temperature by dropping simultaneously 50 ml of 1 M solution of $CdSO_4$ and 50 ml of 1M solution of Na_2S into 200 ml of distilled water containing 50 ml of 0.1M solution of EDTA, which was vigorously stirred using a magnetic stirrer. The high insolubility of CdS formed out of the chemical reaction caused the formation of a number of new nuclei

while preventing the growth of already existing ones, thus limiting the particle size. The role of EDTA was to stabilize the particles against aggregation which may lead to an increase in the particle size. The doping of copper has been done by adding 2 wt% of metal sulphate ($CuSO_4$) to $CdSO_4$ (at the beginning of the reaction) for the formation of CdS:Cu NPs. The precipitate was separated from the reaction mixture and was dried at room temperature. After sufficient drying, the precipitate was crushed to fine powder with the help of mortar and pestle.

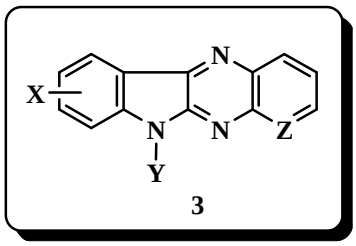
3.2.2 Synthesis of indolo[2,3-*b*]quinoxaline derivatives (3a-j)

Indole-2,3-dione **1** (0.147 g, 1 mmol) and 1,2-diamine **2** (0.108 g, 1 mmol) and 5 mol% of Cu doped CdS NPs in ethylene glycol (10 ml) was introduced in a 50 mL round-bottomed flask. The flask was placed in the microwave cavity and subjected to irradiation for appropriate time at 80 $^{\circ}$C using a maximum power of 300W. When the reaction was complete, ethyl acetate was used to extract the reaction mixture and the organic layer was gradually removed under reduced pressure to obtain the crude product. The pure products were obtained by crystallization from ethanol (Scheme **3.2**). All the synthesized compounds are summarized in **Table 3.2**.



Scheme 3.2: Synthesis of indolo[2,3-*b*]quinoxaline derivatives

Table 3.2: Synthesis of indolo[2,3-*b*]quinoxaline derivatives (**3a-j**) under microwave irradiation



3

Entry	Product	X	Y	Z	Time (min.)	Yield* (%)	Mp (°C)	Color
1	**3a**	H	H	CH	10	95	292-294[20g]	Yellow
2	**3b**	CH_3	H	CH	12	93	296-298[20g]	Yellow
3	**3c**	Cl	H	CH	10	96	287-289	Yellow
4	**3d**	5,7-diCH_3	H	CH	14	91	>300	Yellow
5	**3e**	Br	H	CH	10	96	289-291[20g]	Yellow
6	**3f**	H	CH_2Ph	CH	11	94	142-144[20f]	Light-brown
7	**3g**	H	H	N	10	96	244-246[20c]	Brown
8	**3h**	CH_3	H	N	12	95	>300[20b]	Brown
9	**3i**	Cl	H	N	12	97	294-296	Brown
10	**3j**	5,7-diCH_3	H	N	15	92	>300[20b]	Brown

* = Isolated Yield

3.2.3 Procedure for the recyclability of the catalyst

Reusability of the catalyst was evaluated by carrying out repeated runs of the reaction on the same batch of the catalyst in the case of the model reaction (**Figure 3.1**). After each run, the catalyst could be recycled easily simply by solvent extraction of the product from the reaction mixture and washed with ethanol. It was then dried and used for the next catalytic cycle. The catalytic activity of the catalyst was observed to be recyclable up to five catalytic cycles (**Figure 3.1**).

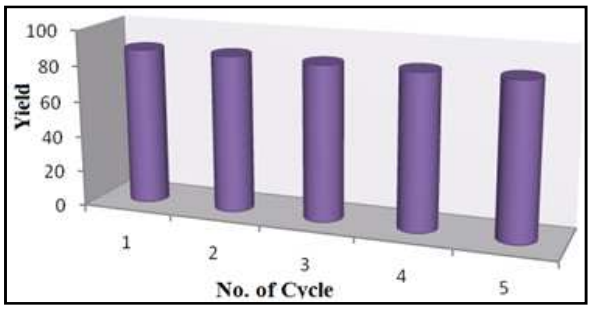


Figure 3.1: Recyclability of NPs

3.3 CHARACTERIZATION OF THE COMPOUNDS SYNTHESIZED

3.3.1 Characterization of the catalyst

X-Ray Diffraction

Figure 3.2 shows the X-ray diffraction patterns of all samples of CdS and Cu doped CdS catalyst prepared by wet chemical method. The samples show intense and broad diffraction peaks at 2θ values of about 26.55, 44.03 and 52.08 correspond to the (111), (220) and (311) planes of cubic phase of CdS. Addition of Cu into the CdS matrix does not cause any significant change in the XRD profile of CdS.[35,36] The average particle size for pure CdS and Cu doped CdS has been found to be 3 nm and 2 nm respectively according to Scherrer's equation.[37]

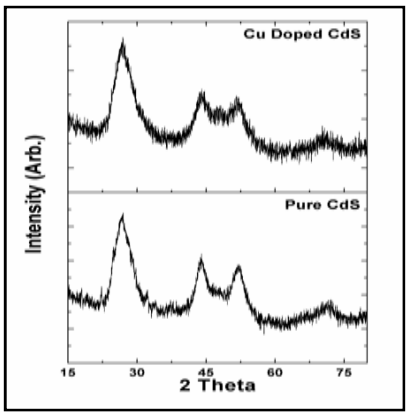


Figure 3.2: XRD patterns of nanoparticles

Transmission Electron Microscopy

Size of the nanoparticles was characterized by Transmission Electron Microscopy (**Figure 3.3**). TEM images confirmed that the particles are in the range of 2-4 nm and they are approximately spherical in shape, whose size distribution is given by the histogram (**Figure 3.4**).

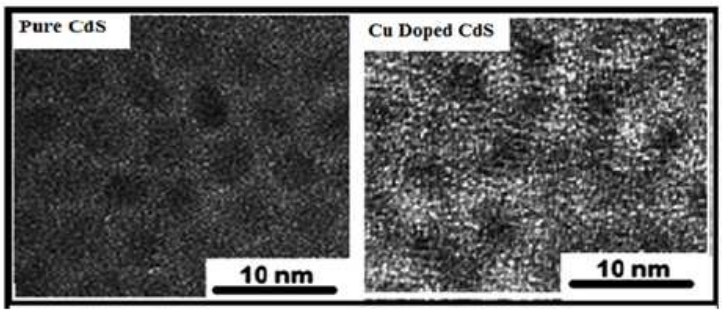


Figure 3.3: TEM image of nanoparticles

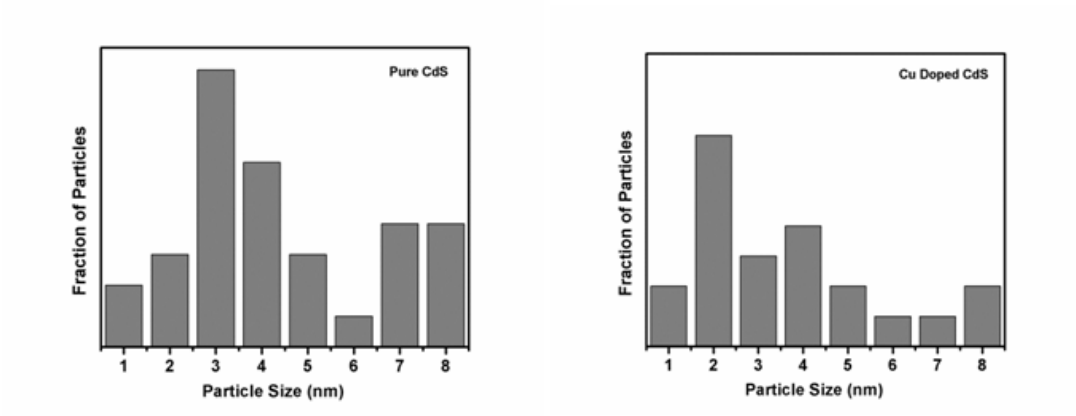


Figure 3.4: Histogram of nanoparticles

Scanning Electron Microscopy

It can be seen that all the nanoparticles have an average size in the 2-4 nm range, which is in consistent with the results obtained through the calculation by Scherrer's equation. This result was further supported by the SEM image (**Figure 3.5**).

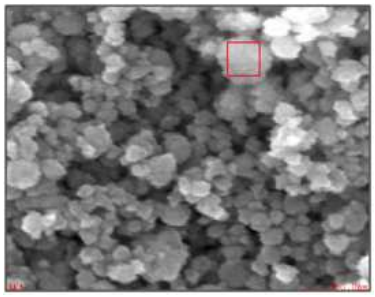


Figure 3.5: SEM image of Cu doped CdS NPs

Energy Dispersive Absorption X-ray

EDAX of both the samples were shown in **Figure 3.6**. The EDAX data substantiate the doping of Cu in CdS matrix. Dopant concentration of the catalyst was also confirmed by ICP-AES analysis.

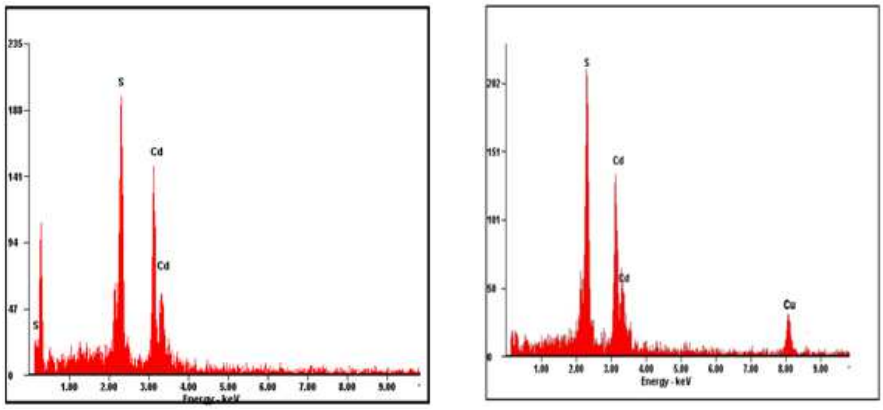
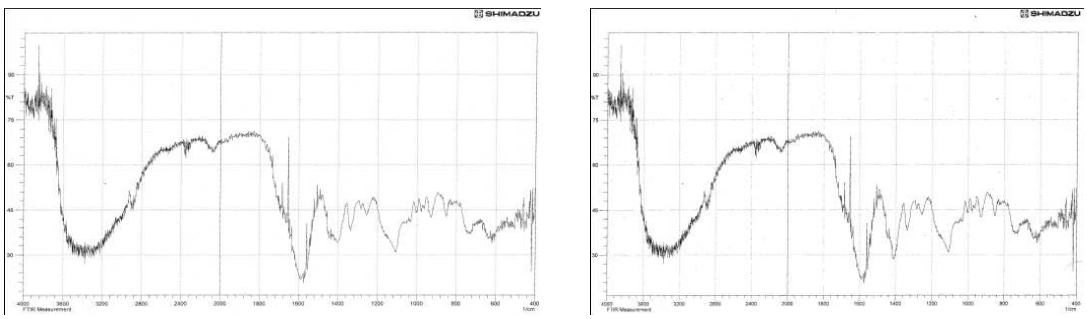


Figure 3.6: EDAX spectrum of the nanoparticles

Surface acidity measurements

The surface Lewis acidity of this material was confirmed through the adsorption of pyridine vapours[38] on the surface of the NPs. FT-IR spectra of pyridine adsorbed on the catalyst are included in spectral information and the band at around 1400 cm^{-1} is attributed to the adsorbed pyridine at the surface Lewis acid site, the intensity of this band increases with Cu doping (**Figure 3.7**). From the results of the acidity measurements, it can be concluded that the incorporation of Cu enhances the surface acidity, which increases the number of exposed metal sites[39,40] and therefore improve the metal-time yields and also increase the reaction rate per exposed metal site, i.e., the turn over frequency.



(a) **(b)**

Figure 3.7: FT-IR spectra of the pyridine-adsorbed samples. (a) CdS NPs (b) Cu doped CdS NPs.

The TEM and XRD analysis of the nanoparticle revealed that the morphology of the recovered NPs remains unaltered during the recycling process, which indicates that the catalyst is stable toward oxidation during the reaction.

3.3.2 Characterization of the compounds synthesized

To confirm the structure of the compounds synthesized and to know the position of various functional groups and presence of heteroatoms in the compounds, they have been well established by their melting points, various spectral studies including IR, Mass, ^{1}H NMR, and ^{13}C NMR analyses.

3.3.2.1 IR and Mass spectral studies

IR spectra of the compounds synthesized were taken on Shimadzu FT-IR 8400S spectrophotometer using KBr pellets. Mass spectra of the representative compound were obtained using JEOL SX-102 and Agilent 1100 LC-MS spectrometer. The structure of the compounds **3a-j** has been confirmed by their melting points and spectroscopic data.

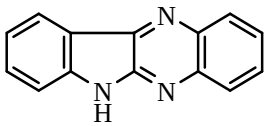
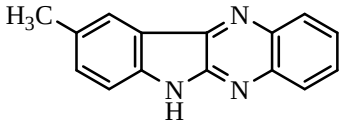
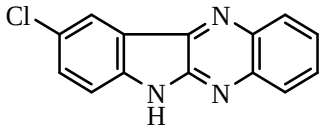
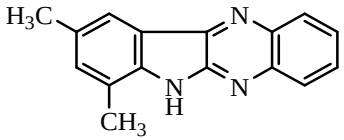
Compound **3a** was isolated as yellow crystalline solid with melting point as 292-294 °C. For instance, the IR spectrum of the product **3a** showed characteristic bands at 1625 cm^{-1} corresponding to the C=N group in the cyclic ring system. In the IR spectrum, the disappearance of two C=O of the oxindole ring along with the appearance of a signal due to C=N further proved the formation of quinoxaline derivatives instead of other products. The mass spectrum of **3a** showed a molecular ion peak at 219.0 $(M+H)^{+}$.

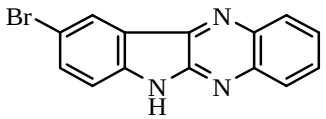
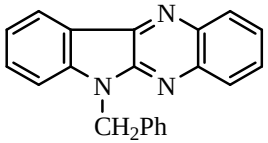
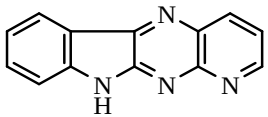
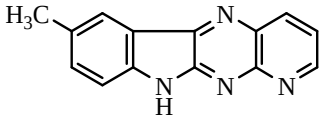
3.3.2.2 ^{1}H NMR and ^{13}C NMR spectral studies

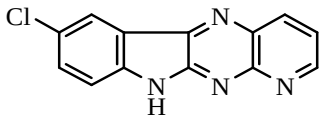
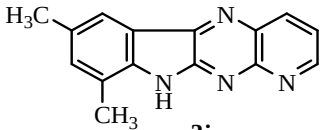
^{1}H NMR and ^{13}C NMR spectra of the compounds synthesized were recorded in DMSO-d_6 and $CDCl_3$ using TMS as an internal standard on a Bruker spectrophotometer at 300, 75 MHz and 400, 100 MHz.

In the ^{1}H NMR spectrum of **3a**, only one hydrogen with chemical shift between 11.94 and 12.21 ppm was observable. This hydrogen can be assigned as the NH of indole moiety of the final structure along with characteristic signals with appropriate chemical shift and coupling constant for the eight protons of the two aromatic moieties. The ^{13}C NMR spectrum of **3a** demonstrated signals at δ 143.9 and 145.7 ppm due to **two** C=N group in the tetra cyclic ring system. The ^{13}C NMR spectrum also confirmed the desired structure of the product as in the spectrum, no signals due to C=O of oxindole ring and spiro carbon was observed.

Table 3.3: IR, Mass, ^{1}H NMR and ^{13}C NMR spectral data of the compounds synthesized **(3a-j)**

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
1	 **3a**	3010, 1625, 1601, 1405, 776	219 m/z as $[M]^+$ for $C_{14}H_9N_3$	7.38 (t, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.71-7.76 (m, 2H), 7.82 (t, *J* = 6.8 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), 8.37 (d, *J* = 8.0 Hz, 1H), 12.06 (s, 1H, NH)	111.9, 118.8, 120.6, 122.1, 125.8, 127.4, 128.6, 128.9, 131.2 (C), 138.5 (C-N), 139.7 (C-N), 140.0 (C-N), 143.9 (C=N), 145.7 (C=N)
2	 **3b**	3095, 1622, 1607, 1418, 765	234 m/z as $[M+H]^+$ for $C_{15}H_{11}N_3$	1.05 (t, *J* = 6.9 Hz, 3H), 7.42-7.53 (m, 2H), 7.67-7.84 (m, 2H), 8.03-8.06 (dd, J_1 = 1.2 Hz, J_2 = 1.2 Hz, 1H), 8.12 (d, *J* = 11.7 Hz, 1H), 8.20-8.24 (dd, J_1 = 1.2 Hz, J_2 = 1.2 Hz, 1H), 11.88 (s, 1H, NH)	20.8 (CH_3), 111.6, 117.2, 119.9, 121.9, 125.7, 127.3, 128.5, 128.9 (C), 129.6 (C), 132.4 (C-N), 138.4 (C-N), 139.7 (C-N), 142.0 (C=N), 146.0 (C=N)
3	 **3c**	3122, 1619, 1604, 1467, 770	253 m/z as $[M]^+$ and 255 as $[M+2]^+$ for $C_{14}H_8ClN_3$	7.61 (d, *J* = 8.4 Hz, 1H), 7.72-7.77 (m, 2H), 7.84 (t, *J* = 6.8 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 8.26 (d, *J* = 8.4 Hz, 1H), 8.36 (s, 1H), 12.02 (s, 1H, NH)	118.3, 119.6, 123.4, 124.6, 126.4, 127.2, 130.2, 130.5 (C), 135.9 (C), 141.8 (C-N), 147.7 (C-N), 149.6 (C-N), 154.3 (C=N), 155.3 (C=N)
4	 **3d**	3187, 1621, 1592, 1498, 762	247 m/z as $[M]^+$ for $C_{16}H_{13}N_3$	2.47 (t, *J* = 11.6 Hz, 3H), 2.55 (s, 3H), 7.35 (s, 1H), 7.71 (t, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 6.8 Hz, 1H), 7.97 (s, 1H), 8.06 (d, *J* = 8.4 Hz, 1H), 8.23 (d, *J* = 8.4 Hz, 1H), 11.94 (s, 1H, NH)	Could not be recorded due to low solubility

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
5	 **3e**	3148, 1614, 1590, 1456, 773	298 m/z as $[M]^+$, and 300 $[M+2]^+$ for $C_{14}H_8BrN_3$	6.64 (t, *J* = 3.6 Hz, 3H), 6.71-6.80 (m, 2H), 8.07-8.10 (dd, J_1 = 1.2 Hz, J_2 = 1.2 Hz, 1H), 8.24-8.27 (dd, J_1 = 1.2 Hz, J_2 = 1.2 Hz, 1H), 12.23 (s, 1H, NH)	112.5, 114.0, 117.3, 120.0, 120.8, 124.3, 126.3, 127.5 (C), 129.1 (C), 133.5 (C-N), 138.6 (C-N), 140.3 (C-N), 142.6 (C=N), 145.8 (C=N)
6	 **3f**	1625, 1610, 1444, 754	310 m/z as $[M+H]^+$ for $C_{21}H_{15}N_3$	4.78 (s, 2H), 6.87 (d, 1H, *J* = 5.7 Hz), 7.08 (d, 1H, *J* = 5.4 Hz), 7.27-7.34 (m, 3H), 7.42 (t, *J* = 5.7 Hz, 1H), 7.63 (d, *J* = 6.0 Hz, 1H), 7.74-7.79 (m, 2H), 7.86 (t, *J* = 5.1 Hz, 1H), 8.11 (t, *J* = 6.3 Hz, 1H), 8.25 (d, *J* = 6.0 Hz, 1H), 8.30 (d, *J* = 6.0 Hz, 1H)	46.1 (CH_2), 110.7, 113.1, 118.7, 122.2, 123.7, 124.9, 125.2, 126.2, 126.5, 127.2, 127.4, 128.6, 129.0, 131.3 (C), 132.4 (C), 136.1 (C-N), 143.5 (C-N), 144.0 (C-N), 146.8 (C=N), 147.0 (C=N)
7	 **3g**	3121, 1618, 1575, 1443, 764	221 m/z as $[M+H]^+$ for $C_{13}H_8N_4$	6.54-6.60 (m, 1H), 6.79-6.82 (dd, J_1 = 0.6 Hz, J_2 = 0.9 Hz, 1H), 7.12-7.18 (m, 1H), 7.34-7.38 (q, *J* = 4.8 Hz, 1H), 8.04-8.07 (dd, J_1 = 1.5 Hz, J_2 = 1.2 Hz, 1H), 8.21-8.24 (dd, J_1 = 1.5 Hz, J_2 = 1.8 Hz, 1H), 8.47-8.49 (dd, J_1 = 1.5 Hz, J_2 = 1.8 Hz, 1H), 12.87 (s, 1H, NH)	114.4, 116.1, 116.8, 119.6, 126.5, 131.0, 131.3, 135.7 (C), 143.2 (C), 148.8 (C-N), 149.3 (C=N), 156.0 (C=N), 156.9 (C=N)
8	 **3h**	3098, 1621, 1522, 1457, 749	234 m/z as $[M]^+$ for $C_{14}H_{10}N_4$	2.50 (t, *J* = 1.8 Hz, 3H), 6.79-6.82 (dd, J_1 = 0.9 Hz, J_2 = 0.9 Hz, 1H), 7.12-7.18 (m, 1H), 7.34-7.38 (dd, J_1 = 4.8 Hz, J_2 = 4.8 Hz, 1H), 8.03-8.07 (dd, J_1 = 1.5 Hz, J_2 = 1.8 Hz, 1H), 8.21-8.24 (dd, J_1 = 1.5 Hz, J_2 = 1.5 Hz, 1H), 8.47-8.49 (dd, J_1 = 1.5 Hz, J_2 = 1.8 Hz, 1H), 12.87 (s, 1H, NH)	23.5 (CH_3), 114.4, 116.1, 116.8, 119.6, 126.5, 131.0, 131.3 (C), 135.7 (C), 143.2 (C), 148.8 (C-N), 149.3 (C=N), 156.0 (C=N), 156.9 (C=N)

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
9	 **3i**	3114, 1626, 1569, 1449, 752	254 m/z as $[M]^+$ and 256 $[M+2]^+$ for $C_{13}H_7ClN_4$	6.72 (d, *J* = 6.8 Hz, 1H), 7.17-7.23 (m, 2H), 8.17 (d, *J* = 8.4 Hz, 1H), 8.26 (d, *J* = 8.4 Hz, 1H), 8.56 (d, *J* = 7.2 Hz, 1H), 12.10 (s, 1H, NH)	116.4, 119.7, 123.5, 126.4, 130.3, 130.4, 135.9 (C), 141.9 (C), 143.2 (C), 147.8 (C-N), 149.7 (C=N), 154.4 (C=N), 155.4 (C=N)
10	 **3j**	3087, 1612, 1546, 1452, 768	266 m/z as $[M+H_2O]^+$ for $C_{15}H_{12}N_4$	2.36 (t, *J* = 11.6 Hz, 3H), 2.59 (s, 3H), 6.46 (s, 1H), 7.23 (t, *J* = 7.6 Hz, 1H), 7.68 (d, *J* = 6.8 Hz, 1H), 7.92 (d, *J* = 8.4 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 11.95 (s, 1H, NH)	21.1 (CH_3), 23.4 (CH_3), 115.4, 117.8, 122.4, 123.5, 124.3, 125.6 (C), 126.3 (C), 127.4 (C), 128.3 (C), 130.1 (C-N), 134.6 (C=N), 140.5 (C=N), 142.9 (C=N)

3.4 RESULTS AND DISCUSSION

3.4.1 Catalytic performance of Cu doped CdS NPs

To examine the catalytic activity of nanoparticles in the chemoselective synthesis of indolo[2,3-*b*]quinoxalines, isatin and ortho-phenylenediamine were chosen as model substrates for the reaction in ethylene glycol (5 ml) under microwave irradiation. A control experiment was conducted in the absence of catalyst. The reaction was incomplete even after 60 min, though formation of a small amount of 6*H*-indolo[2,3-*b*]quinoxaline **3a** (31%) was observed. Thus the initial efforts were focused on the systematic evaluation of various catalyst systems. As shown in Table **3.4**, when the reaction was preceded with the use of catalytic amount of CdS NPs, the desired product **3a** was isolated with 71% yield. Further, the study of catalytic ability of Cu doped CdS nanoparticles showed that doping increases the product yield up to 95% and reduces the reaction time (10 min.) with the decreased catalyst loading (5 mol %). It was found that the best result in terms of turnover frequency (TOF) could be achieved by using Cu doped CdS NPs catalyst (5 mol% loading). It shows that the CdS NPs were active in reaction with a turn-over frequency (TOF) of 767 h^{-1} is observed. When the Cu doped CdS NPs were used, the activity was septupled to (TOF) 5415 h^{-1}. This finding indicates that doping of Cu promotes the activity and selectivity of CdS NPs and higher concentration of acidic sites gives more products in the reaction. Therefore, lower catalyst loading is required for this transformation as compared to other catalytic systems, thus showing that this method is superior to the other methods in terms of yield and reaction time.

Table 3.4: Comparison of catalytic activity of catalyst

Entry	Catalyst	Time (min.)	Yield (%)*	TOF (h^{-1})
1	-	60	28	-
2	$CdSO_4$ (10 mol%)	38	42	298
3	$CdCl_2$ (10 mol%)	36	44	330
4	$CdNO_3$ (10 mol %),	34	47	373
5	Powder CdS (10 mol%)	30	56	504
6	CdS NPs (10 mol%)	25	71	767
7	Cu doped CdS NPs (10 mol %)	10	95	2565
8	Cu doped CdS NPs (5 mol %)	10	95	5415

* = Isolated Yield

3.4.2 Effect of solvent

In order to ascertain the effect of solvent, the reaction was carried out using different solvents. The superiority of ethylene glycol as a solvent as compared to commonly employed solvents is quite evident from the results summarized in Table **3.5**.

Table 3.5: Effect of solvent for the synthesis of **3a**

Entry	Solvent	Time (min.)	Yield (%)*
1	Ethanol	10	69
2	Methanol	10	54
3	Acetonitrile	10	46
4	Water	10	74
5	Ethylene glycol	10	95

* = Isolated Yield

3.4.3 Comparison of catalytic activity of catalyst under different reaction conditions

The model reaction was also investigated under different nonconventional and conventional conditions and the overall findings are given in Table **3.6**. Under MW, the catalytic activity of Cu doped CdS NPs was found to be 18 times higher than the conventional method (Table **3.6**). The enhancement of catalytic activity in MW might be due to the fact that nanocatalyst act as a susceptor and absorb microwave irradiation,[41] thus they can serve as an internal heat source for the reactions which enhance the overall capacity to absorb MW in the reaction mixture and prevented the deactivation of nanocatalyst during the reaction. The model reaction was also studied by varying microwave power and temperature. It was concluded that 300 W power output at 80°C was required to accomplish maximum conversion to product. Furthermore, to take advantage of the highly efficient green protocol, the reaction was scaled up to 10 mmol.

Table 3.6: Dependency of catalytic activity of catalysts under different nonconventional and conventional conditions

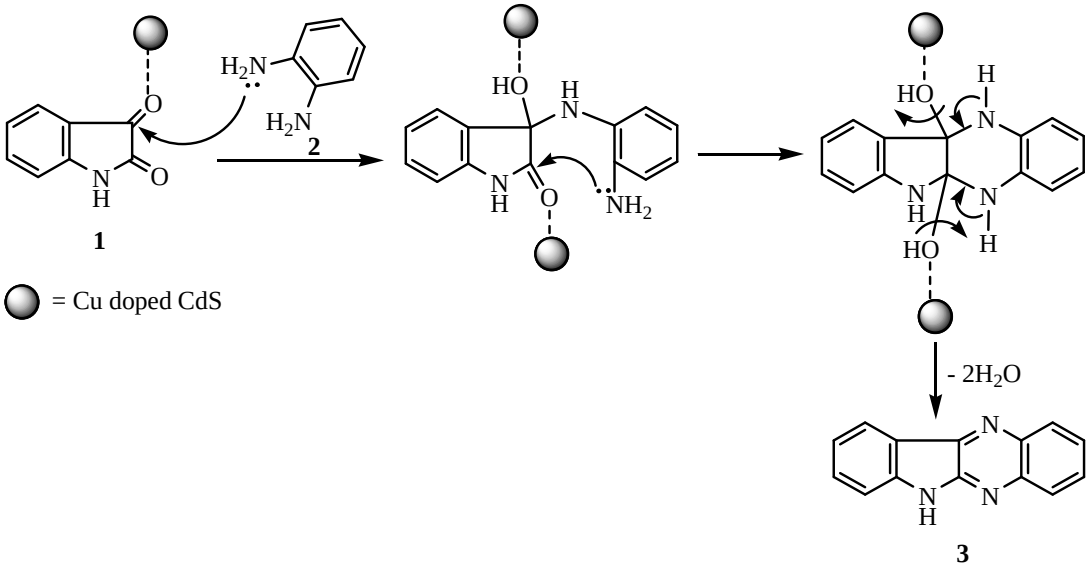
Entry	Condition	Catalyst	Temp. (°C)	Time (min.)	Yield (%)*	TOF (h^{-1})
1	Conventional	Cu doped CdS NPs (5 mol%)	80	90	48	304
2	Ultrasound	Cu doped CdS NPs (5 mol%)	80	60	54	513
3	Microwave	Cu doped CdS NPs (5 mol%)	60	10	42	2394
4	Microwave	Cu doped CdS NPs (5 mol%)	70	10	68	3876
5	Microwave	Cu doped CdS NPs (5 mol%)	80	10	95	5415
6	Microwave	Cu doped CdS NPs (5 mol%)	90	10	95	5415

* = Isolated Yield

In order to study the generality of this procedure, a variety of substituted isatins were subjected to this reaction (Table **3.2**). Isatins bearing electron withdrawing groups reacted faster with slightly improved yields as compared to the isatins having electron donating counter parts. The application of the reaction to heteroatomic diamines has also been explored. The results suggest that, the reaction proceeds well in the optimized conditions.

3.4.4 Mechanism

A possible mechanism for the formation of products **3a-j** is shown in Scheme **3.3**. The Lewis acid sites of NPs are coordinated to the oxygen of carbonyl groups. The coordination of NPs with the carbonyl oxygen of **1** induces electrophilic activation of isatin, which benefits the initial addition of **2** with **1** to give an amino-1,2-diol. The resultant amino-1,2-diol undergoes dehydration to give indolo[2,3-*b*] quinoxaline **3** as the end product.



Scheme 3.3: Plausible mechanism for the synthesis of indolo[2,3-*b*]quinoxaline derivatives

3.5 CONCLUSION

The present chapter describes the synthesis, characterization, and catalytic activity of Cu doped CdS NPs. Cu doped CdS NPs showed good catalytic activity for the chemoselective synthesis of indolo[2,3-*b*]quinoxalines by the reaction of isatins with 1,2-diamines in ethylene glycol under microwave irradiation. Under microwave irradiation (MW), the catalytic activity of doped CdS NPs was about 18 times higher as compared to the conventional method. Catalytic processes with shorter reaction times safeguard the catalyst from deactivation and decomposition. The positive effect of Cu doping in catalyst CdS NPs on reaction rate acceleration was attributed to an increase in surface acidity. This method offers several advantages, including high yield, short reaction time, simple work-up procedure, ease of separation, and recyclability of the nanocatalyst, as well as the easier scaling up for large scale synthesis while avoiding the use of high temperature, pressure and toxic chemicals.

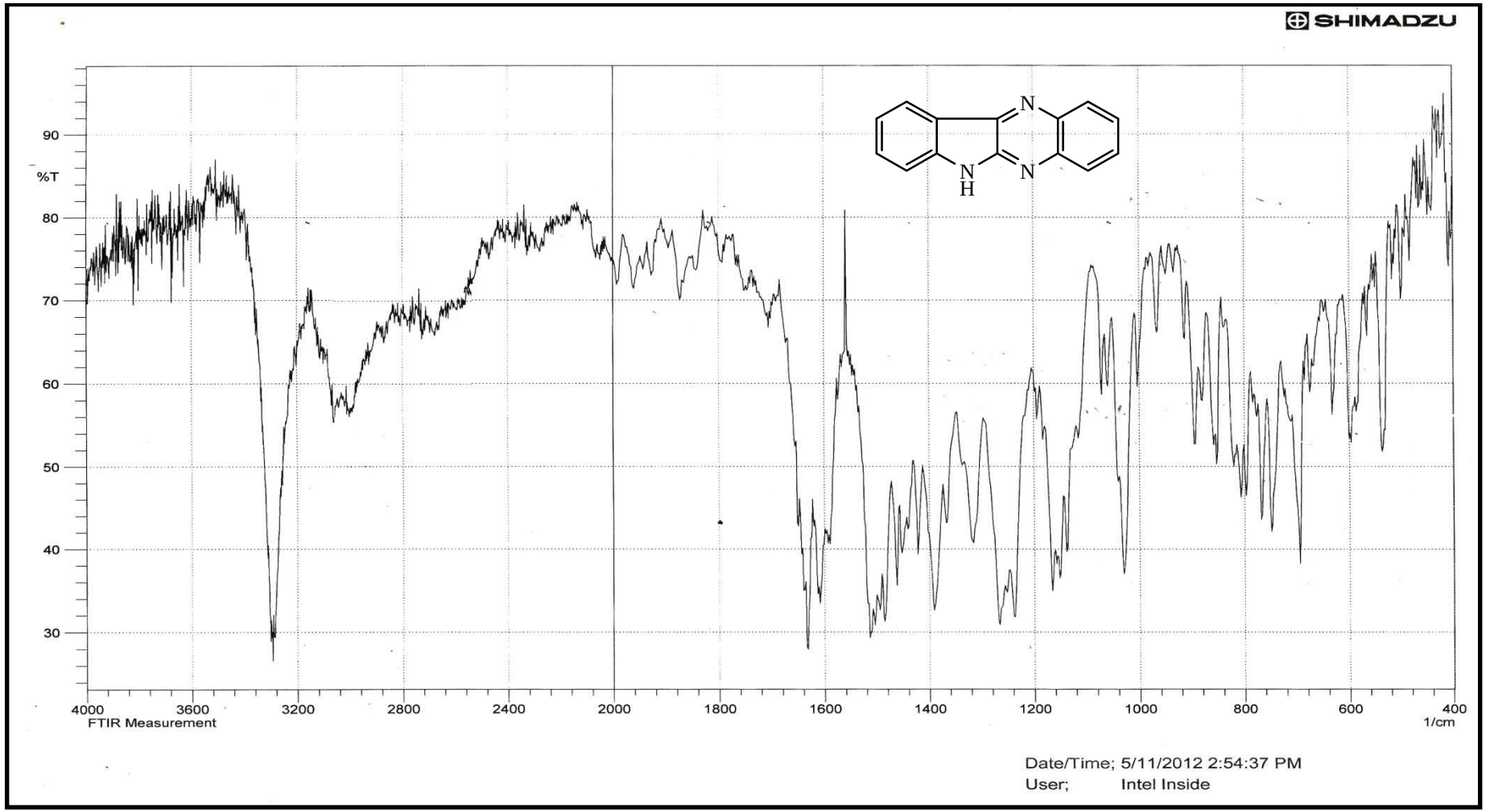


Figure 3.8: FT-IR spectrum of **6*H*-indolo[2,3-*b*]quinoxaline (3a)**

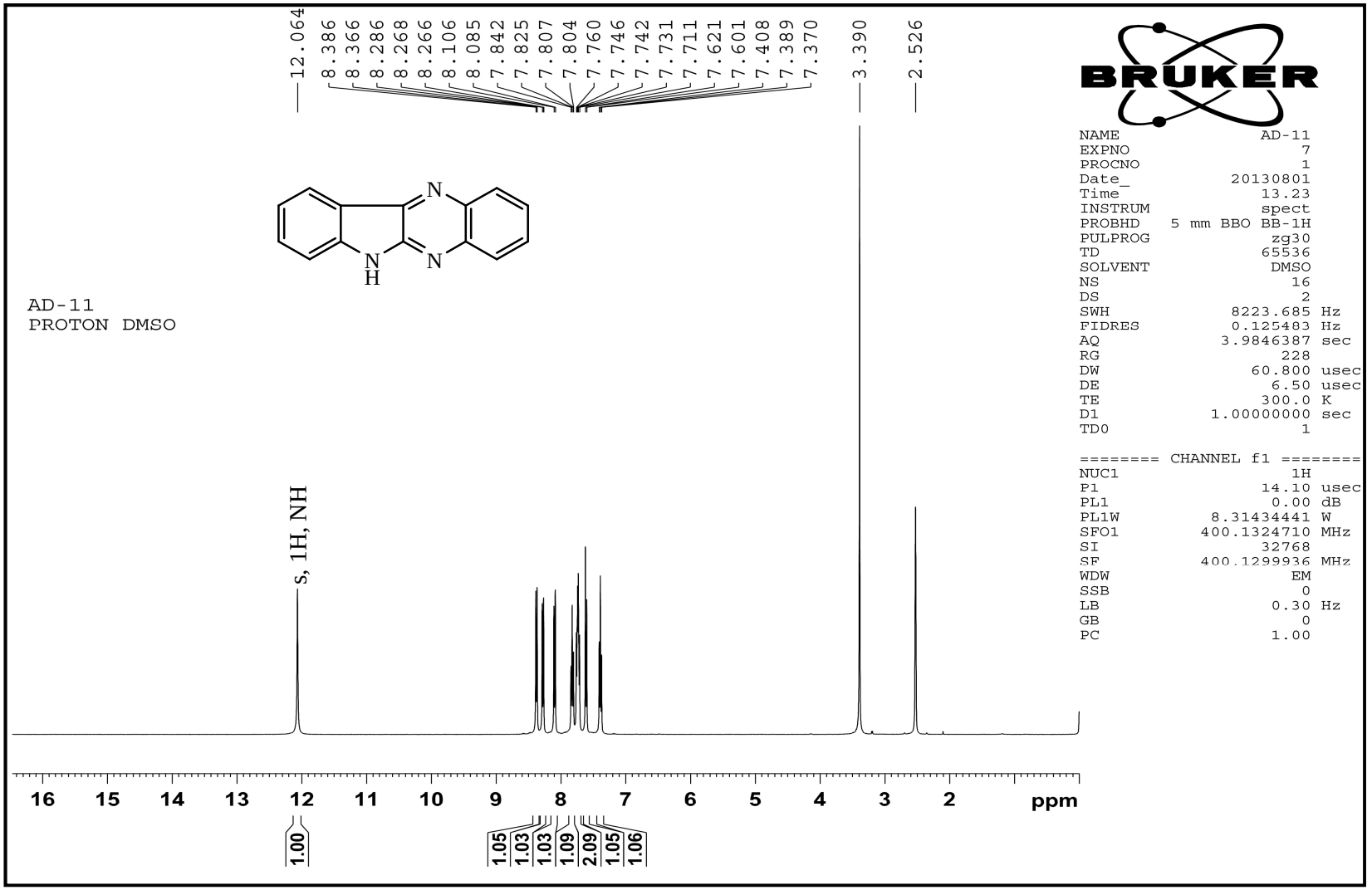


Figure 3.9: ^{1}H NMR spectrum of **6*H*-indolo[2,3-*b*]quinoxaline (3a)**

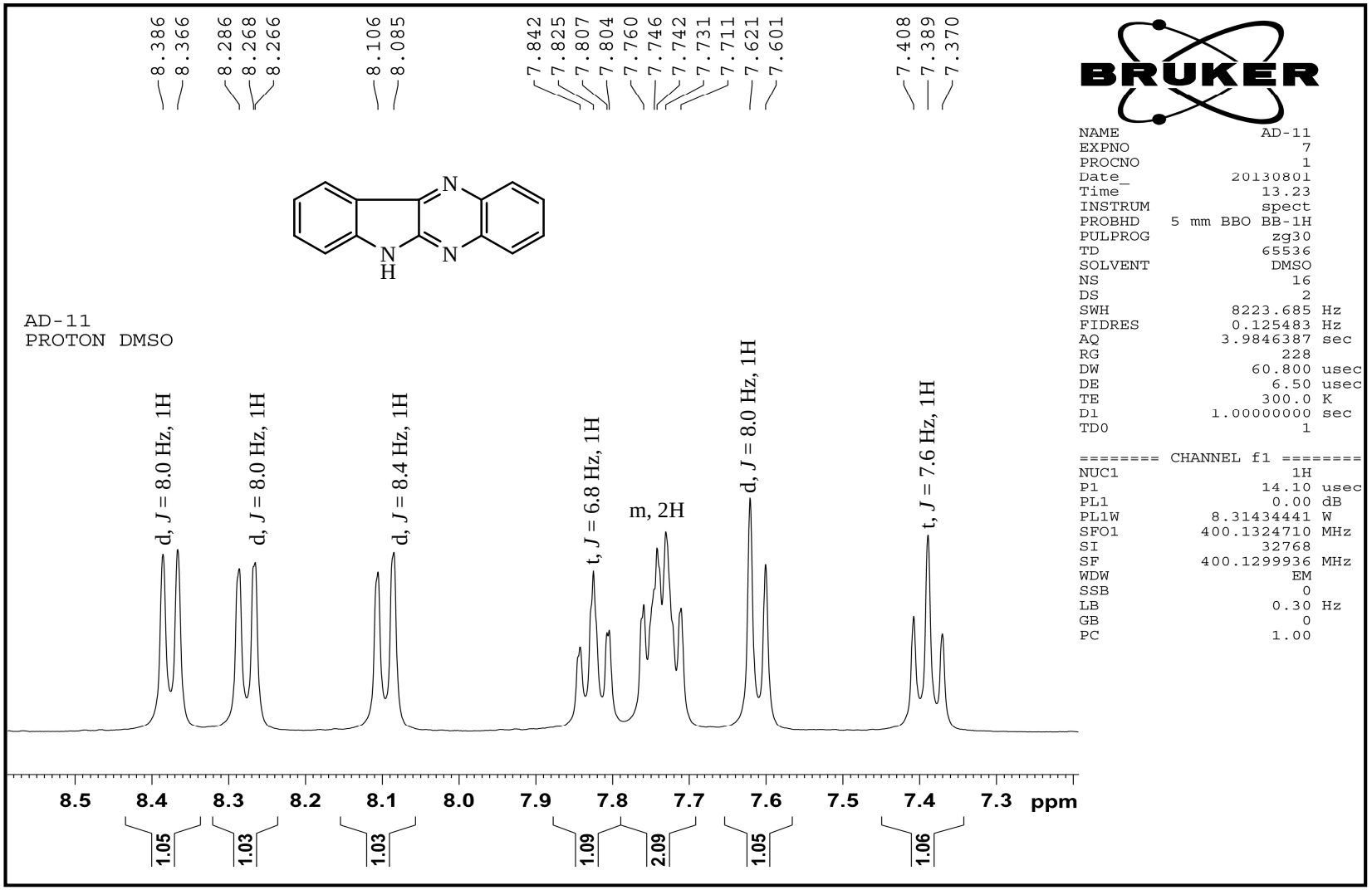


Figure 3.10: Extended ^{1}H NMR spectrum of **6*H*-indolo[2,3-*b*]quinoxaline (3a)**

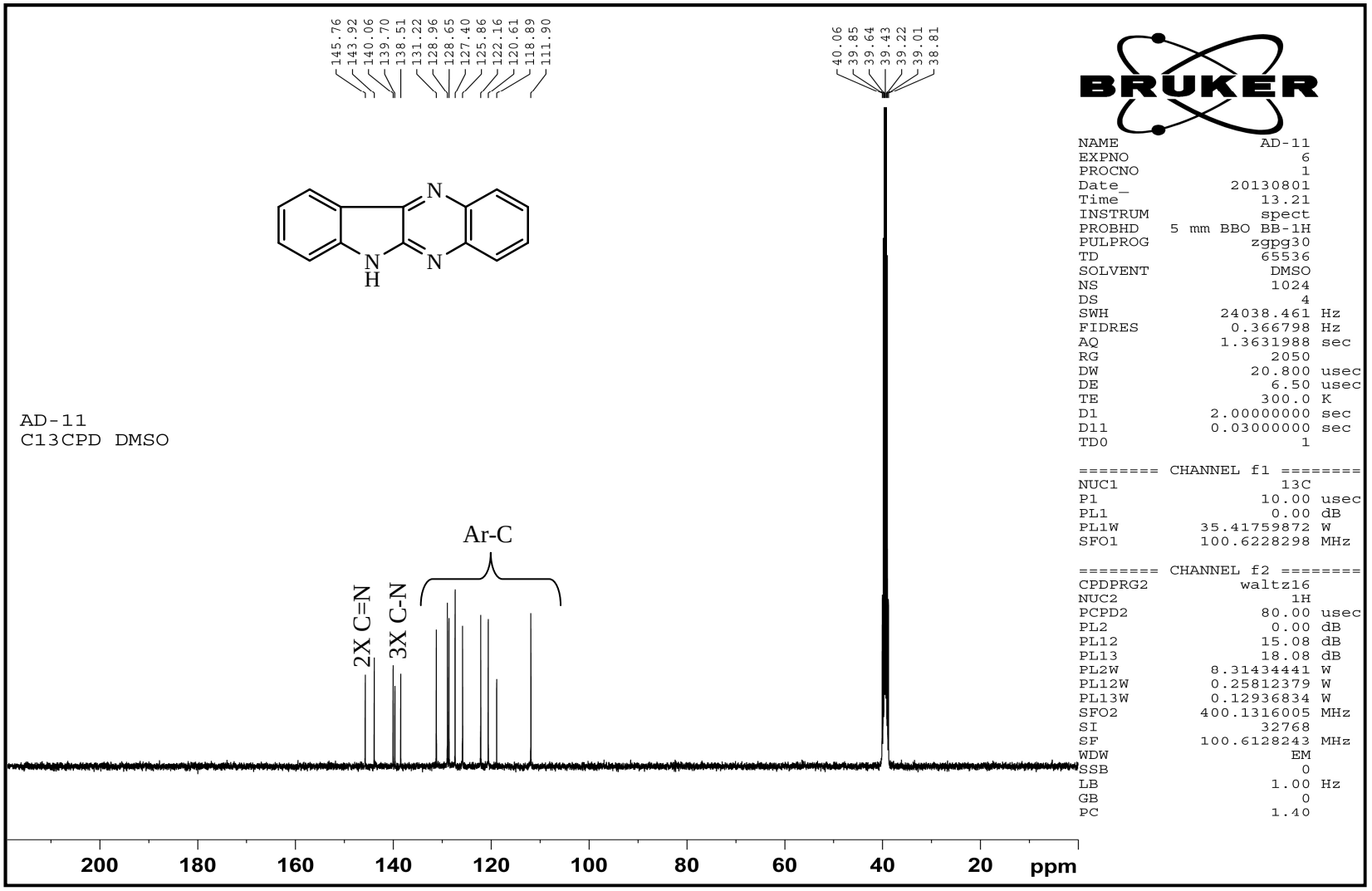


Figure 3.11: ^{13}C NMR spectrum of **6*H*-indolo[2,3-*b*]quinoxaline (3a)**

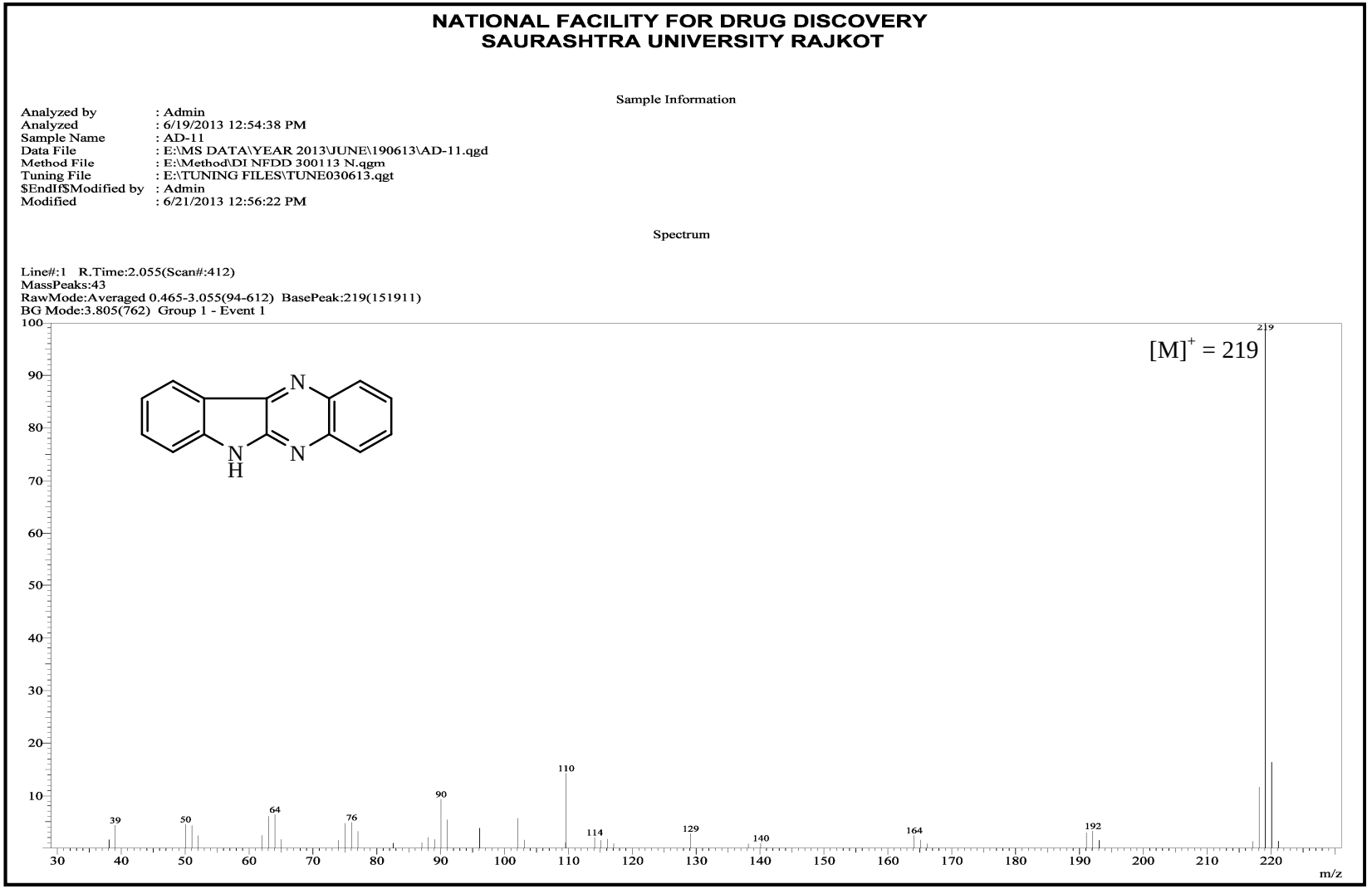


Figure 3.12: Mass spectrum of **6*H*-indolo[2,3-*b*]quinoxaline (3a)**

3.6 References

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CHAPTER 4

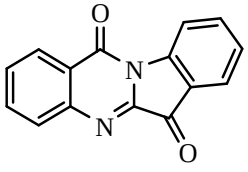
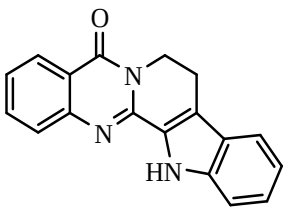
Synthesis and Characterization of Quinazolinone Derivatives catalyzed by highly efficient Magnetically Separable Copper Ferrite Nanoparticles

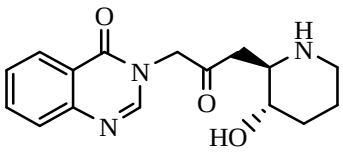
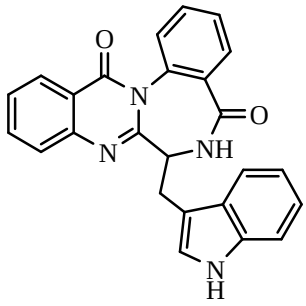
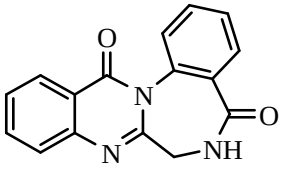
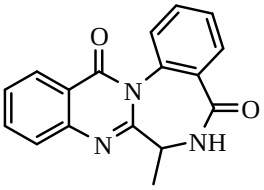
4.1 INTRODUCTION

Synthetic strategies that allow the creation of natural product based diverse molecular architectures,[1] present an interesting and demanding challenges to the art of organic synthesis.[2] In this regard, cascade reactions can be considered to fall under the banner of "green chemistry" to constructs bioactive scaffolds.[3,4] Many natural products are heterocyclic compounds, and a good number of them are quinazolinone alkaloids.[5] In recent years, numerous natural products with the quinazolinone core structure have been isolated and purified, exhibited various pharmacological and biological activities.[6,7] In addition, many synthetic quinazolinones show useful biological activities, such as histamine H_4 receptor inverse agonists,[8] antitumor, anticonvulsant,[9] antiviral,[10] antihypertensive,[11] anti-inflammatory,[12] analgesic,[13] antihyperglycemic,[14] cytotoxicity,[15] antibacterial[16] and angiotensin II AT1 receptor anatagonists.[17] Moreover, they also have plant growth regulating abilities and act as powerful inhibitors of the epidermal growth factor (EGF) receptors of tyrosine kinase.[18]

Quinazolinone containing alkaloids include trypanthrin, rutaecarpine and febrifugine (**Table 4.1**) have interesting biological activity and have therefore been extensively investigated for the treatment of microbial infections, headache and malaria.[19-22]

Table 4.1: Structure of alkaloids incorporating quinazolinone framework

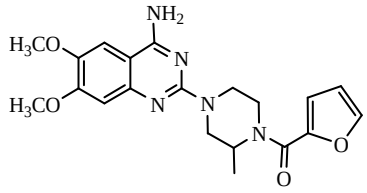
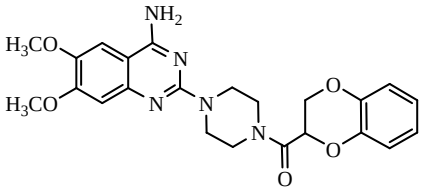
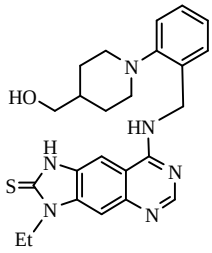
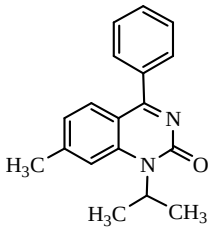
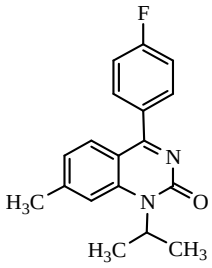
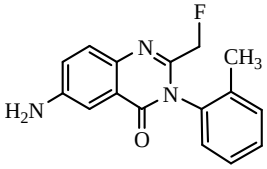
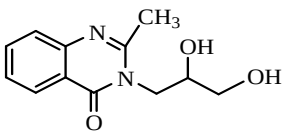
S. No.	Name of the compound	Structure of the compound	Use of the compound[19-22]
1	Trypanthrin		Tryptanthrin has been reported to have various pharmacological activities, including anti-microbial, anti-inflammatory, immuno-modulatory and anti-tumor effects.
2	Rutaecarpine		Rutaecarpine has been reported to exhibit anti-inflammatory, anti-nociceptive effects, and strongly inhibit prostaglandin E2 synthesis.

S. No.	Name of the compound	Structure of the compound	Use of the compound[19-22]
3	Febrifugine		Febrifugine is a quinazolinone alkaloid and it is used in treatment of malaria and stomach cancer.
4	Asperlicin C		Asperlicin C is a mycotoxin which is used to act as a selective antagonist and also found to be useful for a variety of CCKA antagonists.
5	Sclerotigenin		Sclerotigenin is a quinazolinone alkaloid isolated from the *Penicillium sclerotigenum.* It acts as an anti-insecticide.
6	Circumdatin F		Circumdatin F is a quinazolinone alkaloid and can be isolated from the fungus *Aspergillus ochraceous*

In addition, quinazolinone is an important pharmacophore and the chemistry of the quinazolinone alkaloids and analogues is well illustrated in the form of comprehensive reviews and is constantly updated in Natural Product Reports.[23]

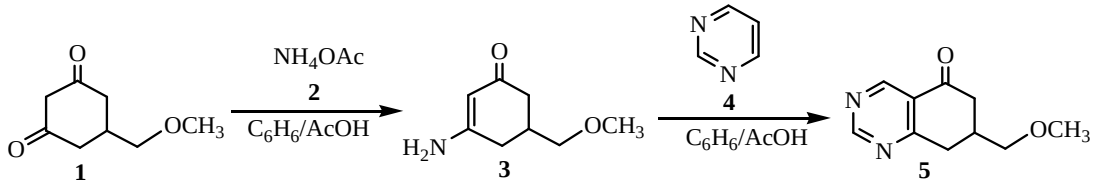
Above and beyond, quinazolinone derivatives are also the structural motifs of various marketed drugs like Prazosin,[24] Doxazosin,[25] etc. which are well known medicines as antihypertensives. Some other drugs, such as Afloqualone as muscle relaxant, Proquazone and Fluproquazone exhibit non-steroidal anti-inflammatory property, and Diproqualone possess sedative analgesic effects.[26] KF31327 [27] is also an important medicine for heart disease.

Table 4.2: Structure of marketed drugs incorporating quinazoline skeleton

S. No.	Name of the compound	Structure of the compound	Use of the compound [24-27]
1	Prazosin		Prazosin is a sympatholytic drug which is used to treat high blood pressure and anxiety.
2	Doxazosin		Doxazosin, a quinazoline compound is an α1-selective blocker used to treat high blood pressure.
3	KF31327		KF31327 has been known as a heart disease remedy and an impotence medicine.
4	Proquazone		Proquazone exhibits non-steroidal anti-inflammatory properties.
5	Fluproquazone		Fluproquazone has potent analgesic and antipyretic effects with anti-inflammatory action.
6	Afloquazone		Afloqualone is a GABAergic class drug and has sedative and muscle-relaxant effects.
7	Diproqualone		Diproqualone is also a GABAergic class drug and has sedative, anxiolytic, anti- histamine and analgesic properties.

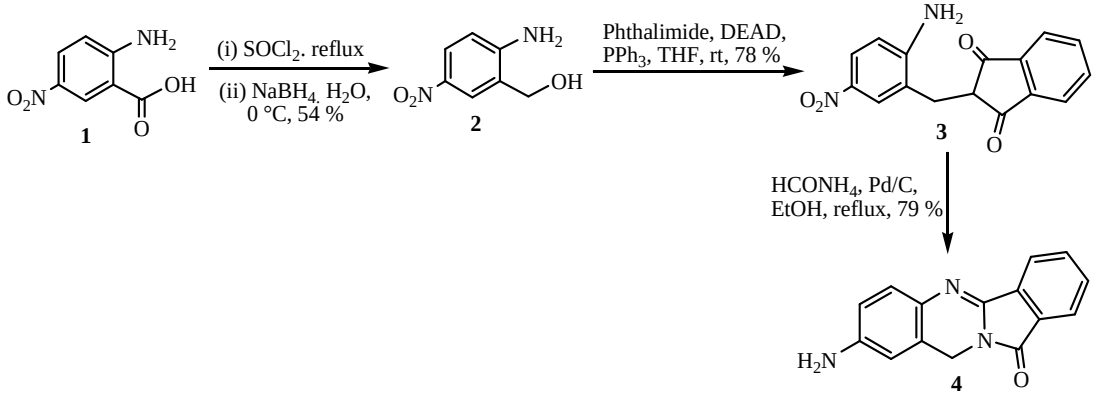
Therefore, preparation of this heterocyclic nucleus has gained great importance in organic synthesis. Several methods have been reported for the synthesis of quinazoline derivatives which involve:

An approach for the multi-step synthesis of functionalised quinazolinones **5** *via* the Diels-Alder reaction of 1,3-diazine **4** with enaminones **3** which were prepared by the reaction of 1,3-dicarbonyls **1** and ammonium acetate **2** had been reported by Masaguer *et al*.[28] (Scheme **1**)



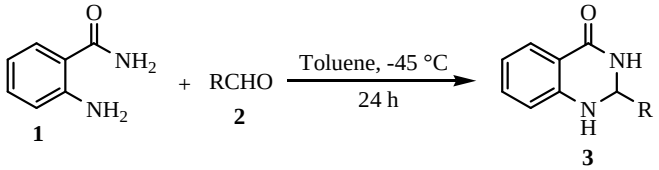
Scheme 1

Dominguez *et al*.[29] described the synthesis of tetracyclic isoindolo[1,2-*b*] quinazolin-12-ones **4** from easily accessible *o*-acylanilines **1** and *o*-aminobenzyl alcohol **2** through a Mitsunobu reaction followed by spontaneous cyclodehydration of substituted indene-1,3-dione **3** (Scheme **2**).



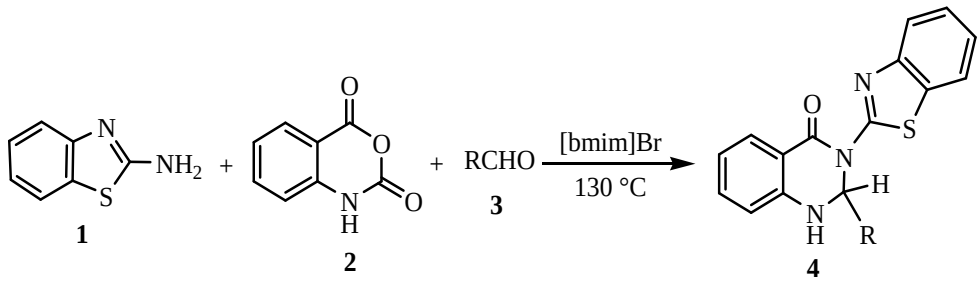
Scheme 2

List *et al*.[30] furnished the dihydroquinazolinone derivatives **3** from aminobenzamide **1** and a variety of aldehydes **2** using toluene as a reaction medium (Scheme **3**).



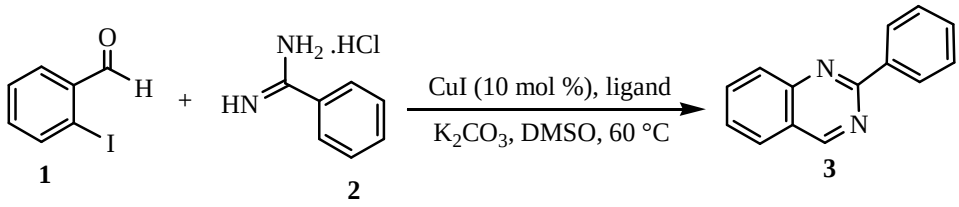
Scheme 3

Shaabani *et al.*[31] prepared the 3-(2′-benzothiazolo)-2,3-dihydroquinazolin-4(1*H*)-one derivatives **4** from the multicomponent reaction of 2-aminobenzothiazole **1**, isatoic anhydride **2** and aldehyde **3** in the presence of 1-butyl-3-methylimidazolium bromide [bmim]Br at 130 °C (Scheme **4**).



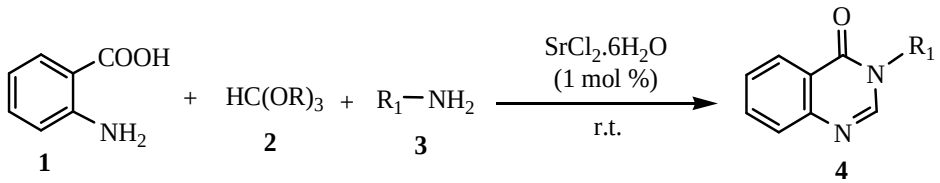
Scheme 4

Truong *et al.*[32] explored the ligand-free copper catalysed Ullmann N-arylation condensation reaction of *o*-iodobenzaldehydes **1** with amidine hydrochlorides **2** to affording the corresponding quinazolines **3** (Scheme **5**).



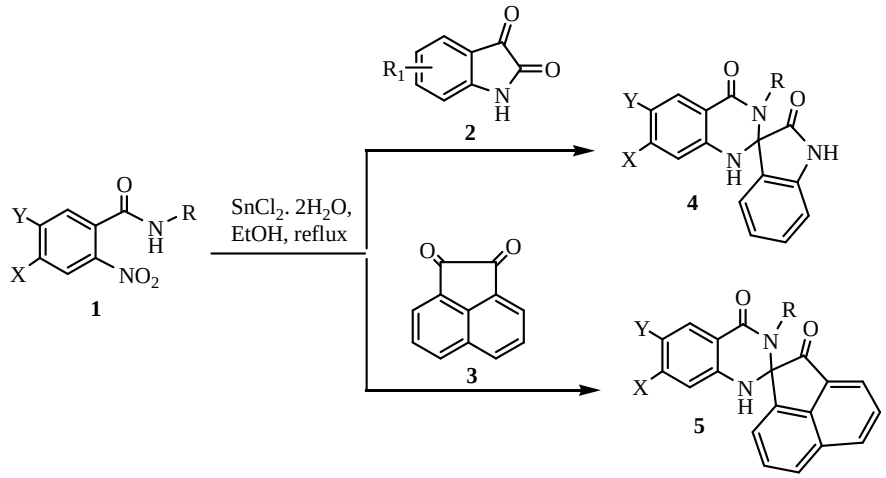
Scheme 5

Wang *et al.*[33] reported an efficient methodology to synthesize 4(3*H*)-quinazolinone derivatives by the three-component condensation reaction of anthranilic acid, ortho esters and amines at room temperature under solvent-free conditions catalysed by strontium chloride (Scheme **6**).



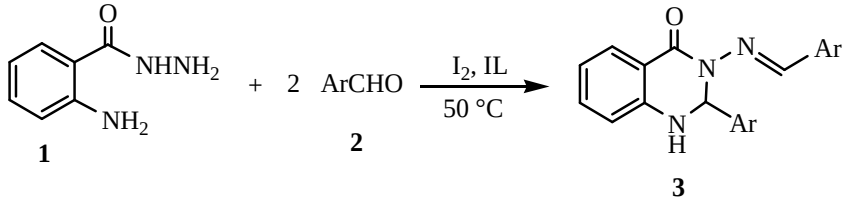
Scheme 6

$SnCl_2.2H_2O$ was efficiently used by Shi *et al.*[34] for the synthesis of 1′*H*-spiro[indoline-3,2′-quinazoline]-2,4′(3′*H*)-dione derivatives **4/5** by the novel reductive cyclization of 2-nitrobenzamides **1** and isatin **2**/acenaphthenequinone **3** (Scheme **7**).



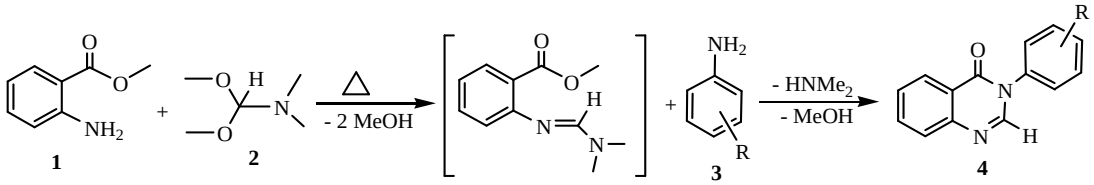
Scheme 7

A combinatorial synthesis of 3-arylideneaminoquinazolin-4(1*H*)-one derivatives **3** was illustrated by Wang *et al*.[35] by the reaction of 2-aminobenzohydrazides **1** with two equivalents of aldehydes **2** in ionic liquids catalyzed by iodine (Scheme **8**).



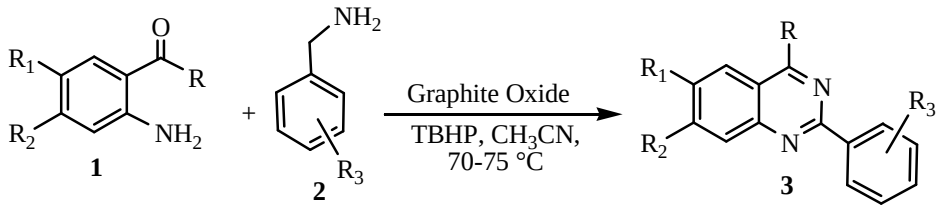
Scheme 8

Vasu *et al*.[36] studied a greener solvent-free protocol for the synthesis of 3-substituted quinazolin-4(3*H*)one derivatives **4** by the three-component condensation reaction of methyl anthranilate **1** with N,N′-dimethyl formamide dimethyl acetal **2** and various anilines **3** (Scheme **9**).



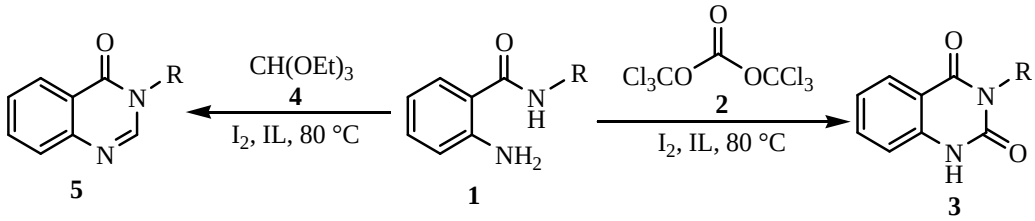
Scheme 9

Two-component condensation reaction of various benzophenones **1** and benzylamines **2** in the presence of *tert*-butyl hydroperoxide as an oxidant catalysed by graphite oxide to afford 2-phenylquinazoline derivatives **3** had been reported by Nageswar *et al*.[37] (Scheme **10**)



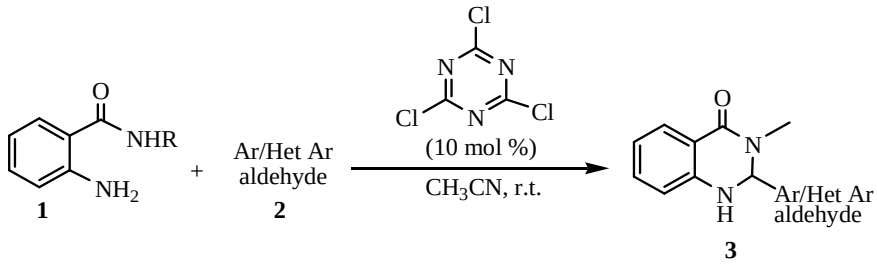
Scheme 10

A simple ionic liquid catalysed methodology for the synthesis of a series of quinazoline derivatives **3/5** by the reaction of 2-aminobenzamides **1** and triphosgene **2** /triethyl orthoformate **4** at 80 °C had been studied by Wang *et al*.[38] (Scheme **11**)



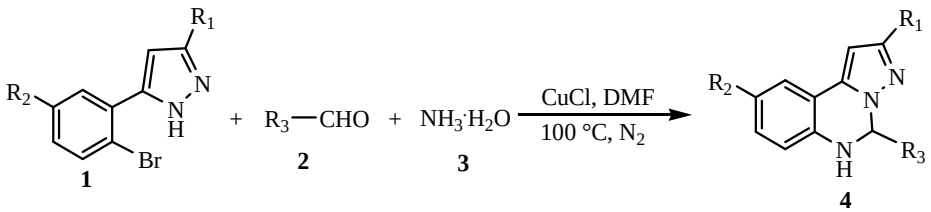
Scheme 11

Chauhan *et al*.[39] elucidated an efficient cyanuric chloride catalysed approach for the synthesis of quinazolinone derivatives **3**. The cyanuric chloride catalysed reaction is followed by the cyclisation of anthranilamide **1** and various benzaldehydes **2** to give skeletal complexity (Scheme **12**).



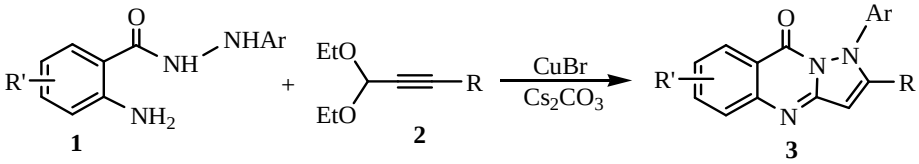
Scheme 12

CuCl catalyzed tandem reaction of 5-(2-bromoaryl)-1*H*-pyrazoles **1** with aldehydes **2** and aqueous ammonia **3** for the synthesis of 5,6-dihydropyrazolo[1,5-*c*]quinazoline derivatives **4** was efficiently demonstrated by Guo *et al*.[40] (Scheme **13**)



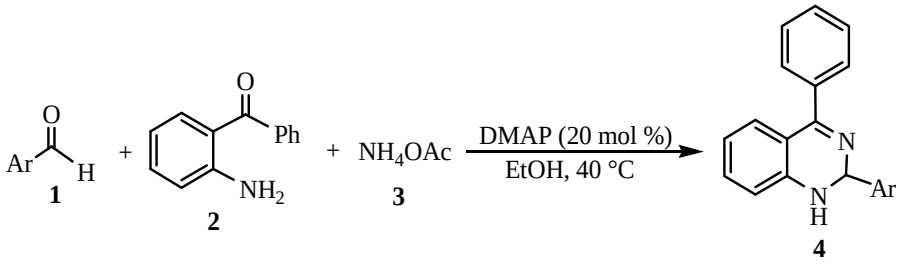
Scheme 13

Wang *et al.*[41] reported a simple $CuBr/Cs_2CO_3$ catalyzed intramolecular alkyne hydroamination reaction of 2-amino-N′-arylbenzohydrazide **1** and alkynyl diethyl acetal **2** to access 1-arylpyrazolo[5,1-*b*]quinazolin-9(1*H*)-one derivatives **3** (Scheme **14**).



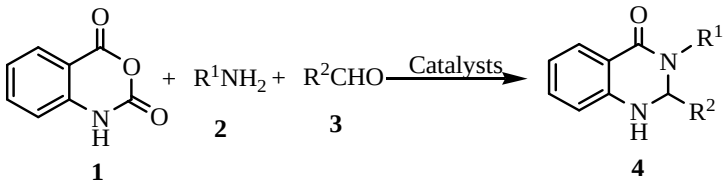
Scheme 14

Boulcina *et al.*[42] focussed on a simple method to synthesize 1,2-dihydroquinazoline derivatives **4** catalysed by 4-(N,N-dimethylamino)pyridine (DMAP) from aromatic or heteroaromatic aldehydes **1**, 2-aminobenzophenone **2**, and ammonium acetate **3** (Scheme **15**).



Scheme 15

The condensation reaction of isatoic anhydride **1**, substituted anilines **2** and various aldehydes **3** is one of the primary routes to construct the quinazoline ring (Scheme **16**).



Scheme 16

The reported procedures for this protocol involves a wide spectrum of reagents including montmorillonite K-10,[43a] silica sulfuric acid,[43b,c] zinc(II) perfluorooctanoate $Zn(PFO)_2$,[43d] $KAl_2(SO_4)_3.12H_2O$,[43e] $Al(H_2PO_4)_3$,[43f] gallium(III) triflate,[43g] molecular iodine,[43h] MCM-41-SO_3H,[43i] 1-butyl-3-methylimidazolium

tetrafluoroborate [bmim]BF_4,[43j,k] and Amberlyst-15,[43l] β-cyclodextrin,[43m] silica-bonded N-propylsulfamic acid,[43n] $Bi(NO_3)_3.5H_2O$,[43o] etc.

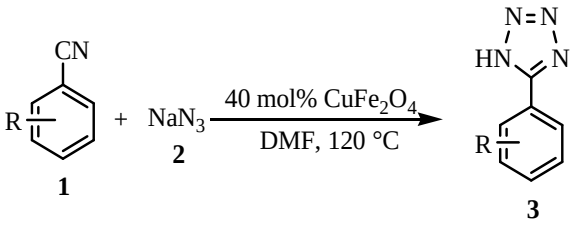
All of these procedures suffer from one or more of the following disadvantages such as harsh reaction conditions, prolonged reaction times, use of hazardous catalysts, excessive amounts of reagents and tedious workup procedures. In addition lower yields are obtained along with the poor selectivity under such harsh conditions. In addition, some of the catalysts and reagents are expensive, toxic, and air sensitive. Therefore, development of uses of nontoxic reagent and a new catalytic route for the synthesis of quinazolinones which could be superior to the existing ones in terms of handling and operational simplicity is an active area of research. Inexpensive and readily available catalysts which can bring the organic transformations in operationally simple way are always an attractive to both organic and medicinal chemists. Careful tuning of catalysts in a synchronized manner will lead to new synthetic transformations possessing significant chemoselectivity in functionally complex systems.

Over the past several decades, there has been an exponential growth towards the new understanding and mastery of catalysis, particularly in the field of nanotechnology.[44] Based on nanotechnology approaches, several new mathods are being develop for the synthesis of pharmaceutical. The combination of catalysis and nanotechnology has opened new possibilities to create controlled structures and geometries to investigate and optimize a broad range of heterogeneous catalytic processes.[45] Easy isolation and purification in organic synthesis is an essential requirement and the simple separation of the catalyst from the reaction mixture is an advantage in the organic synthesis, particularly for the pharmaceutical industry.

There has been a growing interest in copper-mediated reactions for organic synthesis as compared to transition metal catalysts, as it is environmentally benign and economically viable.[46] Copper compounds exist in variable oxidation states of copper such as 0, +1, +2 and +3.[47,48] Thus, due to their higher reactivity, low cost, easy availability, efficient selectivity, variable oxidation states, copper compounds are best suited for organic synthesis.[49,50] Further, copper is apparently more versatile catalyst which leads to high-yielding reactions and are established in numerous industrial and academic applications.[51]

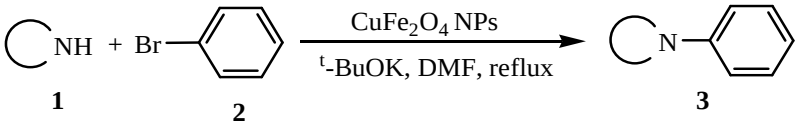
Some of the reactions using the $CuFe_2O_4$ NPs are discussed below:

Sreedhar *et al.*[52] discussed an efficient method for the synthesis of 5-substituted 1*H*-tetrazole derivatives **3** using substituted benzonitriles **1** and sodium azides **2** catalysed by $CuFe_2O_4$ nanoparticles (Scheme **17**).



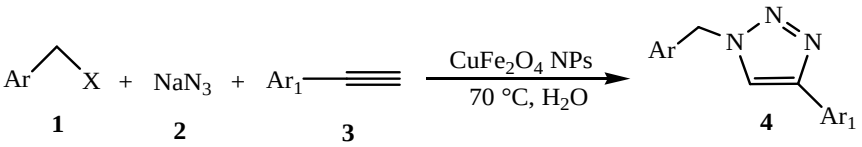
Scheme 17

Panda *et al.*[53] attempted the synthetic utility of copper ferrite nanoparticles for the N-arylation of nitrogen containing heterocyclic compounds **3** by the reaction of a variety of secondary amines **1** and aryl halides **2** (Scheme **18**).



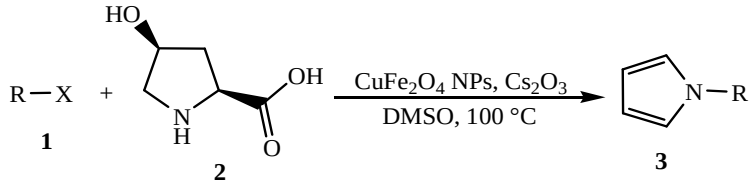
Scheme 18

Nageswar *et al.*[54] achieved the synthesis of 1,4-disubstituted 1,2,3-triazole derivatives **4** using magnetically separable and reusable copper ferrite nanoparticles. This one-pot method for the synthesis of 1,2,3-triazoles entails the initial substitution of benzyl halides **1** with sodium azide **2** to generate *in situ* benzyl azides followed by copper ferrite catalyzed cycloaddition reaction with alkynes **3** in water at 70 °C (Scheme **19**).



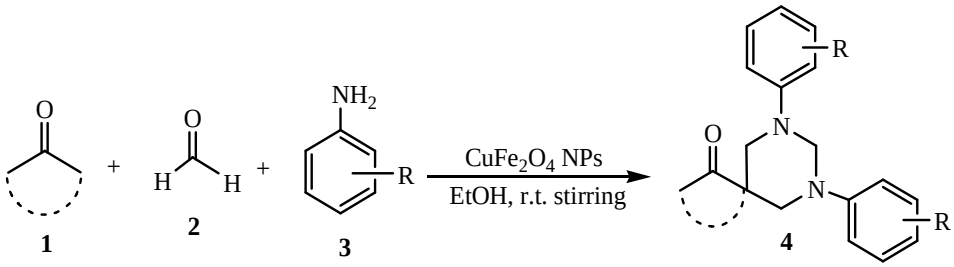
Scheme 19

Nageswar *et al.*[55] presented a simple ligand-free C–N cross-coupling reaction of aryl halides/benzyl halides **1** with trans-4-hydroxy-L-proline **2** to afford aromatized N-substituted pyrroles **3** catalyzed by magnetically separable and recyclable $CuFe_2O_4$ nanoparticles in the presence of Cs_2CO_3 in DMSO at 100 °C (Scheme **20**).



Scheme 20

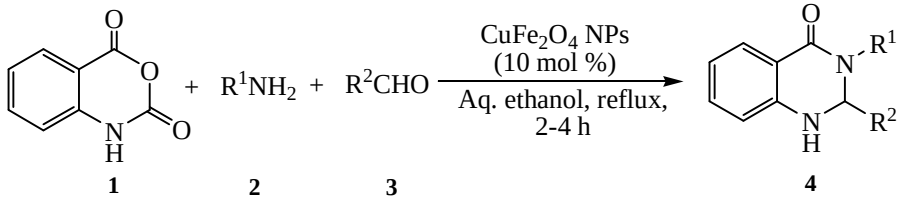
Dandia *et al.*[56] reported an efficient method to synthesize spirohexahydropyrimidine derivatives **4** by the multi-component reaction of cyclic ketones **1**, formaldehyde **2** and aromatic amines **3** in the presence of catalytic amount of magnetically separable $CuFe_2O_4$ nanoparticles in ethanol at room temperature (Scheme **21**).



Scheme 21

4.2 EXPERIMENTAL

As a part of our continued interest towards the development of greener methodologies for synthesis of medicinally important heterocyclic moieties and nanoparticles,[57] herein, we dwell on the use of magnetically separable $CuFe_2O_4$ nanoparticles as a catalyst for the synthesis of quinazolinone derivatives by the multicomponent condensation reaction of isatoic anhydride, substituted anilines and aldehydes in aqueous ethanol under reflux conditions. (**Scheme 4.1**)



Scheme 4.1: Synthesis of substituted-2,3-dihydroquinazolin-4(1*H*)-one derivatives

4.2.1 Synthesis of Catalyst

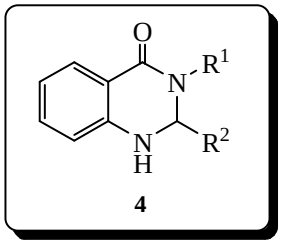
$CuFe_2O_4$ nanoparticles were prepared without the use of any capping agent or surfactant by the combined sonochemical and co-precipitation method in aqueous

medium.[53] Due to their magnetic nature, $CuFe_2O_4$ nanoparticles were easily collected in the side walls of the reaction vessel by using a hand-held magnet during the separation and washing process. Simple aqueous chemical method has been used to prepare $CuFe_2O_4$ nanoparticles. $CuFe_2O_4$ nanoparticles of size 15-50 nm were prepared by thermal decomposition of $Cu(NO_3)_2$ and $Fe(NO_3)_3$ in water in the presence of sodium hydroxide. Briefly, to a solution of $Fe(NO_3)_3.9H_2O$ (3.34 g, 8.2 mmol) and $Cu(NO_3)_2.3H_2O$ (1 g, 4.1 mmol) in 75 ml of distilled water, 3 g (75 mmol) of NaOH dissolved in 15 ml of water was added at room temperature over a period of 10 min. during which reddish-black precipitate was formed. Then the reaction mixture was warmed to 90 °C and stirred under ultrasonic irradiation for two hours. After 2 h, it was cooled to room temperature and the magnetic particles so formed were separated by a magnetic separator. It was then washed with water (3 X 30 ml) and catalyst was kept in an air oven for overnight at 80 °C. Then the catalyst was ground in a mortar-pestle and kept in a furnace at 700 °C for 5 h (step up temperature 20 °C/min) and then cooled to room temperature slowly. 820 mg of magnetic $CuFe_2O_4$ particles of size 35-50 nm were obtained. Copper and iron content in $CuFe_2O_4$ nanoparticles were also estimated from ICP-AES which was found to be 27.5% and 47.2% respectively.

4.2.2 Synthesis of substituted-2,3-dihydroquinazolin-4(1*H*)-one derivatives (4a-k)

To a solution of isatoic anhydride **1** (0.326 g, 2 mmol) and aniline **2** (0.244 g, 2.2 mmol) and benzaldehyde **3** (0.212 g, 2 mmol) and a catalytic amount of $CuFe_2O_4$ nanoparticles (10 mol%) in aqueous ethanol (5 mL) was refluxed for the stipulated times. After the reaction was completed as monitored by TLC, 10 mL ethanol was added to the reaction mixture and the catalyst $CuFe_2O_4$ was separated magnetically using an external magnet. The reactants were given an overnight time period for completion of the reaction. The solid material was filtered off, washed with water (2 X 10 mL), dried and recrystallized from ethanol to furnish pure quinazolinone derivatives (Scheme **4.1**). All the synthesized compounds are summarized in **Table 4.3**.

Table 4.3: Synthesis of quinazolinone derivatives **4a-k**



4

Entry	Product	R^1	R^2	Time (h)	Yield (%)*	Mp (^oC)	Color
1	**4a**	4-F-C_6H_4	C_6H_5	2	95	229-231	White
2	**4b**	4-F-C_6H_4	3,4-diOCH_3-C_6H_3	3	88	214-216	Off-white
3	**4c**	3-Cl,4-F-C_6H_3	C_6H_5	3	90	200-202	White
4	**4d**	3-Cl-C_6H_4	C_6H_5	3	92	185-187	White
5	**4e**	4-CH_3-C_6H_4	C_6H_5	3	94	210-212[43d]	Off-white
6	**4f**	C_6H_5	4-OCH_3-C_6H_4	2	87	218-220[43d]	Off-white
7	**4g**	4-Br-C_6H_4	C_6H_5	2	86	250-252[43e]	Off-white
8	**4h**	3,4-diCH_3-C_6H_3	C_6H_5	3	89	218-220[43f]	White
9	**4i**	3-CF_3-C_6H_4	C_6H_5	3	86	142-144[39]	Off-white
10	**4j**	4-Cl-C_6H_4	C_6H_5	2	90	220-222[43d]	White
11	**4k**	C_6H_5	3,4-diOCH_3-C_6H_3	3	87	245-247[43n]	Off-white

***** = isolated yield.

4.3 CHARACTERIZATION OF THE COMPOUNDS SYNTHESIZED

4.3.1 Characterization of the catalyst

The catalyst was fully characterised using various physicochemical techniques including XRD (X-ray Diffraction), SEM (Scanning Electron Microscopy), FT-IR (Fourier Transform Infra-red) and EDAX (Energy Dispersive Absorption X-ray) analysis. The wide angle X-ray diffraction pattern of the sample was obtained using Bragg-Brentanno geometry on PANalytical X'pert pro diffractometer in 2θ range of 20-70° with Cu-K_α radiation source (λ = 1.5406 Å). The X-ray tube was operated at 45 kV and 40 mA. Formation of copper ferrite nanoparticles was ascertained by electron dispersive absorption X-ray (EDAX) analysis combined with scanning

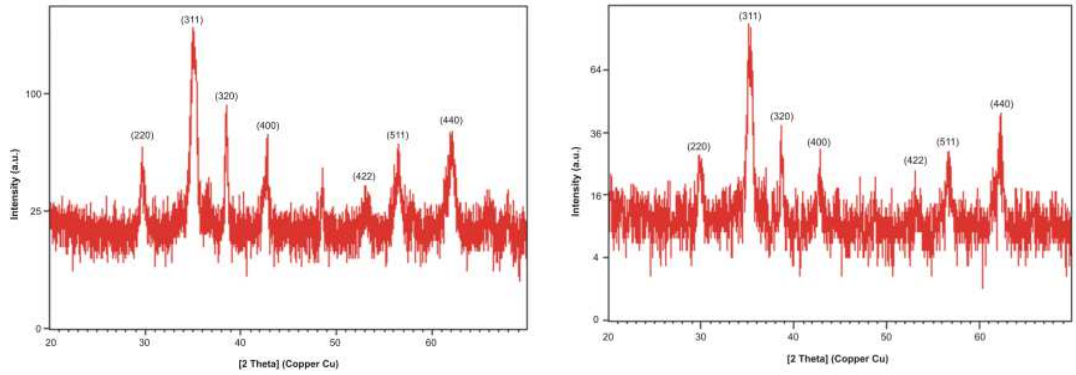
electron microscope (SEM). SEM micrographs were obtained using 'JEOL JSM-6610LV' Scanning Electron Microscope combined with EDAX system (INCA Analyzer). For SEM analysis, the sample was dispersed on the aluminium stub used for sample mounting. The sample was scanned at an accelerating voltage of 20 kV at a working distance of 15 mm. The particle size was measured at a magnification of 5 kX. IR spectra (KBr) were recorded on a Shimadzu FT IR–8400S spectrophotometer.

X-Ray Diffraction

Figure 4.1 shows the powder X-ray diffraction patterns of the sample $CuFe_2O_4$, obtained at room temperature using Cu-K_α radiation. The X-ray powder diagram clearly confirms the presence of single phase with high crystallinity. The XRD pattern of the as-produced $CuFe_2O_4$ nanoparticles was indexed as the standard tetragonal structure (JCP card no. 34-0425).[58] In addition, the strong and sharp peaks observed in the XRD pattern prove the crystalline nature of the prepared $CuFe_2O_4$ nanoparticles. The crystallite size was estimated from the Scherrer equation which is as:

$$D = 0.9\,\lambda/\beta\cos\theta$$

Where, D is the average size of the crystallite (nm), λ is the wavelength of incident X-ray (1.540 Å), β is the full width at half maximum (rad.), and θ is the angle between the incident and diffracted beams (deg.). The average crystallite size was found to be in the range of 15-50 nm.

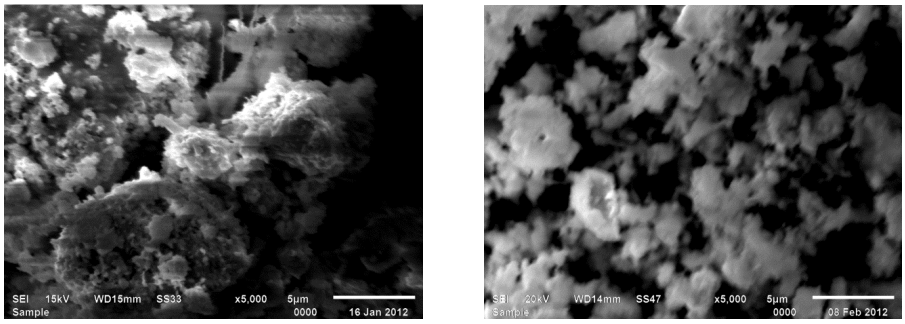


(a) **(b)**

Figure 4.1: X-ray diffraction pattern of (a) fresh $CuFe_2O_4$ nanoparticles and (b) reused $CuFe_2O_4$ nanoparticles after third cycle.

Scanning Electron Microscopy

The structural morphology of the nano-sized particles was investigated using scanning electron microscopy (SEM). SEM micrographs of the $CuFe_2O_4$ nanoparticles are shown in **Figure 4.2**. It is quite evident from the micrographs that $CuFe_2O_4$ have almost uniform structural morphology. The particle size estimated using SEM micrograph was found to be in consistent with the particle size as determined from XRD analysis.

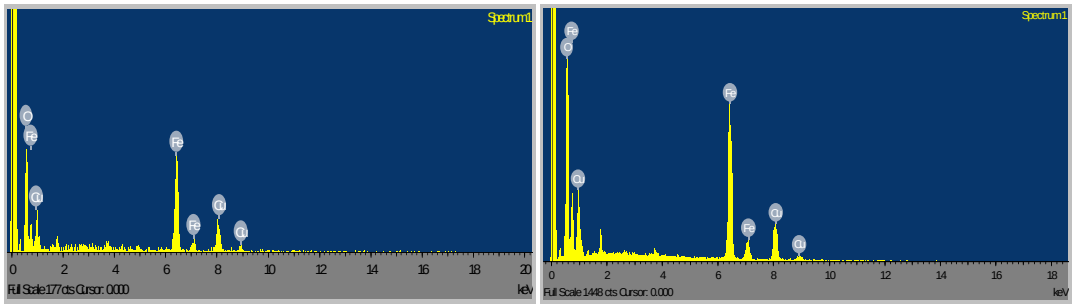


(a) **(b)**

Figure 4.2: SEM micrographs of the (a) fresh $CuFe_2O_4$ nanoparticles and (b) reused $CuFe_2O_4$ nanoparticles after third cycle.

Energy Dispersive Absorption X-ray

The compositional analysis was carried out using EDAX (**Figure 4.3**). The analysis of the nanoparticles showed that Fe, Cu and O are the main elemental components. With the use of the two precursors at 2:1 (Fe: Cu), a typical array of the $CuFe_2O_4$ nanoparticles is analysed as Fe = 14.28 %, Cu = 28.95 % and O = 56.77 %. The Fe/Cu ratio of as-produced $CuFe_2O_4$ nanoparticles is only 2:1, which confirms the initial composition as $CuFe_2O_4$.

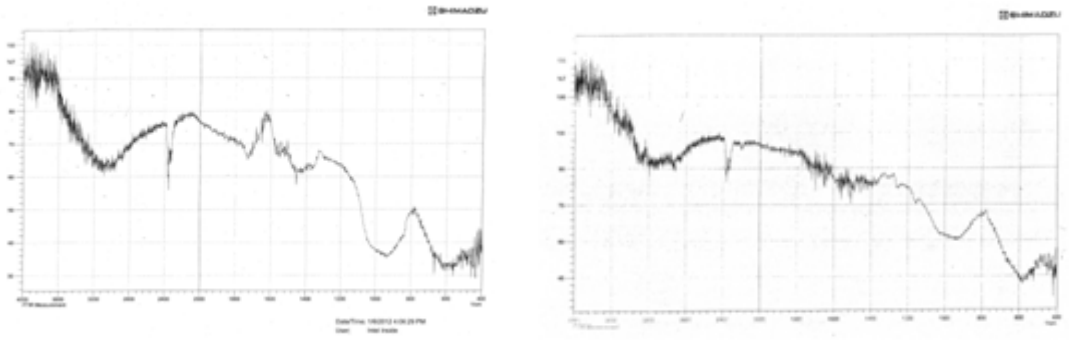


(a) **(b)**

Figure 4.3: EDAX spectra of the (a) fresh $CuFe_2O_4$ nanoparticles and (b) reused $CuFe_2O_4$ nanoparticles after third cycle.

Fourier Transform-Infra Red

The structure of the $CuFe_2O_4$ magnetic nanoparticles was further characterised with FT-IR spectroscopy (**Figure 4.4**) which was recorded in the range of 400-4000 cm^{-1}. Two significant absorption bands in the range of 500-700 cm^{-1} are observed in the spectra which correspond to the Fe-O and Cu-O stretching. Hence, confirmed the structure of the prepared $CuFe_2O_4$ nanoparticles.



(a) **(b)**

Figure 4.4: FT-IR spectra of the (a) fresh $CuFe_2O_4$ nanoparticles and (b) reused $CuFe_2O_4$ nanoparticles after third cycle.

From XRD, SEM, EDAX and FT-IR spectral studies, it was revealed that the $CuFe_2O_4$ nanoparticles remained in the same state, even after the three cycles. From the entire experimental data presented, it can be conclusively proven that there was no considerable change in the catalytic activity of the $CuFe_2O_4$ nanoparticles before and after the utilization in the reaction. Further, the analysis of the final product by ICP-AES showed that there was no nanocatalyst present in the final product.

4.3.2 Characterization of the compounds synthesized

To confirm the structure of the compounds synthesized and to know the position of various functional groups and presence of heteroatoms in the compounds, they have been well established by their melting points, various spectral studies including IR, Mass, ^1H NMR, ^{13}C NMR and single crystal X-ray analyses.

4.3.2.1 IR and Mass spectral studies

IR spectra of the compounds synthesized were taken on Shimadzu FT-IR 8400S spectrophotometer using KBr pellets. Mass spectra of the representative compound were obtained using Waters UPLC-TQD Mass spectrometer. The

structure of the compounds **4a-k** has been confirmed by their melting points and spectroscopic data.

Compound **4a** was isolated as white crystalline solid with melting point as 229-231 °C. The IR spectrum showed absorptions at 3312 (-NH), 1619 (-C=O) cm^{-1} which clearly indicated the formation of quinazolinone framework. Further, the presence of molecular ion peak at m/z 319 $(M+1)^+$ in the mass spectrum confirmed the formation of the compound **4a**.

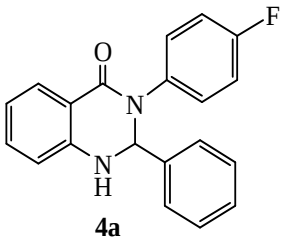
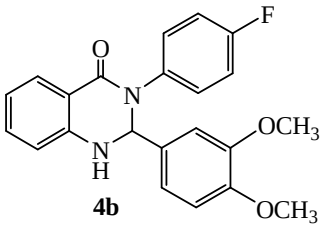
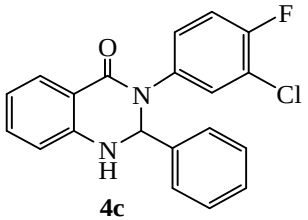
4.3.2.2 ^{1}H NMR and ^{13}C NMR spectral studies

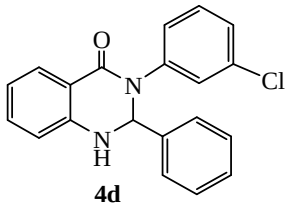
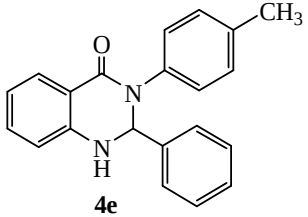
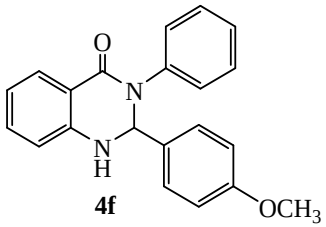
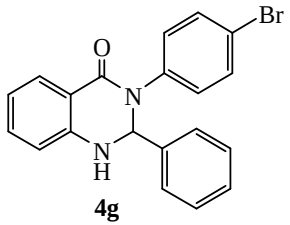
^{1}H NMR and ^{13}C NMR spectra of the compounds synthesized were recorded in DMSO-d_6 and $CDCl_3$ using TMS as an internal standard on a Bruker Avance III spectrophotometer at 400 and 100 MHz.

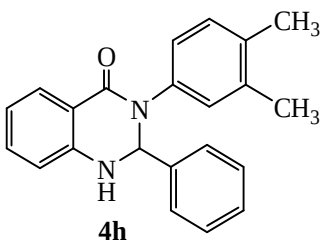
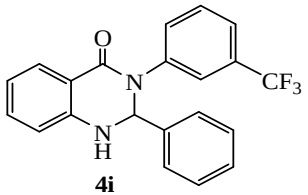
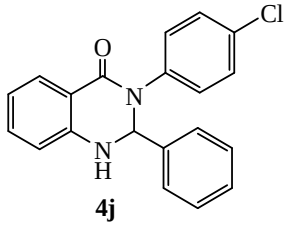
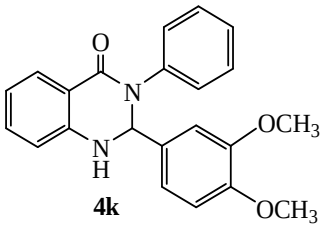
The ^{1}H NMR spectrum of **4a** revealed a sharp double doublet at δ 7.71-7.74 due to the proton present next to the –NH group. The –NH protons in the compound showed one doublet peak at δ 7.58 and the thirteen aromatic protons appeared in the region δ 6.27-7.38 ppm. In ^{13}C NMR spectrum of **4a**, the carbonyl carbon atom is shown to be resonated at δ 162.4. Signals at δ 161.0 can be attributed to the carbon atom of the aniline ring attached to fluorine group and the signals from δ 114.7-158.6 confirmed the presence of aromatic carbons while the carbon atom between the two nitrogen atoms in the pyrimidine ring appeared at δ 72.9 ppm. The relative configuration was also unambiguously confirmed by X-ray crystallography[59] of the compound **4a**.

In the single crystal X-ray structure of the compound **4a**, the quinazoline ring system (N2/C12/C13/C14/C15/C16/C17/C18/N1/C5) is in the plane. The two benzene ring systems attached to the N1 and C5 are perpendicular to the plane of the quinazoline ring system.

Table 4.4: IR, Mass, ^{1}H NMR and ^{13}C NMR spectral data of the compounds synthesized **(4a-k)**

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
1	 **4a**	3312 (-NH), 1619 (-C=O), 1523	319 m/z as $[M+H]^+$ for $C_{20}H_{15}FN_2O$	6.27 (d, *J* = 2.4 Hz, 1H), 6.70-6.74 (m, 1H), 6.74-6.77 (m, 1H), 7.11-7.15 (m, 2H), 7.23-7.31 (m, 6H), 7.36-7.38 (dd, J_1 = 1.6 Hz, J_2 = 1.2 Hz, 2H), 7.58 (d, *J* = 2.4 Hz, 1H), 7.71-7.74 (dd, J_1 = 1.6 Hz, J_2 = 1.2 Hz, 1H)	72.9, 114.7, 115.0, 115.1, 115.4, 117.5, 126.7, 127.9, 128.3, 128.4, 128.7, 128.7, 133.7, 136.8, 136.8, 140.3, 146.7, 158.6, 161.0, 162.4 (C=O)
2	 **4b**	3319 (-NH), 1638 (-C=O), 1507	379 m/z as $[M+H]^+$ for $C_{22}H_{19}FN_2O_3$	3.66 (s, 3H), 3.67 (s, 3H), 6.19 (d, *J* = 2.0 Hz, 1H), 6.72 (t, *J* = 8.0 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.81-6.86 (dd, J_1 = 8.8 Hz, J_2 = 8.8 Hz, 2H), 7.00 (d, *J* = 0.8 Hz, 1H), 7.11-7.15 (m, 2H), 7.25-7.30 (m, 3H), 7.47 (d, *J* = 2.0 Hz, 1H), 7.70-7.72 (dd, J_1 = 1.2 Hz, J_2 = 1.2 Hz,1H)	55.3 (OCH_3), 55.4 (OCH_3), 72.85, 110.7, 111.0, 114.7, 115.1, 115.3, 117.4, 119.2, 127.8, 128.8, 132.3, 133.6, 136.9, 136.9, 146.9, 148.5, 148.8, 158.6, 161.0, 162.6 (C=O)
3	 **4c**	3305 (-NH), 1645 (-C=O), 1473	353 m/z as $[M+H]^+$ for $C_{20}H_{14}ClFN_2O$	6.34 (d, *J* = 1.2 Hz, 1H), 6.73 (t, *J* = 7.6 Hz, 1H), 6.77 (d, *J* = 8.0 Hz, 1H), 7.24-7.32 (m, 5H), 7.34-7.39 (m, 3H), 7.49-7.51 (dd, J_1 = 2.0 Hz, J_2 = 2.4 Hz, 1H), 7.60 (s, 1H), 7.72 (d, *J* = 7.6 Hz, 1H)	72.8, 114.7, 116.4, 116.7, 117.6, 118.9, 119.1, 126.9, 127.5, 127.9, 128.4, 128.5, 128.9, 133.9, 137.4, 139.8, 146.9, 162.5 (C=O)

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
4	 **4d**	3291 (-NH), 1652 (-C=O), 1496	335 m/z as $[M+H]^+$ for $C_{20}H_{15}ClN_2O$	6.30 (s, 1H), 6.70-6.78 (m, 2H), 7.26-7.31 (m, 6H), 7.37 (d, *J* = 8.0 Hz, 4H), 7.63 (s, 1H), 7.72 (d, *J* = 7.6 Hz, 1H)	72.5, 114.8, 115.0, 117.5, 126.6, 127.9, 128.1, 128.4, 128.5, 130.1, 133.8, 139.5, 140.2, 146.6, 162.3 (C=O)
5	 **4e**	3309 (-NH), 1642 (-C=O), 1511	315 m/z as $[M+H]^+$ for $C_{21}H_{18}N_2O$	2.63 (s, 3H), 4.73 (s, 1H), 5.98 (s, 1H), 6.57 (d, *J* = 7.6 Hz, 1H), 7.22-7.29 (m, 1H), 7.33-7.37 (m, 1H), 7.39-7.44 (m, 7H), 7.46-7.49 (m, 2H), 8.19 (d, *J* = 8.0 Hz, 1H)	21.2 (CH_3), 73.9, 114.3, 116.2, 118.7, 125.3, 126.8, 127.5, 127.8, 128.1, 128.2, 128.4, 128.6, 129.4, 135.4, 141.7, 142.6, 148.4, 162.4 (C=O)
6	 **4f**	3296 (-NH), 1628 (-C=O), 1508	331 m/z as $[M+H]^+$ for $C_{21}H_{18}N_2O_2$	3.39 (s, 3H), 5.74 (s, 1H), 6.41-6.69 (m, 2H), 6.92-6.99 (m, 2H), 7.02-7.09 (m, 3H), 7.23-7.41 (m, 4H), 7.47-7.52 (m, 2H), 9.52 (d, *J* = 8.0 Hz, 1H)	55.5 (OCH_3), 69.9, 114.2, 114.7, 121.9, 123.2, 125.0, 126.2, 127.4, 128.7, 129.3, 132.0, 133.8, 142.7, 152.2, 156.4, 161.7, 163.2, 190.8 (C=O)
7	 **4g**	3312 (-NH), 1641 (-C=O), 1520	380 m/z as $[M+H]^+$ for $C_{20}H_{15}BrN_2O$	6.04 (s, 1H), 6.54-6.58 (m, 2H), 6.88-7.02 (m, 2H), 7.25-7.30 (m, 2H), 7.49-7.54 (m, 5H), 7.82-7.87 (m, 2H), 8.19 (d, *J* = 8.0 Hz, 1H)	71.1, 120.9, 121.6, 125.0, 125.7, 126.1, 126.5, 127.2, 127.4, 128.6, 128.8, 131.6, 131.8, 142.3, 148.7, 151.4, 154.6, 162.2 (C=O)

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
8	 **4h**	3296 (-NH), 1634 (-C=O), 1510	329 m/z as $[M+H]^+$ for $C_{22}H_{20}N_2O$	2.15 (s, 3H), 2.15 (s, 3H), 6.20 (d, *J* = 2.4 Hz, 1H), 6.70 (t, *J* = 8.0 Hz, 1H), 6.74 (d, *J* = 8.0 Hz, 1H), 6.92-6.95 (dd, J_1 = 2.0 Hz, J_2 = 2.0 Hz, 1H), 7.05-7.08 (m, 2H), 7.23-7.31 (m, 4H), 7.36 (d, *J* = 6.8 Hz, 2H), 7.56 (d, *J* = 2.4 Hz, 1H), 7.70-7.72 (dd, J_1 = 0.8 Hz, J_2 = 0.8 Hz, 1H)	18.8 (CH_3), 19.3 (CH_3), 72.7, 114.6, 115.4, 117.4, 123.5, 126.4, 127.3, 127.8, 128.2, 128.3, 129.4, 133.5, 134.0, 136.3, 138.5, 140.9, 146.4, 162.0 (C=O)
9	 **4i**	3312 (-NH), 1641 (-C=O), 1527	369 m/z as $[M+H]^+$ for $C_{21}H_{15}F_3N_2O$	4.72 (s, 1H), 5.86 (s, 1H), 6.61 (d, *J* = 8.0 Hz, 2H), 6.73-6.79 (m, 2H), 6.88 (d, *J* = 8.2 Hz, 2H), 7.30-7.32 (m, 3H), 7.42-7.47 (m, 2H), 7.68 (s, 1H), 7.92 (d, *J* = 7.6 Hz, 1H)	74.1, 115.6, 118.9, 124.4, 125.4, 126.0, 126.1, 127.3, 128.0, 129.2, 129.4, 130.8, 131.5, 132.7, 135.3, 137.6, 142.3, 148.7, 162.9 (C=O)
10	 **4j**	3315 (-NH), 1618 (-C=O), 1523	335 m/z as $[M+H]^+$ for $C_{20}H_{15}ClN_2O$	6.10 (s, 1H), 6.62-6.67 (m, 2H), 6.76-6.79 (m, 1H), 7.25-7.30 (m, 10H), 9.76 (d, *J* = 7.6 Hz, 1H)	71.1, 120.9, 121.6, 125.0, 125.7, 126.1, 126.5, 127.2, 127.4, 128.6, 128.8, 131.6, 131.8, 142.3, 148.7, 151.4, 154.6, 162.2 (C=O)
11	 **4k**	3327 (-NH), 1632 (-C=O), 1511	361 m/z as $[M+H]^+$ for $C_{22}H_{20}N_2O_3$	3.59 (s, 3H), 3.61 (s, 3H), 6.09 (d, *J* = 2.0 Hz, 1H), 6.67 (t, *J* = 8.0 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 7.00 (d, *J* = 0.8 Hz, 1H), 7.11-7.15 (m, 4H), 7.25-7.30 (m, 4H), 7.47 (d, *J* = 2.0 Hz, 1H), 7.49-7.52 (dd, J_1 = 1.2 Hz, J_2 = 1.2 Hz, 1H)	55.3 (OCH_3), 55.4 (OCH_3), 71.1, 120.9, 121.6, 125.0, 125.7, 126.1, 126.5, 127.2, 127.4, 128.6, 128.8, 131.6, 131.8, 142.3, 148.7, 151.4, 154.6, 162.2 (C=O)

4.4 RESULTS AND DISCUSSION

4.4.1 Catalytic performance of $CuFe_2O_4$ nanoparticles

In our initial study, we examine the reaction for the synthesis of **4a** using various catalysts such as CuI, CuO, $FeCl_3$, Fe_3O_4 NPs and magnetically separable $CuFe_2O_4$ NPs using aqueous ethanol under refuxing conditions and the results are summarized in Table **4.5**. Among all the transition metal catalysts screened, it was found that the bimetallic copper ferrite nanoparticles successfully promoted this reaction with high isolated yields. Therefore, this catalyst appears to be superior to any of the other catalysts tested.

In the absence of catalyst, lower yield of the product was obtained under the same reaction condition. The quantity of the catalyst plays a vital role for the formation of the desired product. In evaluating the effects of catalyst concentration, the best yields were found in the presence of 10 mol % of copper ferrite NPs. A higher amount of catalyst did not improve the results to an appreciable extent.

Table 4.5: Effect of different catalysts in aqueous ethanol under refluxing conditions

Entry	Catalyst	Time (h)	Yield (%)*
1	-	4	25
2	CuI	2	68
3	CuO	2	70
4	$FeCl_3$	2	63
5	Fe_3O_4 NPs	2	63
6	$CuFe_2O_4$ NPs (5 mol %)	2	89
7	$CuFe_2O_4$ NPs (10 mol %)	2	95
8	$CuFe_2O_4$ NPs (20 mol %)	2	95

* = Isolated Yield

4.4.2 Effect of solvent

Next, for the optimization of the reaction conditions, a screening was performed with a variety of different solvents like methanol, ethanol, toluene, acetonitrile and water (Table **4.6**). We noticed that the polar protic solvents afforded better yield than other solvents and the best catalytic activity of nano copper ferrite (10 mol %) was observed in aqueous ethanol (Entry **2**, Table **4.6**). Hence, the effect of nature of solvent on the reactivity is predominant in the present reaction.

Table 4.6: Optimization for the synthesis of **4a**

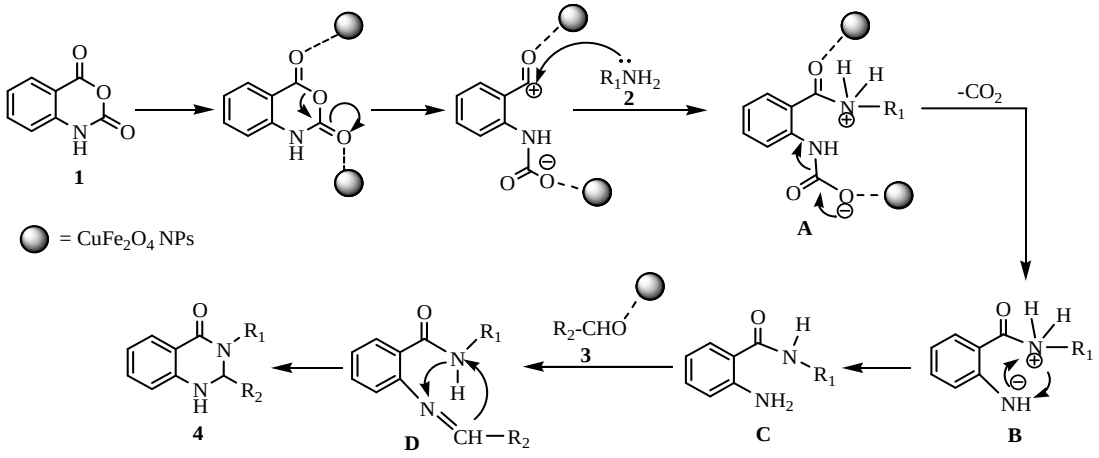
Entry	Solvent	Time (h)	Yield (%)*
1	Methanol	2	79
2	Aqueous Ethanol	2	95
3	Toluene	2	74
4	Acetonitrile	2	82
5	Water	2	80

* = Isolated Yield

To demonstrate the versatility of this technique for the generation of quinazolinone framework, a multicomponent reaction of isatoic anhydride, a variety of anilines and various aldehydes was employed in the presence of copper ferrite nanoparticles as a catalyst in aqueous ethanol under refluxing conditions. (Table **4.3**) The results suggested that the reaction proceeds well in the optimized conditions.

4.4.3 Mechanism

A possible mechanism for the formation of products **4a-k** is shown in Scheme **4.2**. In the first step, the Lewis acidic sites of NPs were coordinated to the oxygen of carbonyl carbon atom which increases its electrophilicity and facilitated the attack of N-nucleophilic amine **2** to form intermediate **A**. Now, the intermediate **A** undergoes decarboxylation reaction to produce another intermediate **B**. The 2-amino-N-substituted amide **C** is formed *via* the proton transfer of the intermediate **B**. Subsequently, the reaction of aldehyde **3** (activated by $CuFe_2O_4$ nanoparticles) with the intermediate **C** proceeds to afford the imine intermediate **D** which is followed by the cyclization to yield the final product **4**.



Scheme 4.2: Proposed mechanism for the synthesis of quinazolin-4(1*H*)-one derivatives

4.5 CONCLUSION

In conclusion, an inexpensive synthetic methodology has been described to synthesize substituted-2,3-dihydroquinazolin-4(1*H*)-one derivatives in refluxing aqueous ethanol. The present methodology uses magnetically separable $CuFe_2O_4$ nanoparticles as a catalyst for the three-component condensation reaction of isatoic anhydride, substituted anilines and various aldehydes affording substituted-2,3-dihydroquinazolin-4(1*H*)-one derivatives. The magnetically separable copper ferrite nanoparticles has been synthesized using a combination of sonochemical and co-precipitation methods in aqueous medium without the use of any surfactant or capping agent. The magnetic nature of $CuFe_2O_4$ nanoparticles is particularly advantageous for easy, quick, and quantitative separation of the catalyst for reuse. The catalyst is mild, magnetically separable and can be easily recyclable for several runs without any noticeable decrease in its efficiency.

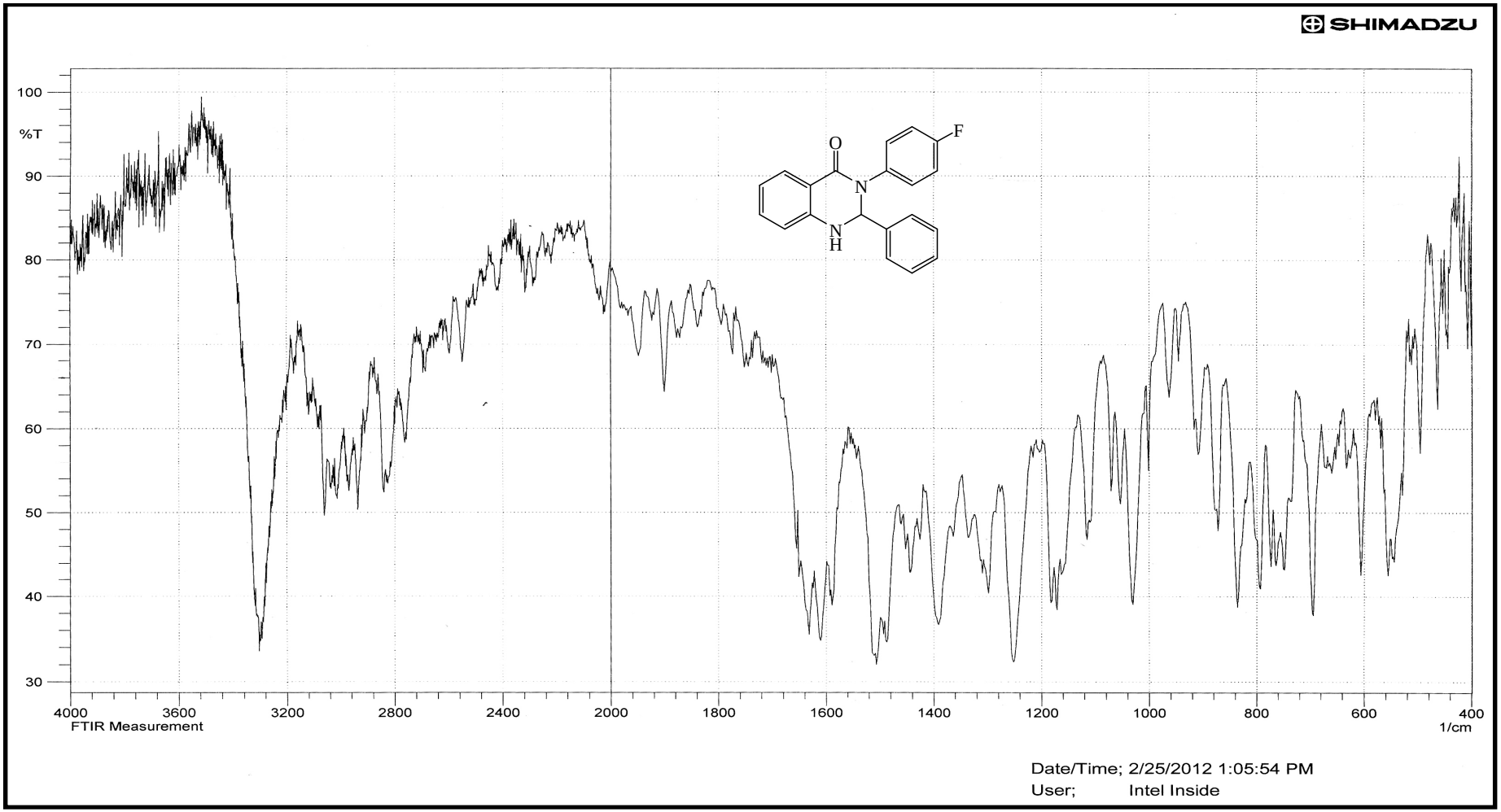


Figure 4.5: FT-IR spectrum of **3-(4-Fluorophenyl)-2-phenyl-2,3-dihydroquinazolin-4(1*H*)-one (4a)**

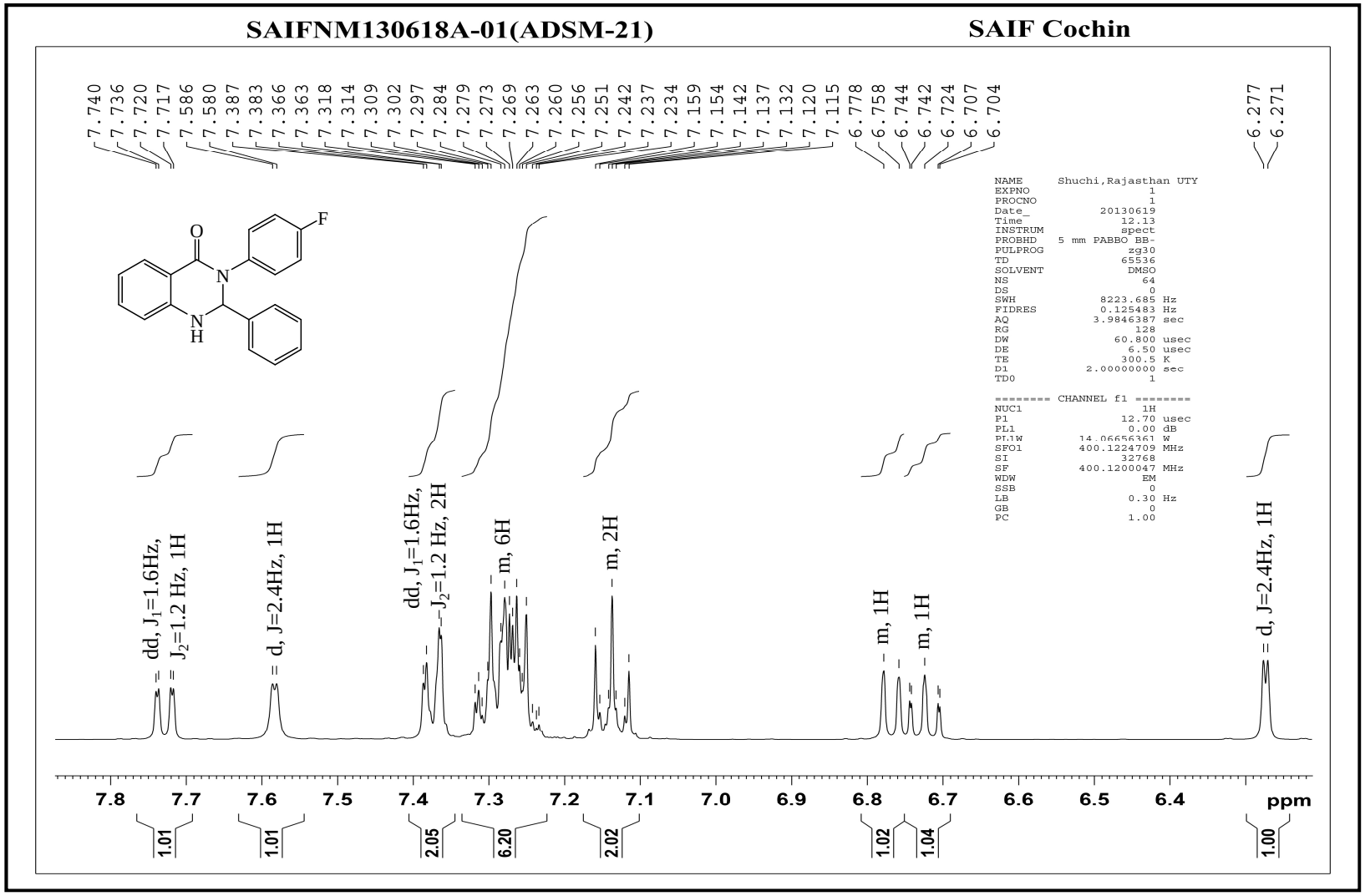


Figure 4.6: ^{1}H NMR spectrum of **3-(4-Fluorophenyl)-2-phenyl-2,3-dihydroquinazolin-4(1*H*)-one (4a)**

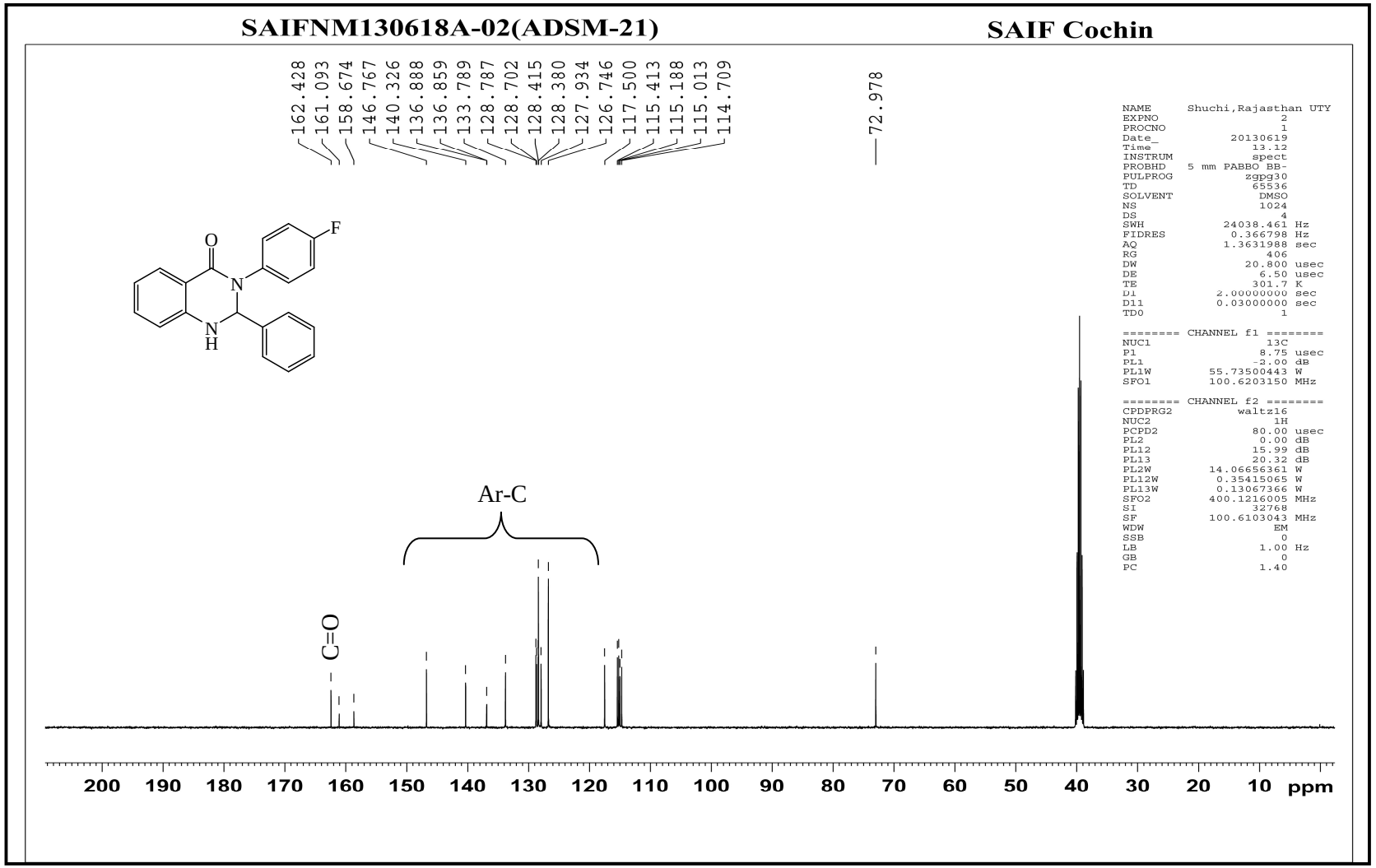


Figure 4.7: ^{13}C NMR spectrum of **3-(4-Fluorophenyl)-2-phenyl-2,3-dihydroquinazolin-4(1*H*)-one (4a)**

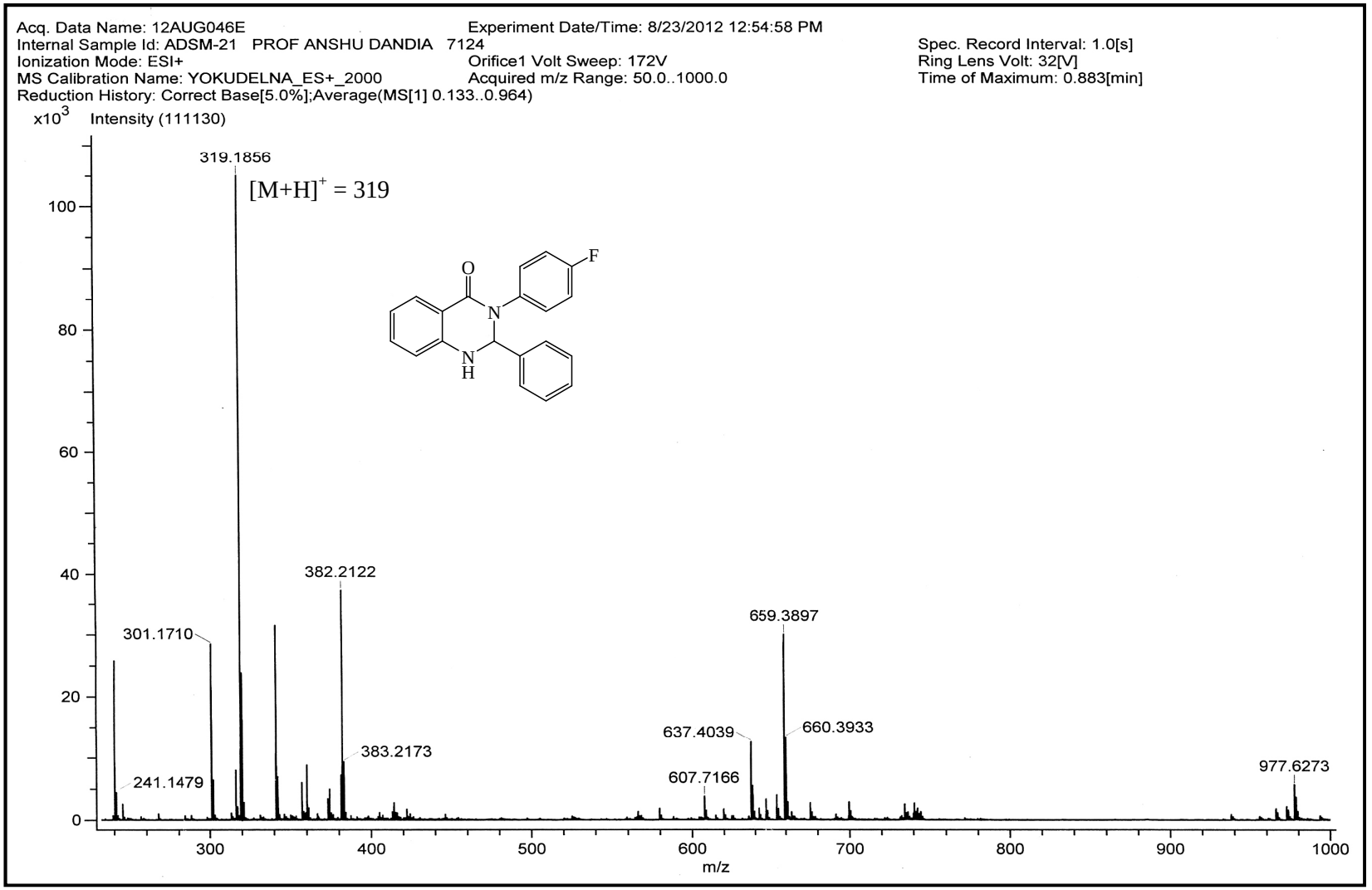


Figure 4.8: Mass spectrum of **3-(4-Fluorophenyl)-2-phenyl-2,3-dihydroquinazolin-4(1*H*)-one (4a)**

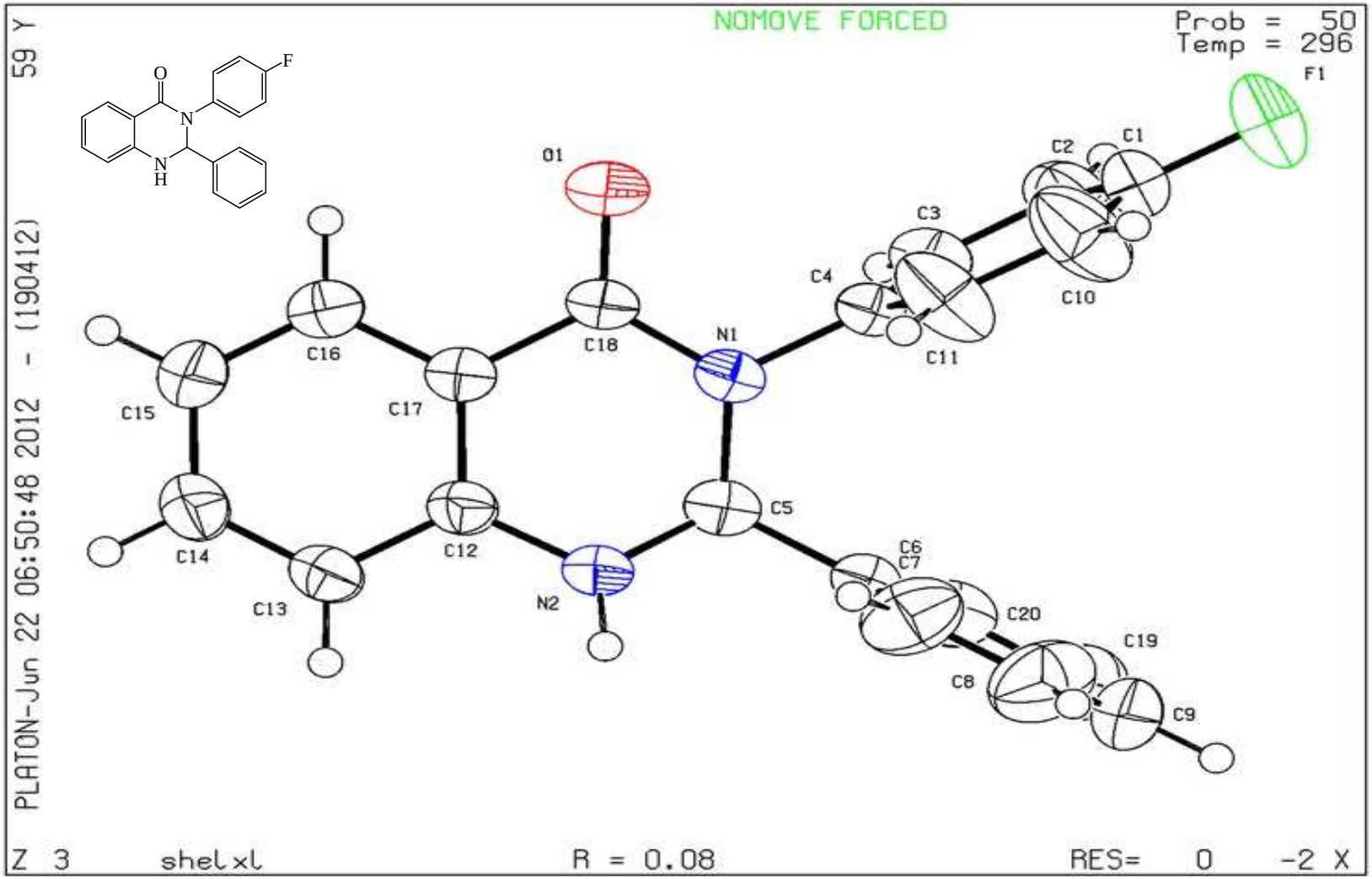


Figure 4.9: ORTEP diagram of **3-(4-Fluorophenyl)-2-phenyl-2,3-dihydroquinazolin-4(1*H*)-one (4a)**

Crystal data and structure refinement for **4a**

Identification code	shelxl	
Empirical formula	$C_{20}\ H_{15}\ F\ N_2\ O$	
Formula weight	318.34	
Temperature	296(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C2/c	
Unit cell dimensions	a = 10.5387(7) Å	α = 90°.
	b = 13.7487(7) Å	β = 96.230(2)°.
	c = 22.3638(13) Å	γ = 90°.
Volume	3221.2(3) $Å^3$	
Z	8	
Density (calculated)	1.313 Mg/m^3	
Absorption coefficient	0.090 mm^{-1}	
F(000)	1328	
Crystal size	0.50 x 0.40 x 0.35 mm^3	
Theta range for data collection	2.54 to 28.33°	
Limiting Index ranges	-13<=h<=14, -18<=k<=17, -29<=l<=29	
Reflections collected	15316	
Independent reflections	4001 [R(int) = 0.0227]	
Completeness to theta = 28.33°	99.4 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9692 and 0.9564	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4001 / 0 / 217	
Goodness-of-fit on F^2	1.030	
Final R indices [I>2sigma(I)]	R1 = 0.0759, wR2 = 0.2276	
R indices (all data)	R1 = 0.0941, wR2 = 0.2494	
Largest diff. peak and hole	0.945 and -0.427 $e.Å^{-3}$	

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59. Crystallographic data for **4a** have been deposited at the Cambridge Crystallographic Data Centre with the deposition number CCDC **1034721**. Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/ deposit (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK; Fax: +44 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk).

CHAPTER 5

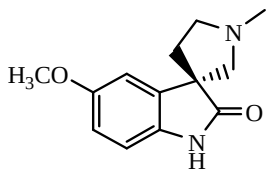
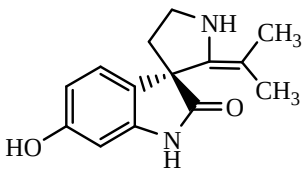
Synthesis and Characterization of Multi-functionalised Spirooxindole Derivatives catalyzed by L-Proline

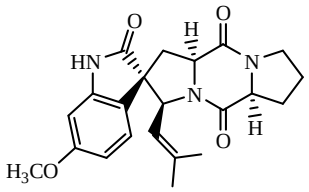
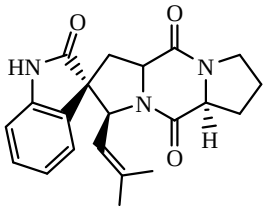
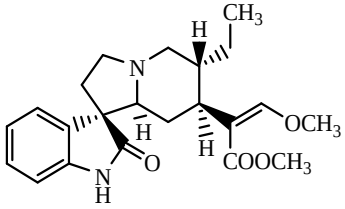
5.1 INTRODUCTION

The chemistry of heterocyclic compounds is one of the most complex branches of chemistry.[1] Heterocyclic compounds are of particular interest in medicinal chemistry. Heterocycles have enormous potential as the most promising molecules as lead structures for the design of new drugs.[2] A vast number of combinations of carbon, hydrogen, and heteroatoms can be designed, providing compounds with the most diverse physical, chemical and biological properties.[3,4]

Spirooxindoles are attractive targets in organic and medicinal chemistry owing to their potency and wide spectrum of biological activities including anti-tumor,[5] antitubercular,[6] anti-microbial,[7] anti-mycobacterial,[8] antifungal,[9] anti-malarial,[10] anti-oxidant,[11] etc. Synthetic or natural heterocyclic compound containing spirooxindole framework is endowed with a wide range of pharmacological activities.[12] Naturally occurring spirooxindole alkaloids, such as horsfiline[13-18] isolated from *Horsfieldia superba* and elacomine[19] isolated from *Elaeagnus commutata* find use as indigenous medicine. Spirotryprostatins A and B[20,21] found in the secondary metabolites of *Aspergillus fugimatus* inhibit mammalian cell cycle at G2/M phase. Rhynchophylline isolated from *Uncaria rhynchophylla* has been used as antipyretic, antihypertensive and anticonvulsant medications for the treatment of headache, vertigo and epilepsy[22] and as noncompetitive antagonists of the NMDA receptor.[23] (**Table 5.1**)

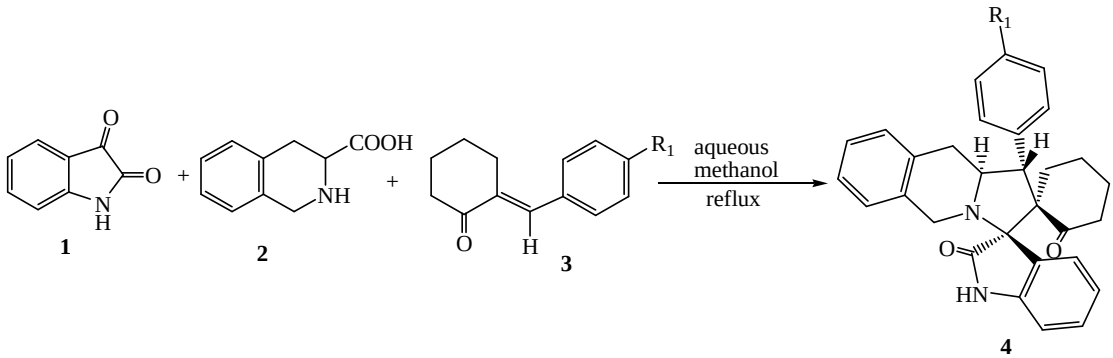
Table 5.1: Some naturally occurring spirooxindole alkaloids

S. No.	Name of the compound	Structure of the compound	Use of the compound[19-22]
1	Horsfiline		Horsfiline is an oxindole alkaloid isolated from the plant *Horsfieldia superba*, is used in traditional herbal medicine. It has analgesic effects.
2	Elacomine		Elacomine, a hemiterpene spirooxindole alkaloid isolated from *Elaeagnus commutata*, has cell cycle inhibition activity.

3	Spirotryprostatin A		Spirotryprostatin A is an indolic alkaloid found in the *Aspergillus fumigatus* fungus. It has been known to have anti-mitotic properties.
4	Spirotryprostatin B		Spirotryprostatin B inhibits mammalian cell cycle at G2/M phase.
5	Rhynchophylline		Rhynchophylline is an alkaloid extracted from the *Uncaria rhynchophylla*. It has been used as antipyretic, antihypertensive and anticonvulsant agent.

The synthetic methods for spirooxindole derivatives have been extensively studied in the past because of the promising biological potential associated with these moieties. This prevalence has led to interests in the development of various methods for the construction of diversely functionalized spirooxindole derivatives which are discussed below:

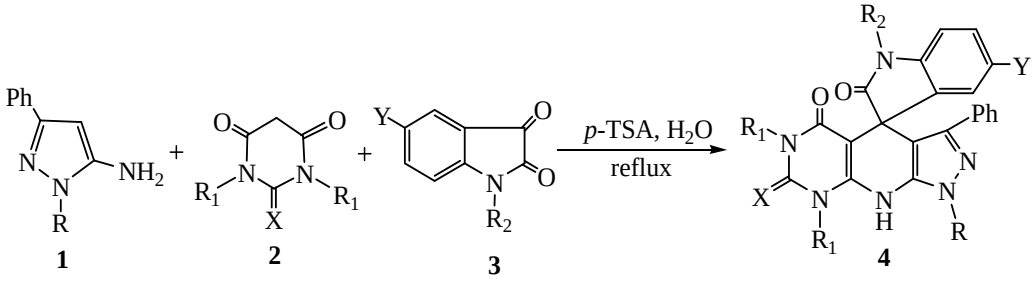
Raghunathan *et al.*[24] has reported an efficient synthesis of dispiro [oxindolecyclohexanone]pyrroloisoquinoline ring system **4** by the 1,3-dipolar cycloaddition reaction of isatin **1**, tetrahydroisoquinoline-3-carboxylic acid **2** and *(E)*-2-arylidene-1-cyclohexanones **3** (Scheme **1**).



Scheme 1

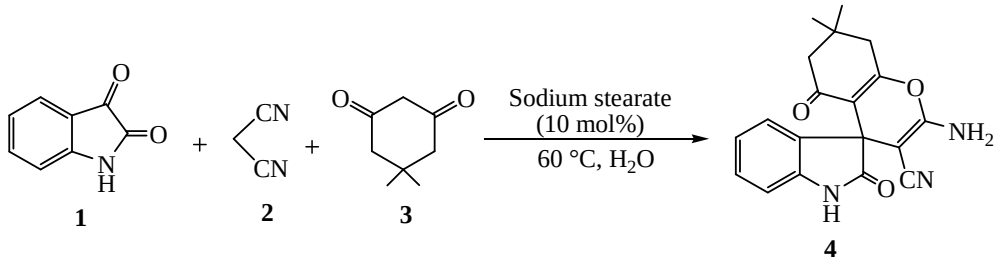
Bazgir *et al.*[25] demonstrated an efficient synthetic protocol to synthesize spiro[indoline-pyrazolopyridopyrimidine]trione derivatives **4** by the three-

component condensation reaction of readily available starting materials such as 1*H*-pyrazol-5-amines **1**, barbituric acids **2** and isatins **3** in aqueous media (Scheme **2**).



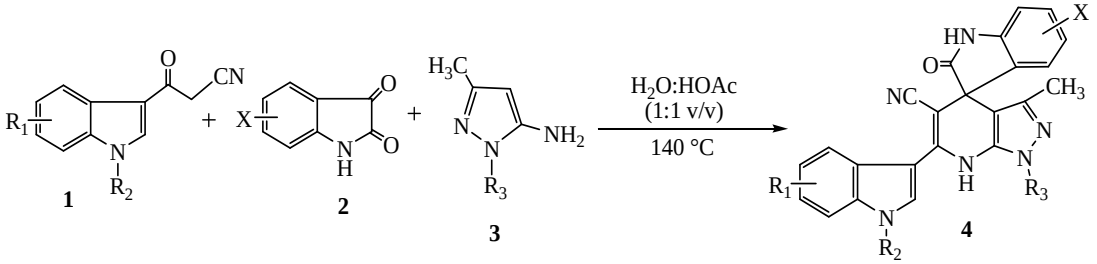
Scheme 2

Wang *et al.*[26] reported a simple and efficient multi-component reaction of isatin **1**, malononitrile **2** and a variety of 1,3-dicarbonyl compounds **3** affording the corresponding spirooxindole pyran derivatives **4** in aqueous micellar media, using sodium stearate as a new type of Lewis base-surfactant-combined catalyst (LBSC) (Scheme **3**).



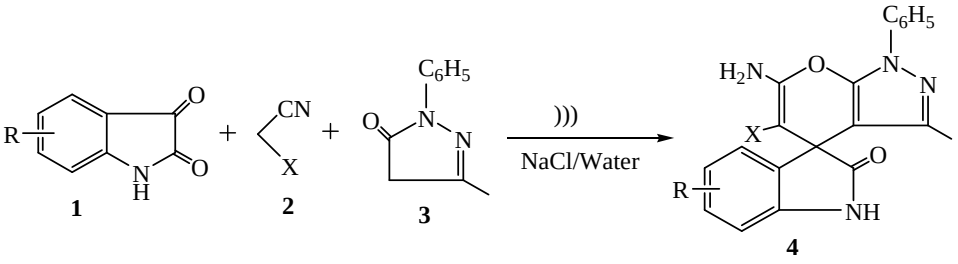
Scheme 3

Ji *et al.*[27] demonstrated a simple and atom economical approach for the synthesis of highly functionalized dihydrospiro[indoline-pyrazolo[3,4-*b*] pyridine]one derivatives **4** from the one-pot, three-component reaction of 3-cyanoacetyl indoles **1**, isatins **2**, and 1*H*-pyrazol-5-amines **3** in H_2O/HOAc in high yields (Scheme **4**).



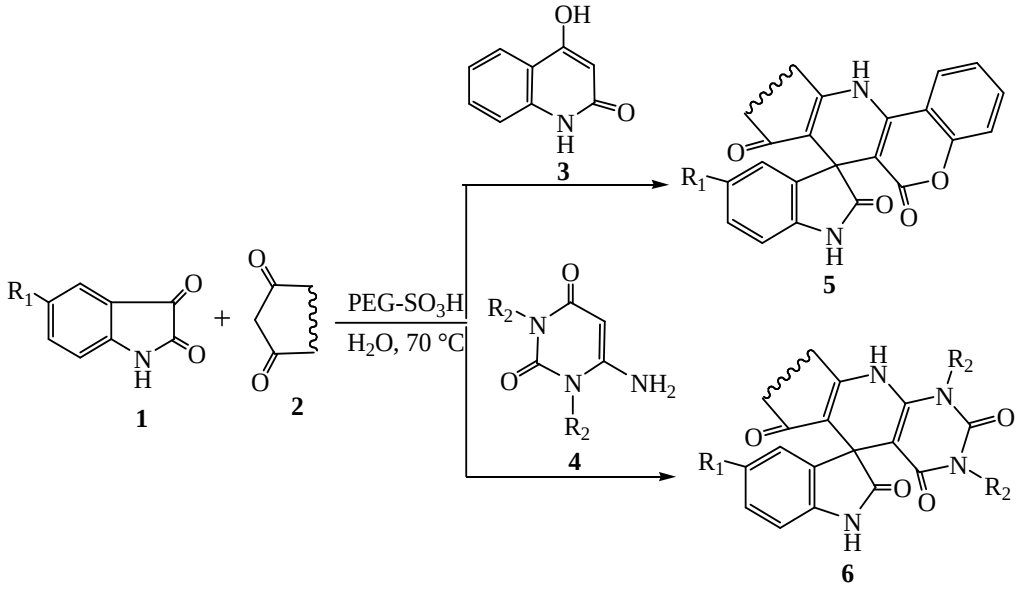
Scheme 4

Dandia *et al.*[28] demonstrated an efficient green method for the synthesis of biologically important spirooxindole pyran derivatives **4** by the one-pot three-component approach involving substituted isatin **1**, activated methylene reagent **2**, and 3-methyl-1-phenyl-2-pyrazolin-5-one **3** in water under sonication catalysed by sodium chloride (Scheme **5**).



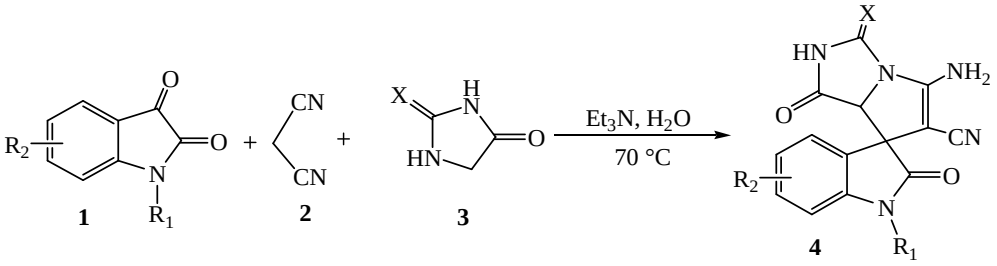
Scheme 5

A highly convergent heteroannulation protocol for the synthesis of a library of coumarin and uracil fused spirooxindole derivatives **5,6** had been developed by Das *et al.*[29] The present reaction involves one-pot three-component domino coupling of a variety of 1,3-diketo compounds such as isatin **1**, a variety of 1,3-dicarbonyl compounds **2**, such as cyclohexane-1,3-dione, indane-1,3-dione, dimedone, and 1,3-dimethylbarbituric acid, etc. and 4-aminocoumarin/6-aminouracil **3,4** respectively mediated by PEG-OSO_3H (Scheme **6**).



Scheme 6

Choudhury *et al.*[30] synthesized pharmacologically proficient spiro[indoline-pyrrolo-imidazole]-6′-carbonitrile derivatives **4** by a sequential one-pot three-component reaction of the readily available starting materials such as isatin **1**, malononitrile **2** and hydantoin or thiohydantoin **3** catalyzed by Et_3N in water (Scheme **7**).



Scheme 7

Although most of the recent methods have their own merits, some methods suffered by some limitations such as low yield, complicated workup procedure and technical intricacy. One of the methods described by Perumal *et al.*[31] also suffers from drawbacks such as use of pre-synthesized isatin malononitrile adducts. In addition scope of cyano substrates have also not been explored. Thus, the development of versatile, eco-friendly multicomponent protocol is highly desirable for an economical synthesis of this class of compounds.

Current research in organic synthesis focuses on economy,[32] the development of rapid and selective synthetic strategies toward focused libraries of functionalized heterocyclic structural units is of great importance to both medicinal as well as organic chemists, and still constitutes a challenge for academic and industrial points of view. In modern organic chemistry, owing to the increasing economic and ecological pressure, investigations are now focused on discovery of methods that largely take into account the criterion of sustainable chemistry.[33]

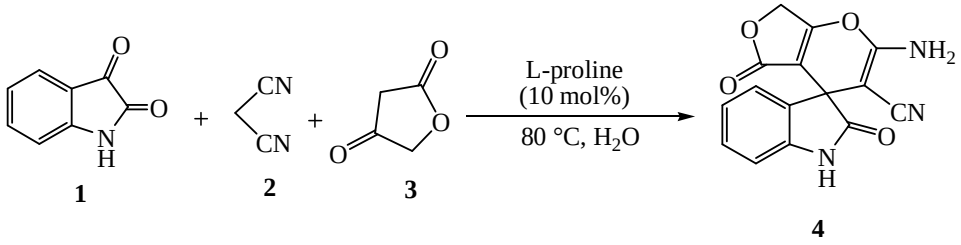
In this context, multicomponent reactions (MCRs)[34,35] which combine at least three or more substrates in a one-pot operation, have emerged as a prominent tool and complementary substrate-directed synthetic alternatives to other known methods.[36-39] Domino reactions have emerged as potent tools to allow the rapid increase of molecular complexity. These processes avoid the excessive handling and isolation of intermediates generated, and reduce waste production.[40] Domino reactions have been reported extensively in the literature and have become ‘state-of-

the-art' in synthetic organic chemistry.[41] Moreover, these transformations amalgamate the various classical concerns such as efficiency, selectivity, molecular complexity and diversity.[42,43]

L-Proline is the more common form of Proline, an α-amino acid, one of the twenty DNA-encoded amino acids. It is unique among the 20 protein-forming amino acids due to the fact that the nitrogen atom of the amine group is bound to two alkyl groups, thus making it a secondary amine. Generally, L-proline has *S* stereochemistry. The characteristic cyclic structure of proline's side chain gives proline an exceptional conformational rigidity as compared to other amino acids. It also affects the rate of peptide bond formation between proline and other amino acids. When proline is bound as an amide in a peptide bond, the nitrogen atom is not bound to any hydrogen atom as it cannot act as a hydrogen bond donor, but it can proceed as hydrogen bond acceptor.[44a]

In recent years, L-proline has drawn a lot of significance in various organic reactions[44b] and has been known to be an eco-friendly and proficient catalyst among the amino acids. The versatile and experimental simplistic nature[44,45] of L-proline has been well illustrated by its considerable catalytic efficiency in many reactions and transformations.[46] Moreover, L-proline is an inexpensive catalyst. Some of the reactions using the L-proline as an organocatalyst[47] are discussed below:

Shi *et al.*[48] reported an efficient L-proline catalyzed multi-component reaction of isatin **1**, malononitrile **2** and a variety of 1,3-dicarbonyl compounds **3** affording the corresponding spirooxindole pyran derivatives **4** in aqueous media (Scheme **8**).

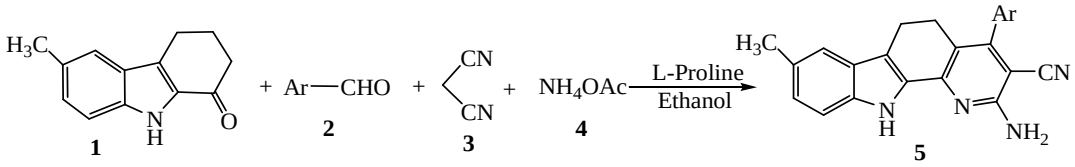


1 **2** **3** **4**

Scheme 8

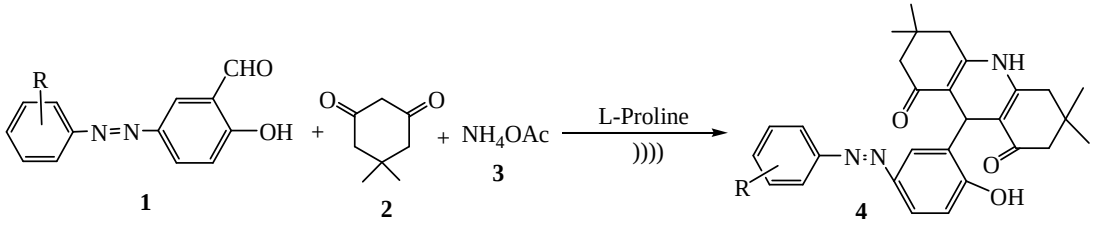
Pyrido[2,3-*a*]carbazoles **4** were prepared by Indumathi *et al.*[49] by the multicomponent reaction of 6-methyl-tetrahydrocarbazol-1-one **1**, benzaldehyde **2**,

malononitrile **3**, and ammonium acetate **4** in dry ethanol using L-proline as a catalyst (Scheme **9**).



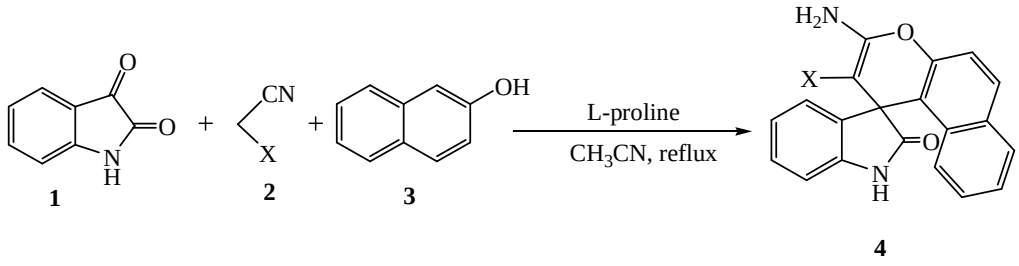
Scheme 9

Ultrasound-assisted multicomponent synthesis of novel derivatives of azo-linked dihydropyridines **4** from azo-linked salicylaldehydes **1**, dimedone **2**, and ammonium acetate **3** in the presence of an inexpensive catalyst, L-proline was reported by Zare *et al*.[50] (Scheme **10**)



Scheme 10

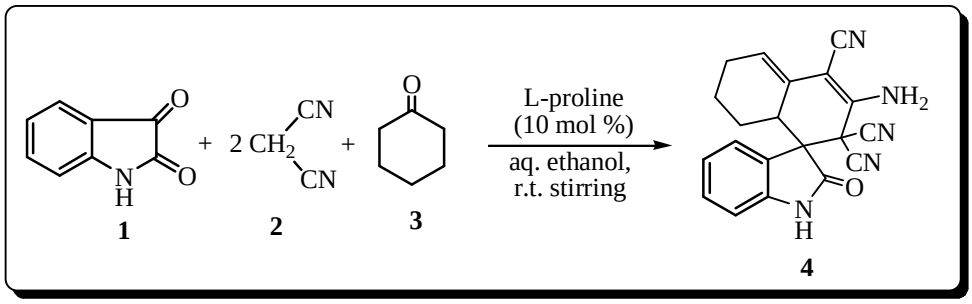
An efficient synthesis of spirooxindoles **4** was accomplished by a one-pot three-component condensation of isatin **1**, malononitrile or cyanoacetic ester **2** and β-naphthol **3** in the presence of L-proline as a catalyst.[51] (Scheme **11**)



Scheme 11

5.2 EXPERIMENTAL

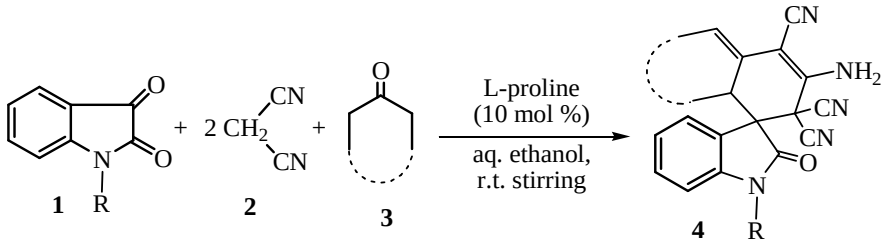
In continuation of our aim to develop new and eco-friendly synthetic methodologies for the synthesis of biologically active spirooxindole derivatives,[52] herein, we report a green and highly efficient L-proline catalyzed synthesis of medicinally important spirooxindole derivatives in aqueous ethanol at room temperature (**Scheme 5.1**).



Scheme 5.1: Synthesis of spirooxindole derivatives

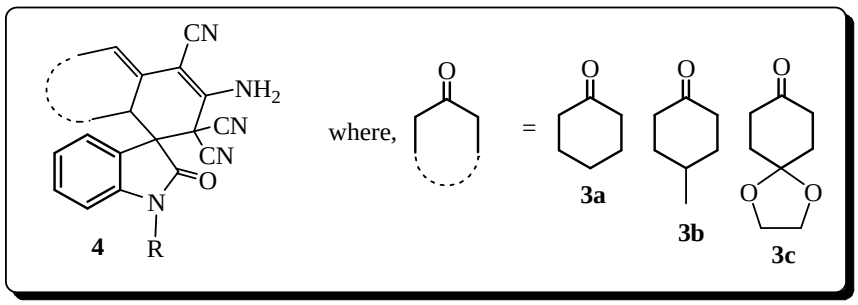
5.2.1 Synthesis of spirooxindole derivatives (4a-l)

A mixture of indole-2,3-dione **1** (0.147 g, 1 mmol), malononitrile **2** (0.132 g, 2 mmol) and cyclohexanone **3** (0.098 g, 1 mmol) was stirred at room temperature in the presence of L-proline (10 mol %) in aqueous ethanol for the appropriate time till a precipitate is formed. Afterwards, when the reaction was complete as shown by TLC, the precipitate was filtered, washed and crystallised from ethanol to afford the pure products **4a-l** (**Scheme 5.2**). All the synthesized compounds are summarized in **Table 5.2**.



Scheme 5.2: Synthesis of spirooxindole derivatives (**4a-l**)

Table 5.2: Synthesis of spirooxindole derivatives (**4a-l**)



Entry	Product	R	Ketone	Time (min.)	Yield (%)*	Mp (^{o}C)	Color
1	**4a**	H	**3a**	75	96	240-242[28]	Off-white
2	**4b**	H	**3b**	70	92	242-244	Off-white
3	**4c**	H	**3c**	60	97	244-246	Off-white
4	**4d**	CH_3	**3a**	45	93	260-262[28]	Off-white
5	**4e**	CH_3	**3b**	40	91	286-288	Off-white
6	**4f**	CH_3	**3c**	33	94	274-276	Off-white
7	**4g**	$CH_2CH=CH_2$	**3a**	56	93	284-286[28]	Off-white
8	**4h**	$CH_2CH=CH_2$	**3b**	44	92	258-260	Off-white
9	**4i**	$CH_2CH=CH_2$	**3c**	35	95	226-228	Off-white
10	**4j**	CH_2Ph	**3a**	42	98	252-254[28]	Off-white
11	**4k**	CH_2Ph	**3b**	34	93	268-270	Off-white
12	**4l**	CH_2Ph	**3c**	25	97	286-288	Off-white

***** = Isolated yield.

5.3 CHARACTERIZATION OF THE COMPOUNDS SYNTHESIZED

To confirm the structure of the compounds synthesized **4a-l** and to know the position of various functional groups in the compounds, they have been well established by their melting points, various spectral studies including IR, Mass, ^{1}H NMR, and ^{13}C NMR analyses.

5.3.1 IR and Mass spectral studies

IR spectra of the compounds synthesized were taken on Shimadzu FT-IR 8400S spectrophotometer using KBr pellets. Mass spectra of the representative compound were obtained recorded on a THERMO Finnigan LCQ Advantage max ion trap mass spectrometer.

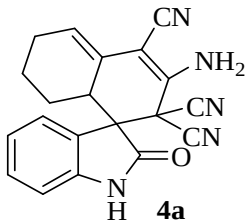
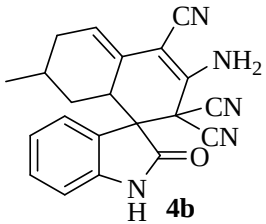
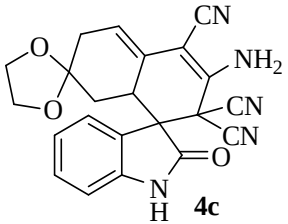
Compound **4e** was isolated as white crystalline solid with melting point as 286-288 °C. The IR spectrum showed absorptions at 3407, 3322 (–NH_2 group), 1629 (–C=O) and 2279 (–C≡N) cm^{-1} which clearly indicated the formation of spirooxindole framework. Further, the presence of peak at m/z 388 as $[M+H_2O]^+$ in the mass spectrum confirmed the formation of the compound **4e**.

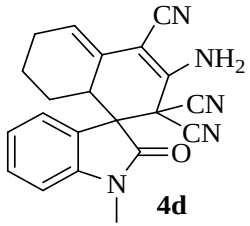
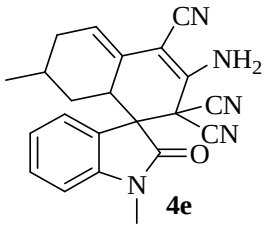
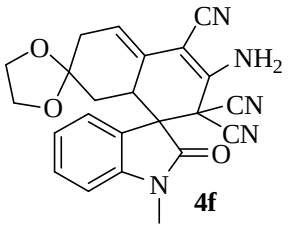
5.3.2 ^{1}H NMR and ^{13}C NMR spectral studies

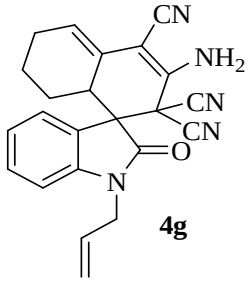
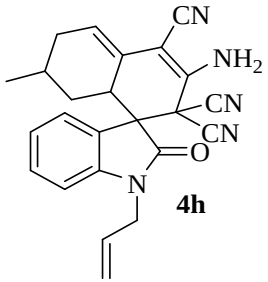
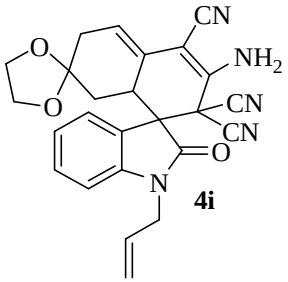
^{1}H NMR and ^{13}C NMR spectra of the compounds synthesized were recorded in DMSO-d_6 using TMS as an internal standard on a Bruker spectrophotometer at 300 and 75 MHz respectively.

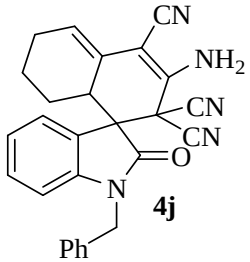
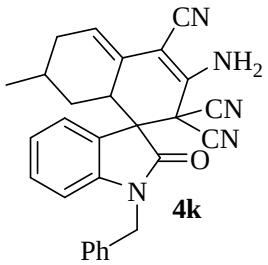
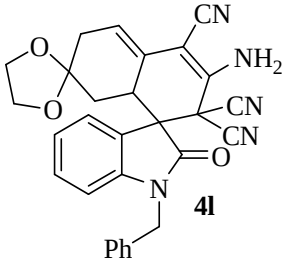
The ^{1}H NMR spectrum of **4e** revealed a sharp singlet at δ 7.57 ppm due to the two protons of the –NH_2 group. The aromatic protons appeared in the region δ 6.90-7.51 ppm in the form of two doublets and two triplets in the spectrum observed. One singlet peak at δ 3.28 ppm indicated the presence of three protons of the methyl group attached to the nitrogen atom of the isatin and the protons of the other methyl group attached to the six-membered ring appeared as doublet at δ 0.77 ppm. In ^{13}C NMR spectrum of **4e**, the carbonyl carbon atom is shown to be resonated at δ 171.9 ppm. Signals from δ 122.2-144.6 ppm confirmed the presence of aromatic carbons while the spiro carbon atom appeared at δ 81.9 ppm. The signals at δ 110.5, 111.0 and 115.9 ppm showed the presence of three cyano groups in the structure of the compound **4e**. And, the signals at δ 21.9 and 23.3 ppm can be attributed to the methyl groups present in the compound.

Table 5.3: IR, Mass, ^{1}H NMR and ^{13}C NMR spectral data of the compounds synthesized **(4a-l)**

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
1	 **4a**	3382, 3207, 2871, 2292, 1739 (C=O), 1592, 776	342 m/z as $[M+H]^+$ for $C_{20}H_{15}N_5O$	0.36 (q, 1H, *J* = 12.3 Hz), 1.68-2.39 (m, 5H), 2.72 (t, 1H, *J* = 10.5 Hz), 5.92 (t, 1H, *J* = 7.8 Hz), 6.92 (d, 1H, *J* = 7.8 Hz), 6.96 (d, 1H, *J* = 7.5 Hz), 7.01 (t, 1H, *J* = 7.8 Hz), 7.30 (t, 1H, *J* = 7.5 Hz), 7.81 (s, 2H, NH_2), 11.14 (s, 1H, NH)	20.0, 26.1, 29.9, 37.0, 52.8, 53.2, 82.3, 107.9, 108.1, 114.1, 115.0, 122.6, 124.9, 125.4, 127.3, 127.6, 140.8, 145.9, 173.2 (C=O)
2	 **4b**	3392, 3215, 2871, 2952, 1741 (C=O), 1589, 753	356 m/z as $[M+H]^+$ for $C_{21}H_{17}N_5O$	0.14 (q, 1H, *J* = 12.2 Hz), 0.75 (d, 3H, *J* = 6.3 Hz), 1.43 (t, 2H, *J* = 13.5 Hz), 2.07- 2.19 (m, 1H), 2.49 (t, 1H, *J* = 1.8 Hz), 2.93 (t, 1H, *J* = 10.2 Hz), 5.74-5.78 (m, 1H), 6.83 (t, 1H, *J* = 6.0 Hz), 6.88 (d, 1H, *J* = 1.5 Hz), 7.17-7.20 (m, 2H), 7.43 (s, 2H, NH_2), 10.82 (s, 1H, NH)	21.8, 28.9, 32.2, 33.8, 49.0, 53.0, 82.2, 110.0, 115.5, 117.1, 121.5, 122.3, 124.5, 127.0, 127.8, 129.9, 142.7, 147.9, 159.5, 174.6 (C=O)
3	 **4c**	3402, 3385, 2922, 2316, 1723 (C=O), 1564, 812	400 m/z as $[M+H]^+$ for $C_{22}H_{17}N_5O_3$	1.47 (t, 1H, *J* = 7.8 Hz), 2.25 (d, 2H, *J* = 18.6 Hz), 3.17 (d, 2H, *J* = 5.1 Hz), 3.72-3.94 (m, 4H), 5.78 (t, 1H, *J* = 2.1 Hz), 6.85 (d, 1H, *J* = 7.8 Hz), 6.96 (d, 1H, *J* = 4.2 Hz), 7.04 (t, 1H, *J* = 7.8 Hz), 7.40 (t, 1H, *J* = 7.8 Hz), 10.8 (s, 2H, NH_2), 11.40 (s, 1H, NH)	23.5, 35.5, 36.5, 42.1, 54.0, 80.6, 105.8, 110.0, 110.6, 110.8, 115.3, 118.3, 120.7, 122.0, 123.8, 124.7, 129.7, 131.1, 147.9, 158.9, 172.8 (C=O)

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
4	 **4d**	3395, 3362, 3207, 2871, 2292, 1729 (C=O), 1605, 1473, 776	356 m/z as $[M+H]^+$ for $C_{21}H_{17}N_5O$	0.29 (q, 1H, *J* = 11.7 Hz), 1.33-2.25 (m, 5H), 3.02 (d, 1H, *J* = 10.2 Hz), 3.28 (s, 3H), 5.90 (t, 1H, *J* = 7.8 Hz), 6.92 (d, 1H, *J* = 7.5 Hz), 7.15 (t, 1H, *J* = 7.5 Hz), 7.25 (d, 1H, *J* = 7.8 Hz), 7.49 (t, 1H, *J* = 7.8 Hz), 7.72 (s, 2H, NH_2)	20.8, 23.6, 25.1, 27.3, 31.9, 36.8, 43.3, 55.7, 83.0, 110.4, 111.3, 112.3, 115.8, 121.7, 122.4, 124.5, 124.9, 125.7, 132.3, 143.7, 171.7 (C=O)
5	 **4e**	3407, 3322, 3211, 2981, 2279, 1731 (C=O), 1629, 1503, 767	388 m/z as $[M+H_2O]^+$ for $C_{22}H_{19}N_5O$	0.16 (q, 1H, *J* = 12.3 Hz), 0.77 (d, 3H, *J* = 6.3 Hz), 1.33-1.70 (m, 4H), 3.02 (d, 1H, *J* = 10.2 Hz), 3.28 (s, 3H), 5.90 (s, 1H), 6.92 (d, 1H, *J* = 7.8 Hz), 7.15 (t, 1H, *J* = 7.5 Hz), 7.25 (d, 1H, *J* = 7.8 Hz), 7.49 (t, 1H, *J* = 7.8 Hz), 7.57 (s, 2H, NH_2)	21.9, 23.3, 27.1, 32.0, 33.8, 35.4, 38.0, 42.7, 54.6, 81.9, 110.5, 111.0, 115.9, 122.2, 124.0, 124.1, 125.1, 125.6, 131.5, 142.7, 144.6, 171.9 (C=O)
6	 **4f**	3369, 3202, 2917, 2318, 1704 (C=O), 1661, 1462, 784	414 m/z as $[M+H]^+$ for $C_{23}H_{19}N_5O_3$	0.68 (q, 1H, *J* = 12.3 Hz), 1.10 (d, 2H, *J* = 7.2 Hz), 2.18 (d, 1H, *J* = 10.7 Hz), 3.19 (s, 3H), 3.69-3.76 (m, 4H), 5.57 (t, 1H, *J* = 7.5 Hz), 6.89 (d, 1H, *J* = 7.8 Hz), 7.12 (t, 1H, *J* = 7.8 Hz), 7.31 (d, 1H, *J* = 7.8 Hz), 7.42 (t, 1H, *J* = 7.8 Hz), 7.89 (s, 2H, NH_2)	23.1, 27.9, 31.1, 34.8, 41.3, 65.7, 107.8, 110.9, 112.5, 113.3, 114.6, 120.8, 122.4, 123.9, 125.6, 127.4, 129.6, 130.7, 132.2, 144.9, 148.5, 173.9 (C=O)

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
7	 **4g**	3374, 3312, 3194, 2903, 2307, 1729 (C=O), 1672, 1498, 779	382 m/z as $[M+H]^+$ for $C_{23}H_{19}N_5O$	0.28 (q, 1H, *J* = 12.3 Hz), 1.53-2.48 (m, 5H), 2.81 (t, 1H, *J* = 10.2 Hz), 4.32-4.47 (m, 2H), 5.22-5.27 (m, 2H), 5.76-5.87 (m, 2H), 6.90 (d, 1H, *J* = 7.2 Hz), 6.99 (t, 1H, *J* = 7.5 Hz), 7.11 (t, 1H, *J* = 7.5 Hz), 7.36 (d, 1H, *J* = 7.5 Hz), 7.67 (s, 2H, NH_2)	24.3, 27.2, 29.7, 31.1, 33.5, 38.9, 42.8, 53.0, 81.6, 110.7, 110.9, 116.2, 118.7, 121.4, 123.9, 124.6, 125.2, 132.1, 143.3, 172.7 (C=O)
8	 **4h**	3381, 3216, 2867, 2291, 1710 (C=O), 1602, 1477, 759	396 m/z as $[M+H]^+$ for $C_{24}H_{21}N_5O$	0.17 (q, 1H, *J* = 11.7 Hz), 0.75 (d, 3H, *J* = 6.3 Hz), 2.47-2.50 (m, 5H), 3.02 (t, 1H, *J* = 10.8 Hz), 4.43-4.48 (m, 2H), 5.18-5.24 (m, 2H), 5.79-5.93 (m, 2H), 6.92 (d, 1H, *J* = 7.2 Hz), 7.14 (t, 1H, *J* = 7.8 Hz), 7.42 (d, 1H, *J* = 6.9 Hz), 7.59 (s, 2H, NH_2)	27.5, 32.1, 33.7, 38.2, 42.7, 54.5, 81.9, 110.6, 110.8, 110.9, 115.9, 117.8, 122.1, 124.1, 124.1, 125.2, 125.5, 131.4, 142.7, 143.7, 171.8 (C=O)
9	 **4i**	3376, 3220, 2903, 2315, 1709 (C=O), 1642, 1381, 749	440 m/z as $[M+H]^+$ for $C_{25}H_{21}N_5O_3$	0.63 (t, 1H, *J* = 12.0 Hz), 1.04 (t, 2H, *J* = 6.9 Hz), 2.19 (d, 2H, *J* = 3.0 Hz), 3.82-3.92 (m, 4H), 4.39-4.45 (m, 3H), 5.18-5.22 (m, 2H), 5.77-5.79 (m, 1H), 6.94-7.02 (m, 4H), 7.70 (s, 2H, NH_2)	32.6, 35.8, 37.2, 42.6, 64.4, 106.1, 110.5, 111.3, 115.7, 118.3, 121.1, 121.7, 123.6, 124.3, 125.0, 125.3, 126.4, 130.1, 131.6, 143.2, 143.6, 171.5 (C=O)

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
10	 **4j**	3354, 3210, 2856, 2311, 1725 (C=O), 1627, 1488, 782	432 m/z as $[M+H]^+$ for $C_{27}H_{21}N_5O$	0.12 (q, 1H, *J* = 11.7 Hz), 1.31-2.84 (m, 5H), 3.01 (t, 1H, *J* = 7.8 Hz), 4.98 (s, 2H), 6.64 (s, 1H), 6.87 (d, 1H, *J* = 7.5 Hz), 7.08 (t, 1H, *J* = 7.5 Hz), 7.24 (d, 1H, *J* = 7.2 Hz), 7.29-7.32 (m, 3H), 7.36-7.45 (m, 3H), 7.64 (s, 2H, NH_2)	26.9, 31.5, 33.7, 38.3, 43.2, 54.7, 81.5, 110.1, 110.9, 115.3, 122.5, 124.2, 125.5, 127.3, 128.8, 129.1, 132.5, 133.7, 135.5, 137.2, 144.6, 173.4 (C=O)
11	 **4k**	3348, 3207, 2831, 2308, 1715 (C=O), 1607, 1479, 1387, 788	446 m/z as $[M+H]^+$ for $C_{28}H_{23}N_5O$	0.07 (q, 1H, *J* = 11.7 Hz), 0.70 (d, 3H, *J* = 6.3 Hz), 1.29 (d, 1H, *J* = 12.0 Hz), 2.47-2.50 (m, 3H), 3.05 (t, 1H, *J* = 10.5 Hz), 5.06 (s, 2H), 5.88 (t, 1H, *J* = 2.7 Hz), 6.92 (d, 1H, *J* = 7.2 Hz), 7.12 (t, 1H, *J* = 8.1 Hz), 7.22 (d, 1H, *J* = 7.8 Hz), 7.27-7.29 (m, 2H), 7.32-7.35 (m, 1H), 7.37-7.44 (m, 3H), 7.60 (s, 2H, NH_2)	27.3, 32.1, 33.6, 38.4, 43.9, 54.4, 81.9, 110.6, 110.8, 111.0, 115.9, 122.2, 124.1, 124.2, 125.2, 125.4, 128.0, 128.2, 129.0, 131.4, 136.0, 142.7, 143.7, 172.2 (C=O)
12	 **4l**	3415, 3316, 2916, 2311, 1713 (C=O), 1618, 1450, 816	490 m/z as $[M+H]^+$ for $C_{29}H_{23}N_5O_3$	0.34 (q, 1H, *J* = 12.3 Hz), 1.14 (d, 2H, *J* = 7.2 Hz), 1.36 (d, 2H, *J* = 10.5 Hz), 3.70-3.76 (m, 4H), 5.02 (s, 2H), 5.64 (t, 1H, *J* = 7.2 Hz), 6.92-6.99 (m, 4H), 7.04-7.40 (m, 5H), 7.88 (s, 2H, NH_2)	21.8, 24.3, 28.1, 41.3, 42.5, 63.8, 108.3, 110.6, 111.4, 115.7, 122.2, 122.8, 123.5, 124.6, 124.7, 125.3, 126.1, 126.9, 128.6, 129.3, 130.2, 130.6, 131.2, 131.7, 145.3, 145.8, 174.5 (C=O)

5.4 RESULTS AND DISCUSSION

The choice of an appropriate reaction medium is of crucial importance for successful synthesis. Initially, the pseudo four component reaction of isatin **1a**, malononitrile **2** and cyclohexanone **3a** using 10 mol % of L-proline as a simple model substrate was investigated to establish the feasibility of the strategy and to optimize the reaction conditions (**Scheme 5.1**). Different solvents such as water, ethanol, tetrahydrofuran, dimethylformamide, acetonitrile, dichloromethane, 1,4-dioxane, and toluene were explored. The results are summarized in **Table 5.3**.

Table 5.4: Optimization of the reaction conditions for the synthesis of **4a**

S. No.	Solvent	L-proline (mol %)	Time (min.)	Yield(%)[a]
1	Water	10	240	44
2	Ethanol	10	90	82
3	Aq. ethanol	10	75	96
4	THF	10	240	38
5	DMF	10	180	62
6	Acetonitrile	10	260	48
7	DCM	10	360	26
8	1,4-dioxane	10	420	39
9	Toluene	10	360	12
10	Aq. ethanol	5	75	89
11	Aq. ethanol	15	75	96

[a] = Isolated yield.

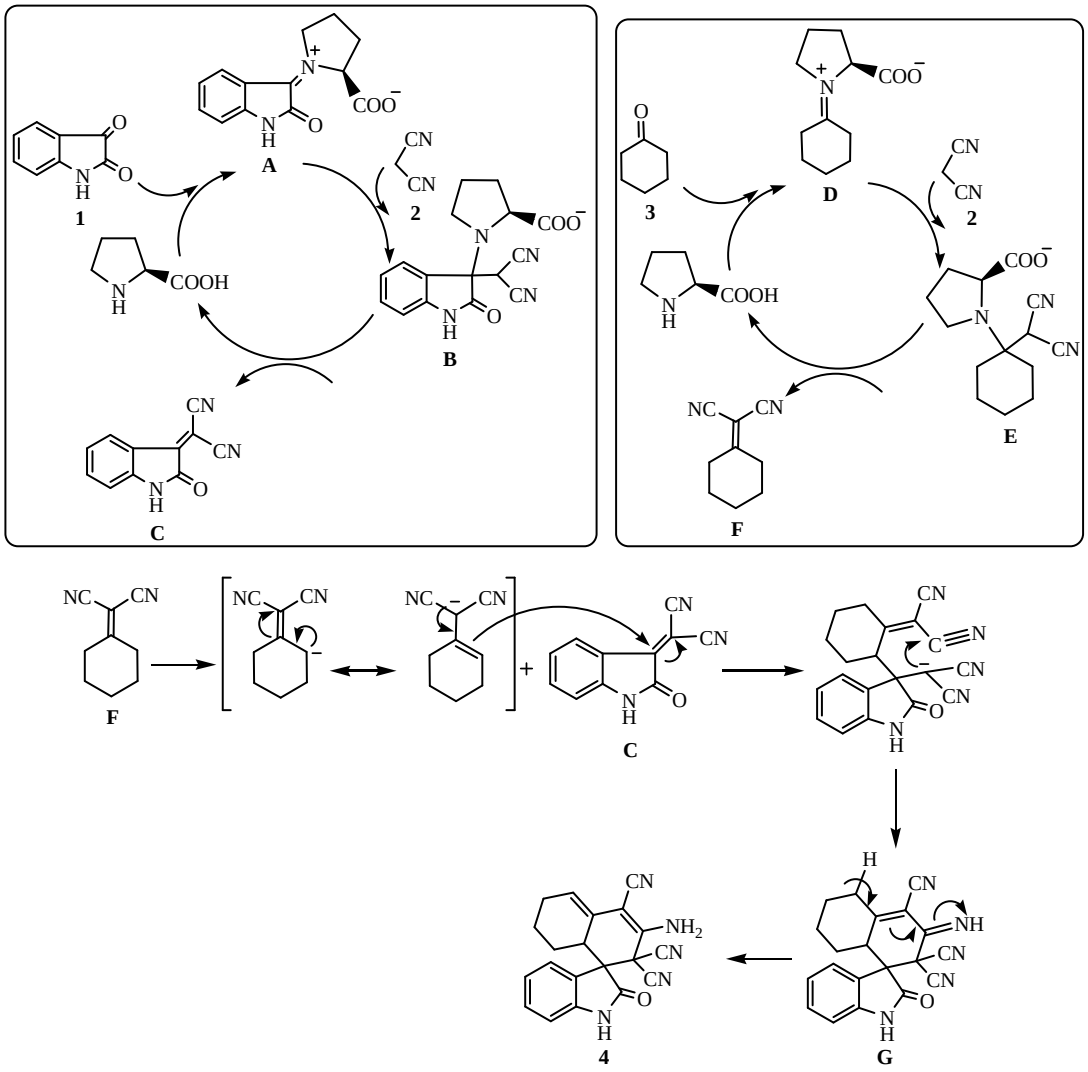
It was observed that a polar protic solvent such as ethanol is found to be very effective for good product yield. However, in case of water, the yield is observed to be lower as compared to ethanol due to the low solubility of the reactants. It is well known that, if the charged species are involved as in case of Knoevenagel reaction, the transition-state is better solvated by polar solvents in homogeneous phase, decreasing the activation free enthalpy and enhancing rate and hence increase the product yield.[53] When the reaction was carried out in 1:4 water-ethanol mixture (aq. ethanol), we obtained 96% isolated yield of the corresponding product. However, the use of other solvents such as THF, DMF, CH_3CN, DCM and toluene did not produce good results.

The reaction was further examined in presence of different amount of catalyst (Entry **10**, **11**; **Table 5.4**). The yield generally increased with the increasing concentration of the catalyst from 5 mol % to 10 mol %. However, further increase of the molar concentration of the catalyst from 10 to 15 mol % did not significantly increase the yield of the product. Hence, a concentration of 10 mol % of L-proline was chosen for the most favorable yield of spirooxindole derivatives.

After optimization of the reaction conditions, to delineate this approach, particularly in regard to library construction, this methodology was evaluated by using different isatins, malononitrile and cyclic ketones under similar reaction conditions to furnish the respective spirooxindole derivatives **(4a-l)** in excellent yields (**Table 5.2**) without the formation of any side products.

5.4.1 Mechanism

A plausible mechanism for the synthesis of spirooxindole derivatives **(4a-l)** is depicted in Scheme **5.3**. In the first step, it is believed that L-proline activated isatin **1** which facilitates the formation of the iminium ion **A**. Due to the higher reactivity of the iminium ion **A**, it is assumed that the Knoevenagel condensation reaction of isatin and malononitrile is facilitated to produce isatylidene malononitrile **C**. Similarly, vinyl malononitrile **F** is produced by the L-proline catalysed Knoevenagel condensation reaction of cyclohexanone and malononitrile. **F** furnishes vinylogous carbanion which attacks on isatylidene malononitrile **C** *via* Michael addition reaction. This step is followed by an intramolecular nucleophilic addition on the cyano group of **F** which produces another intermediate **G**. The intermediate **G** undergoes isomerisation to afford the desired product **4**.



Scheme 5.3: Plausible mechanism for the synthesis of spirooxindole derivatives **(4a-l)**

5.5 CONCLUSION

In conclusion, a facile, efficient and eco-friendly methodology has been built up to synthesize spirooxindole derivatives using L-proline as an organocatalyst in aqueous ethanol. Easy separation of the catalyst eliminates the requirement of catalyst filtration after completion of the reaction, which is an additional greener aspect of this reaction. Preliminary *in vitro* results of various biological screening of the title compounds evidenced that some of the compounds have shown good to significant inhibitory activity.

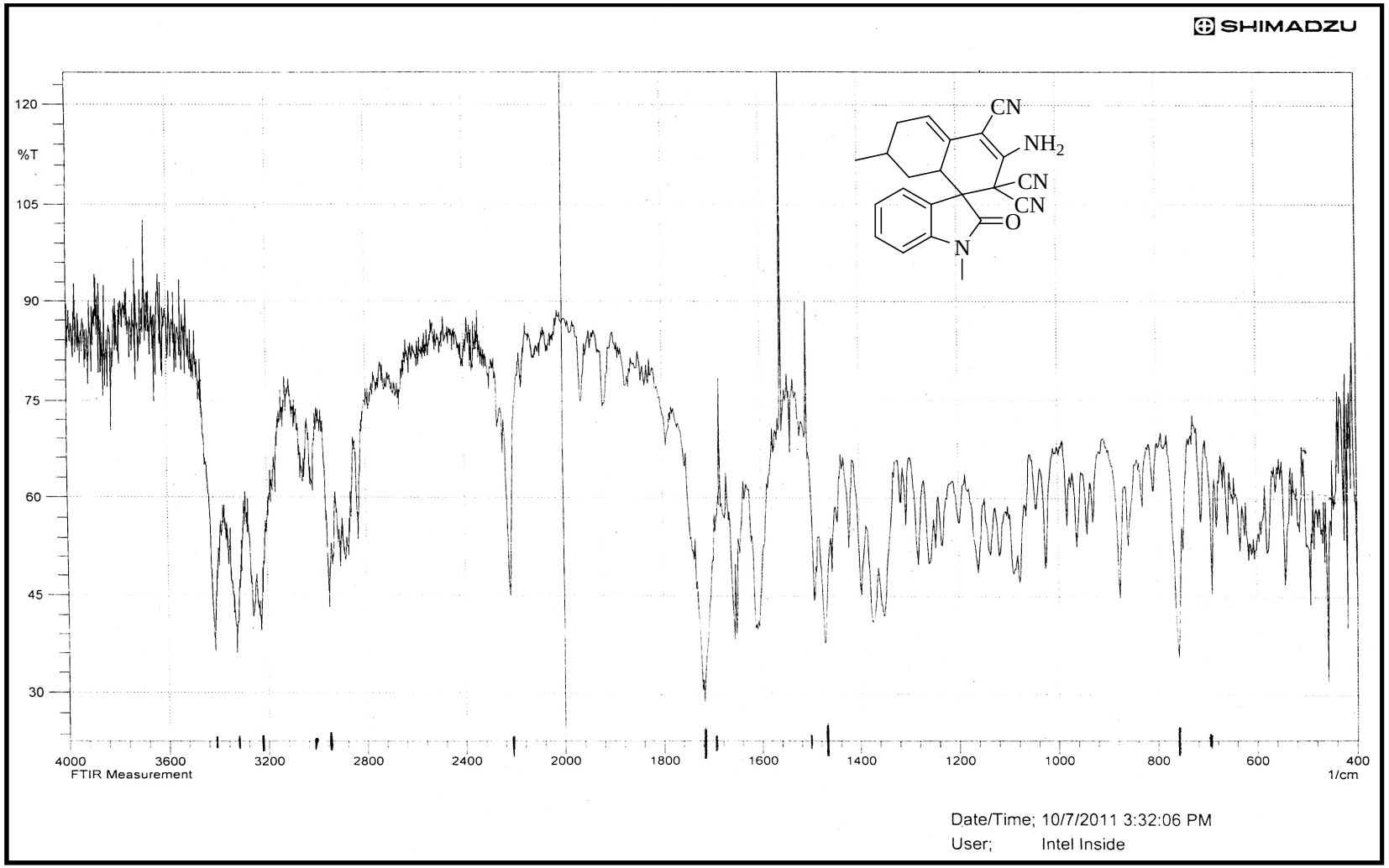


Figure 5.1: FT-IR spectrum of **3′ amino-1,7′-dimethyl-2-oxo-6′,7′,8′,8′a-tetrahydro-2′*H*-spiro[indoline-3,1′-naphthalene]-2′,2′,4′-tricarbonitrile (4e)**

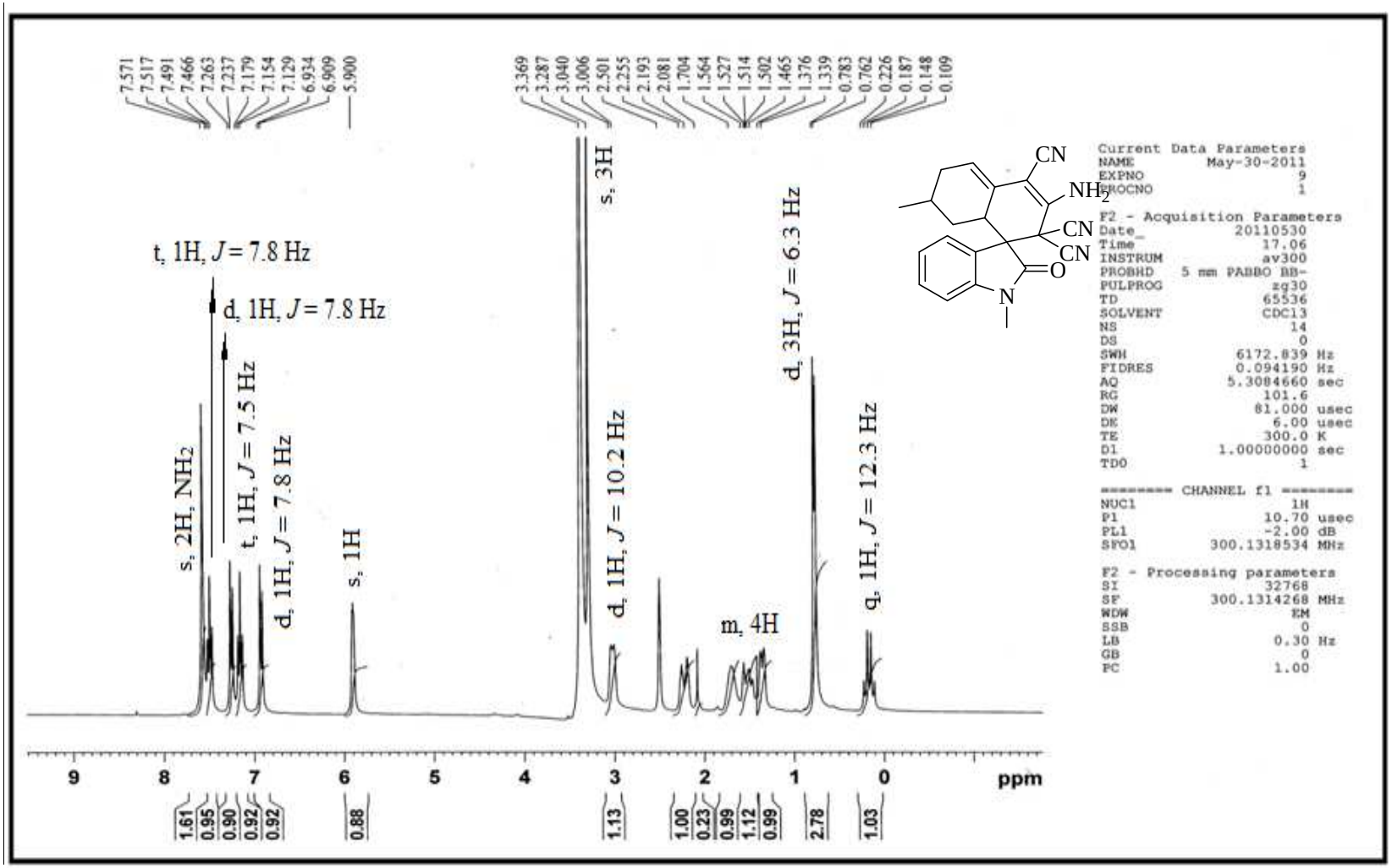


Figure 5.2: ^{1}H NMR spectrum of **3´ amino-1,7´-dimethyl-2-oxo-6´,7´,8´,8´a-tetrahydro-2´*H*-spiro[indoline-3,1´-naphthalene]-2´,2´,4´-tricarbonitrile (4e)**

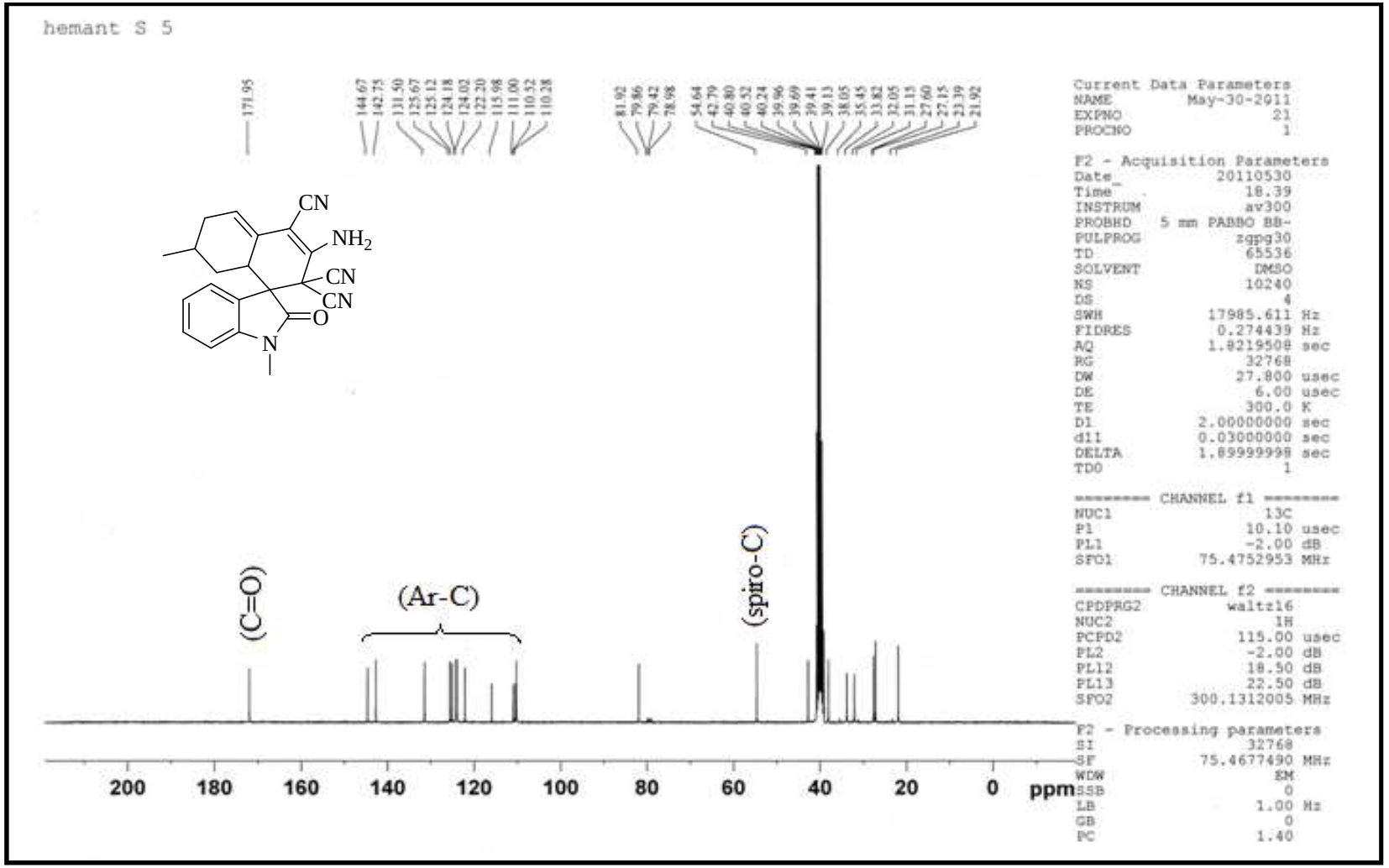


Figure 5.3: ^{13}C NMR spectrum of **3′ amino-1,7′-dimethyl-2-oxo-6′,7′,8′,8′a-tetrahydro-2′*H*-spiro[indoline-3,1′-naphthalene]-2′,2′,4′-tricarbonitrile (4e)**

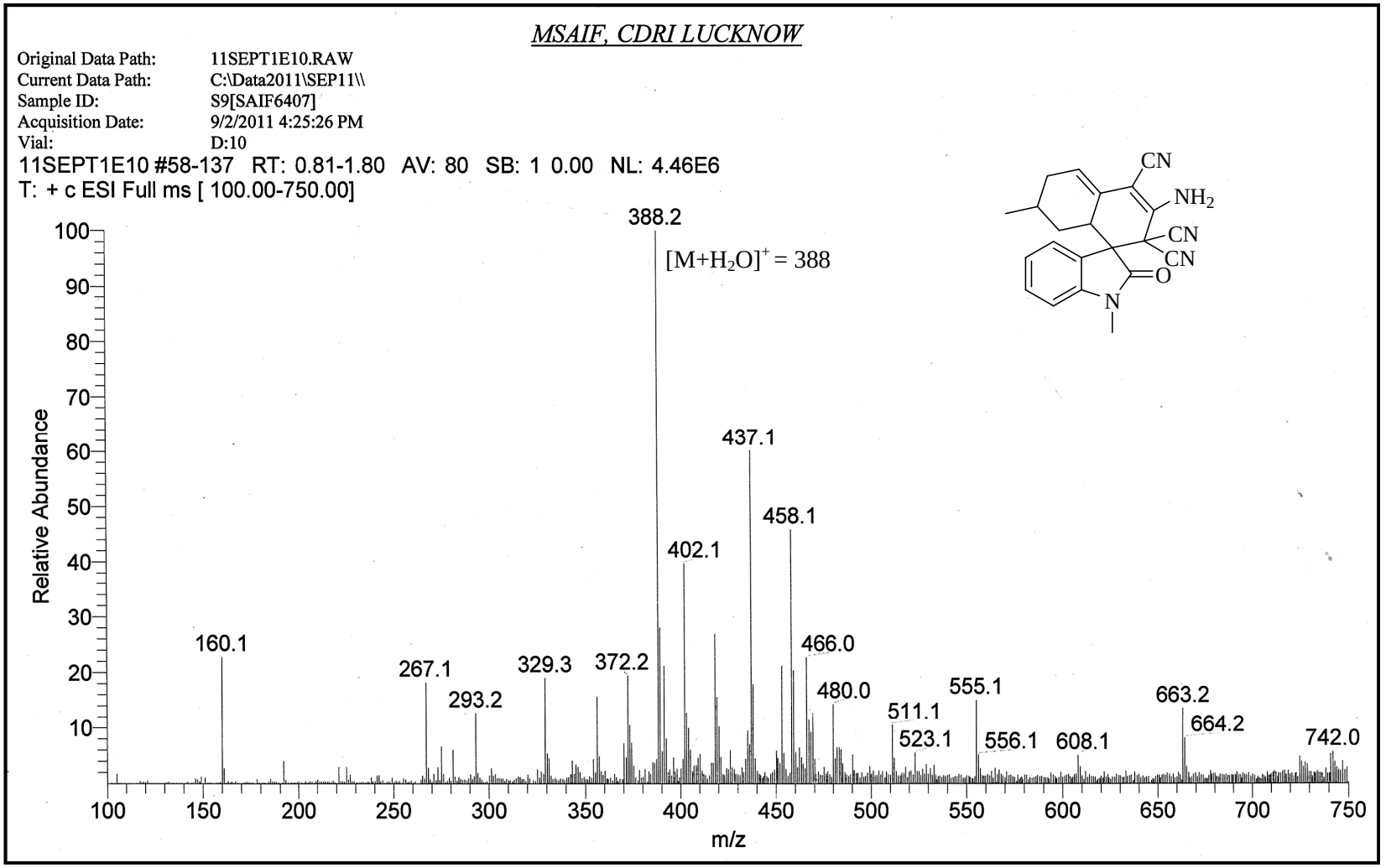


Figure 5.4: Mass spectrum of **3′ amino-1,7′-dimethyl-2-oxo-6′,7′,8′,8′a-tetrahydro-2′*H*-spiro[indoline-3,1′-naphthalene]-2′,2′,4′-tricarbonitrile (4e)**

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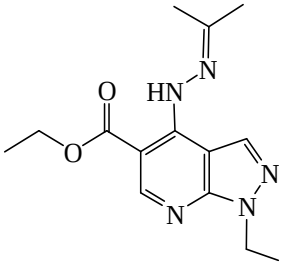
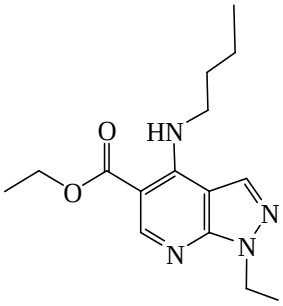
CHAPTER 6

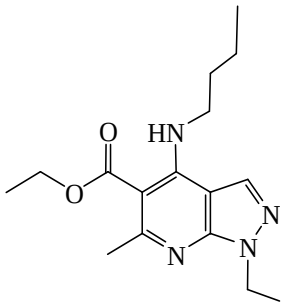
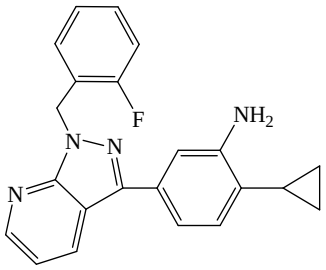
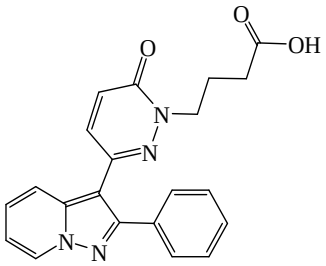
Synthesis and Characterization of Pyrazolo[3,4-b]pyridine Derivatives using 2,2,2-Trifluoroethanol under Microwave Irradiation

6.1 INTRODUCTION

A single molecular framework integrating various heterocyclic moieties is quite relavant to its enhanced biocidal profile. Various heterocycles, such as pyrazole, pyridine, and pyrimidine with fused heterocyclic compounds are observed in numerous bioactive molecules have been used as key pharmacophores.[1] Pyrazolopyridine is an important class of fused heterocyclic compounds. Pyrazolopyridines are familiar substructural motif of drug-like molecules and potential drugs. BAY 41-2272, containing the fused pyrazolopyridine base, is a stimulator of soluble guanylate cyclase (sGC) and induces vasodilation.[2] 6-Aryl pyrazolo[3,4-*b*]pyridines are also reported as potent inhibitors of glycogen synthase kinase-3 (GSK-3).[3] Pyrazolopyridines are also known to exhibit a variety of biological activities such as potent cyclin dependent kinase 1 (CDK1) inhibitor,[4] HIV reverse transcriptase inhibitors,[5] CCR1 antagonists,[6] protein kinase inhibitors,[7] and cGMP degradation inhibitors, alongwith several herbicidal and fungicidal activities.[8] (**Table 6.1**).

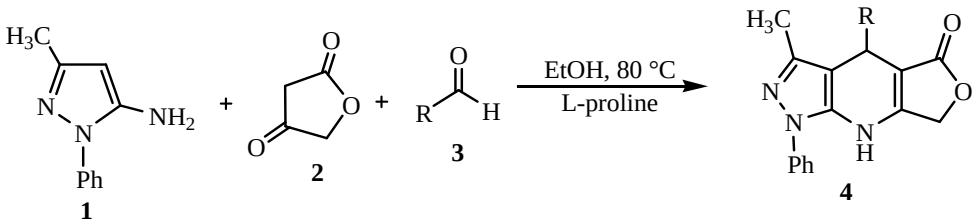
Table 6.1: Medicinally relevant pyrazolopyridine derivatives

S. No.	Name of the compound	Structure of the compound	Use of the compound[2,9-12]
1	Etazolate		Etazolate is an anxiolytic drug and has unique pharmacological properties. It acts as a positive allosteric modulator of the $GABA_A$ receptor.
2	Cartazolate		Cartazolate acts as a $GABA_A$ receptor positive allosteric modulator and known to act as an adenosine antagonist. It also has anxiolytic effects.

3	Tracazolate		Tracazolate has primarily anxiolytic and anticonvulsant effects, with sedative and muscle relaxant effects and is quite selective for $GABA_A$ receptors.
4	BAY 41-2272		BAY 41-2272 is a stimulator of soluble guanylate cyclase (sGC) and induces vasodilation.
5	FK-838		FK-838 has been known to be effective adenosine A1 antagonists.

These examples underline the importance of fused heterocycles, pyrazolopyridines, as key constituents in bioactive molecules. As a consequence, literature survey has been extensively studied by the various workers involving the synthesis of pyrazolopyridine derivatives which is summarized as follows:

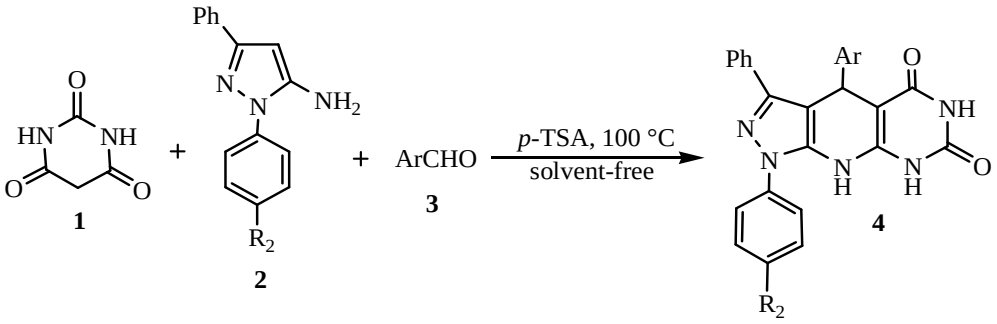
An organocatalyzed Knoevenagel condensation of pyrazol-5-amines **1**, tetronic acid **2** and aldehyde **3**, providing a new approach to synthesize dihydrofuropyrido[2,3-*c*]pyrazole derivatives **4** was reported by Shi *et al.*[13] (Scheme **1**)



1 **2** **3** **4**

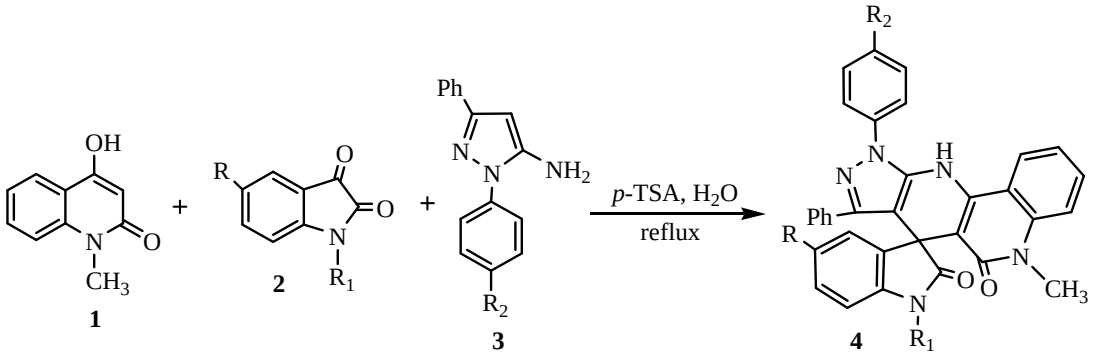
Scheme 1

Bazgir *et al.*[14] described a simple method to synthesize pyrazolopyrido [2,3-*d*]pyrimidin-dione derivatives **4** by the condensation reaction of barbituric acids **1**, 1*H*-pyrazol-5-amines **2** and aldehydes **3** under solvent-free conditions (Scheme **2**).



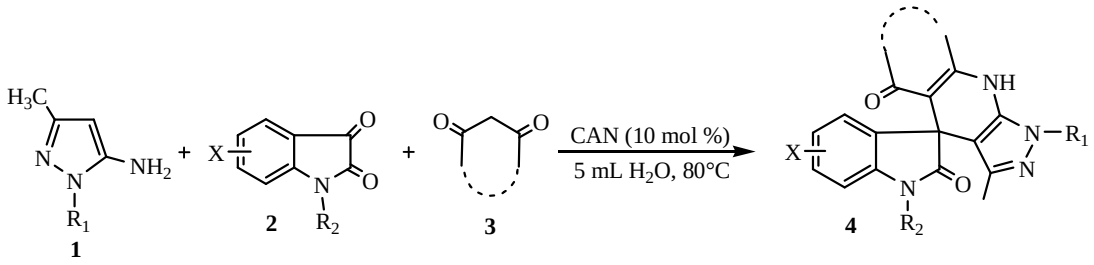
Scheme 2

Bazgir *et al.*[15] also reported an efficient methodology to synthesize spiro[benzo[*h*]pyrazolo[3,4-*b*][1,6]naphthyridine-indoline]-dione derivatives **4** *via* a one-pot, three component reaction of 4-hydroxy-1-methylquinolin-2(1*H*)-one **1**, a variety of isatins **2** and 1*H*-pyrazol-5-amines **3** in water (Scheme **3**).



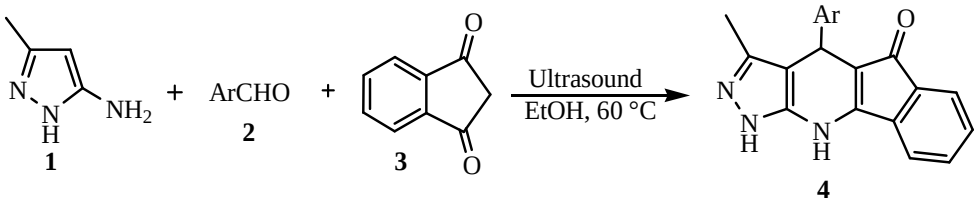
Scheme 3

Shi *et al.*[16] reported a simple one-pot ceric ammonium nitrate catalysed three component reaction involving 5-amino-3-methylpyrazole **1**, isatin **2** and a variety of 1,3-dicarbonyl compounds **3** for the synthesis of spirooxindole pyridine **4** derivatives in aqueous medium (Scheme **4**).



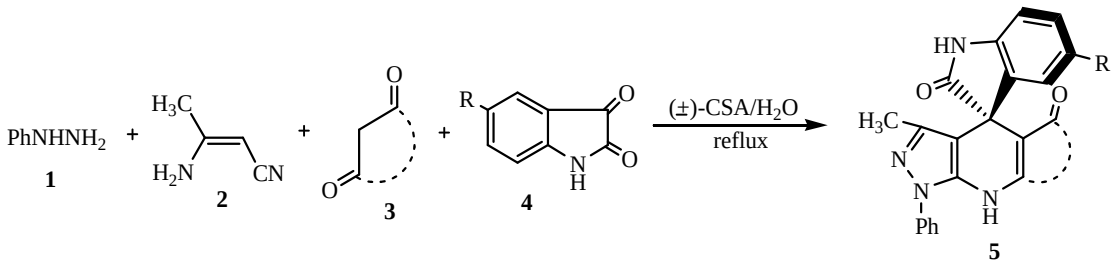
Scheme 4

A regioselective three-component reaction of 5-amino-3-methyl-1*H*-pyrazole **1**, arylaldehydes **2** and 2*H*-indene-1,3-dione **3** to afford fused polycyclic pyrazolo[3,4-*b*]pyridines **4** in ethanol under ultrasound irradiation was developed by Mamaghani *et al*.[17] (Scheme **5**)



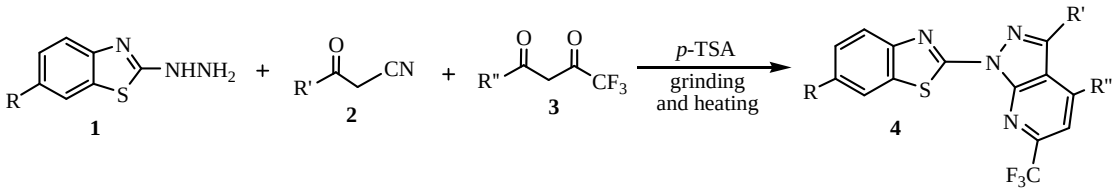
Scheme 5

New four-component domino reaction was described by Perumal *et al*.[18] that allow the one-pot synthesis of spiro[indoline-3,4′-pyrazolo[3,4-*b*]pyridine derivatives **5** from the reaction of phenylhydrazine **1**, 3-aminocrotononitrile **2**, cyclic 1,3-dicarbonyl compounds **3** and isatin **4** in the presence of (±)-camphor-10-sulfonic acid (CSA) (Scheme **6**).



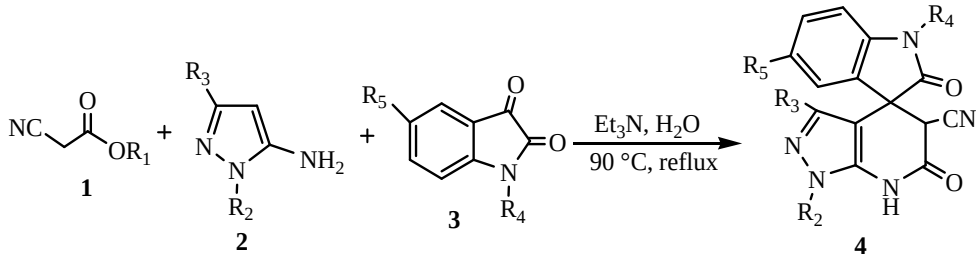
Scheme 6

Aggarwal *et al*.[19] explained a simple and efficient regiospecific synthesis of 6-trifluoromethyl-1*H*-pyrazolo[3,4-*b*]pyridine derivatives **4** *via* a three-component reaction of 2-hydrazinobenzothiazoles **1**, α-cyanoacetophenones **2** and trifluoromethyl-β-diketones **3** under solvent-free conditions (Scheme **7**).



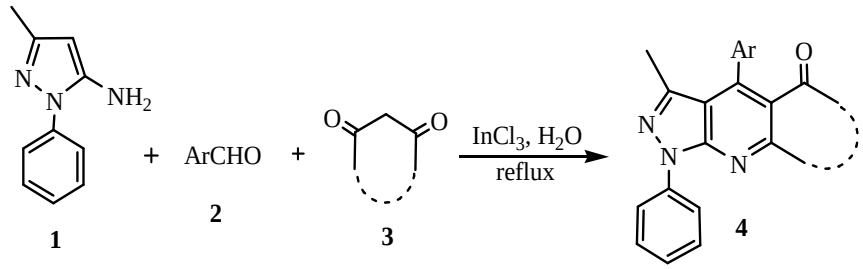
Scheme 7

Rahmati *et al.*[20] described a green and efficient one-pot, three-component condensation reaction of an alkyl cyanoacetate **1**, aminopyrazole **2**, and isatin **3** in water to give 2,6′-dioxo-tetrahydrospiro[indoline-3,4′-pyrazolo[3,4-*b*]pyridine]-carbonitrile derivatives **4** at 90 °C using Et_3N as a catalyst with good yields (Scheme **8**).



Scheme 8

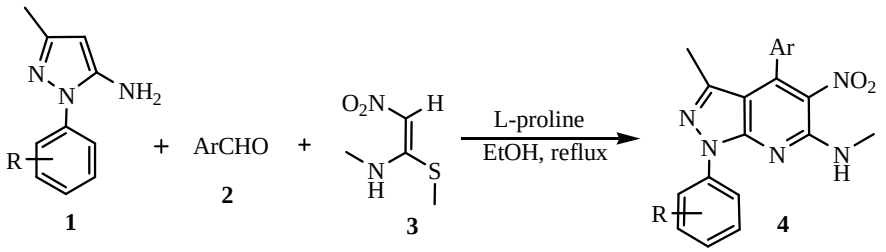
Khurana *et al.*[21] developed a facile one-pot three-component combinatorial synthetic protocol of a variety of substituted pyrazolo[4,3-*e*]pyridinone derivatives **4** from 3-methyl-1-phenyl-pyrazol-5-amine **1**, aldehydes **2** and 1,3-dicarbonyl compounds **3** using $InCl_3$ as catalyst in water (Scheme **9**).



Scheme 9

Inspired by the ‘medicinal’ scaffold of pyrazolo[3,4-*b*]pyridinones, Perumal *et al.*[22] documented a simple and efficient L-proline catalyzed synthetic strategy to assemble substituted pyrazolo[3,4-*b*]pyridin-6-amine framework **4** from the three-component domino reaction of 3-methyl-1-aryl-1*H*-pyrazol-5-amines **1**, aromatic

aldehydes **2**, and N-methyl-1-(methylthio)-2-nitropropenamine **3** in ethanol (Scheme **10**).



1 **2** **3** **4**

Scheme 10

Although the discussed methods had their own advantages there are still some of the disadvantages such as lower yield of the products, longer reaction times, tedious work-up procedures, use of harsh reaction conditions and solvents which is required to be overcome. Hence, there is an utmost requirement to develop a protocol for the synthesis of fused heterocycles, pyrazolopyridine derivatives using a much simpler and greener approach.

Multicomponent reactions (MCRs) are defined as one-pot chemical transformations in which at least three or more reactants form a product derived from all of the inputs. MCRs have high proficiency which facilitate the rapid assembly to increase the molecular diversity for the synthesis of complex molecules.[23-28] The use of MCRs have several significant advantages such as convergence, operational simplicity, reduction in the number of workup steps, extraction and purification processes, and hence minimize waste generation.[29] One-pot MCRs shorten the reaction time to a large extent, increase the overall yield of the products rendering the processes green. Therefore, the design and development of newer MCRs has attracted great attention in the fields of organic synthesis, drug discovery and material sciences.[30]

Conventional methods for various chemical syntheses are very well documented and still in practice.[31] The methodological techniques for the synthesis of organic compounds have been continuously modified through the decades. And, the microwave technique has become an established tool in organic synthesis, being a significant approach towards the development of green chemistry. Microwave assisted technique has opened up new opportunities in organic synthesis, because of

the rate enhancements, higher yields, and very often, the improved selectivity which is not feasible using conventional reaction conditions.[32,33]

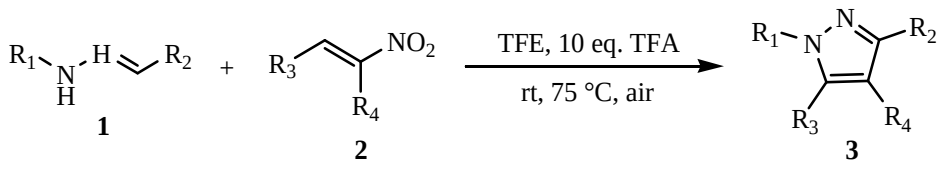
The advantages of this emerging technology have been employed in the fields of multistep synthesis,[34] drug discovery,[35] and recently gone through some related fields, such as polymer synthesis,[36] biochemical processes,[37] material sciences,[38] and nanotechnology.[39]

Recently, the remarkable properties of fluorine have gained extensive industrial concern in various significant fields such as pharmaceutical, chemical, agricultural, electronic materials and many more.[40] Since last decade, fluorous solvents have gained recognition as green reaction medium in organic synthesis and in various catalytic processes.[41] This eco-friendly and clean solvent significance of fluorous solvents are owed to their various unique physicochemical properties, being non volatile, nonflammable, highly polar, and high ionizing power.

In addition, their utilization in organic reactions has proven to be very useful due to some other distinguished properties such as lower boiling points and higher melting points than their non-fluorinated counterparts, strong hydrogen bond donation properties and the ability to solvate water. The high acidity to the hydrogen atom of the hydroxyl group was furnished by the electron-withdrawing character of CF_3 group.[42] Besides these, reactions in fluorinated alcohols generally have intense effects on reaction rates and product selectivity and can be carried out without the use of any reagent or catalyst, thus allowing a simplistic isolation of the product and afterwards, can be easily separated from the reaction mixture for subsequent use. Further, fluorinated alcohols are poor nucleophiles and hence, the property of auto-association is low facilitating the easy isolation from the reaction mixture.[43]

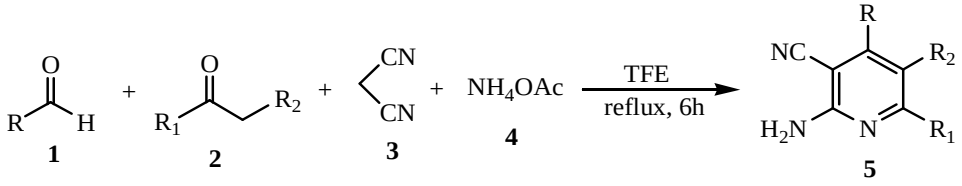
Some of the reactions using the 2,2,2-trifluoroethanol as a reaction medium are discussed below:

Deng *et al.*[44] presented an efficient synthesis of substituted pyrazoles **3** by the reaction of electron-deficient N-arylhydrazones **1** with nitroolefins **2** using trifluoroethanol at room temperature (Scheme **11**).



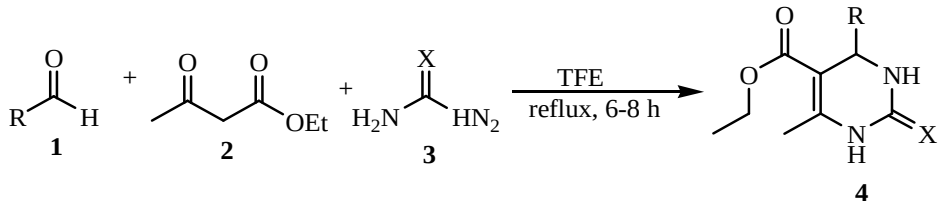
Scheme 11

Khaksar *et al.*[45] reported the synthesis of 2-amino-3-cyanopyridine derivatives **5** by the multi-component reaction of aldehydes **1**, ketones **2**, malononitrile **3**, and ammonium acetate **4** using trifluoroethanol (Scheme **12**).



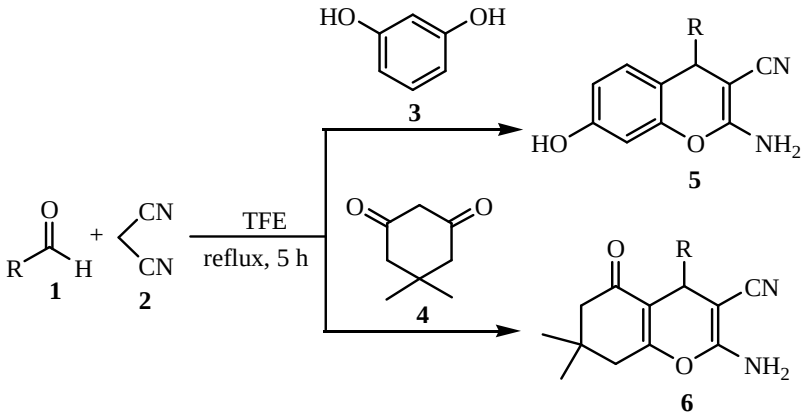
Scheme 12

Rashmi *et al.*[46] synthesized aryl substituted 3,4-dihydropyrimidin-2-ones **4** by the multi-component reaction of aldehydes **1**, aromatic β-ketoesters **2**, and urea (or thiourea) **3** without any catalyst in trifluoroethanol under reflux conditions (Scheme **13**).



Scheme 13

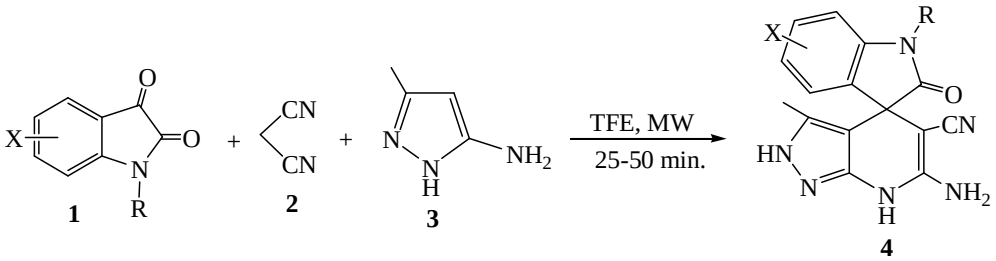
Khaksar *et al.*[47] developed a simple and efficient regio-selective synthesis of 2-amino-3-cyano-4*H*-chromene **5** and tetrahydrobenzo[*b*]pyran derivatives **6** by the one-pot multi-component reaction of aldehydes **1**, malononitrile **2**, and resorcinol **3** or dimedone **4** in trifluoroethanol (Scheme **14**).



Scheme 14

6.2 EXPERIMENTAL

Hence, as a part of our program towards the design and development of new greener strategies for the synthesis of bioactive heterocyclic moieties,[48] we have investigated a three-component reaction of isatin, malononitrile and 5-amino-3-methylpyrazole using 2,2,2-trifluoroethanol as a reusable green solvent under microwave irradiation. (**Scheme 6.1**)

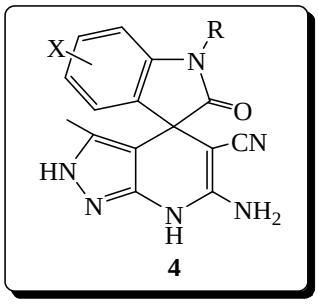


Scheme 6.1: Synthesis of spiro[indoline-3,4′-pyrazolo[3,4-*b*]pyridine] derivatives

6.2.1 Synthesis of spiro[indoline-3,4′-pyrazolo[3,4-*b*]pyridine] derivatives (4a-j)

An equimolar mixture of Indole-2,3-dione **1** (0.147 g, 1 mmol), malononitrile **2** (0.066 g, 1 mmol) and 5-amino-3-methylpyrazole **3** (0.097 g, 1 mmol) in 2,2,2-trifluoroethanol (10 ml) was introduced in a 50 mL round-bottomed flask. The flask was placed in the microwave cavity and subjected to irradiation for appropriate time at 150 °C using a maximum power of 250 W. After the completion of the reaction, the reaction mixture was checked with TLC and the flask was detached from the microwave cavity and the content was transferred into a beaker to afford the crude product. The pure products were obtained by crystallization from ethanol (**Scheme 6.1**). All the synthesized compounds are summarized in **Table 6.2**.

Table 6.2: Synthesis of spiro[indoline-3,4′-pyrazolo[3,4-*b*]pyridine] derivatives (**4a-j**) under microwave irradiation



4

Entry	Product	X	R	Time (min.)	Yield[a] (%)	Mp (^{o}C)	Color
1	**4a**	5-H	H	45	95	>300[48g]	White
2	**4b**	5-CH_3	H	35	86	>300[48g]	White
3	**4c**	5-Cl	H	45	89	>300[48g]	White
4	**4d**	5-Br	H	40	91	>300[48g]	White
5	**4e**	5-NO_2	H	50	84	>300[48g]	White
6	**4f**	5-F	H	40	90	284-286	White
7	**4g**	5,7-diCH_3	H	32	86	>300	White
8	**4h**	H	CH_2-CH=CH_2	35	87	262-264	Light-brown
9	**4i**	H	CH_2-Ph	25	92	>300	White
10	**4j**	H	CH_3	30	82	276-278	White

[a] = Isolated Yield

6.3 CHARACTERIZATION OF THE COMPOUNDS SYNTHESIZED

To confirm the structure of the compounds synthesized and to know the position of various functional groups and presence of heteroatoms in the compounds, they have been well established by their melting points, various spectral studies including IR, Mass, ^{1}H NMR, and ^{13}C NMR analyses.

6.3.1 IR and Mass spectral studies

IR spectra of the compounds synthesized were recorded on Shimadzu FT-IR 8400S spectrophotometer using KBr pellets. Mass spectra of the representative compound were obtained using JEOL SX-102 spectrometer. The structure of the compounds **4a-j** has been confirmed by their melting points and spectroscopic data.

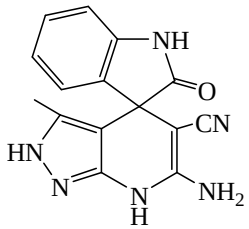
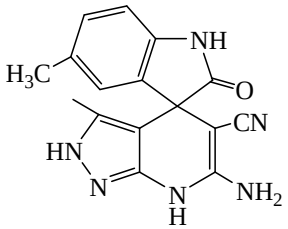
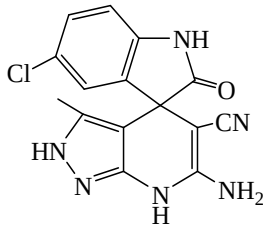
Compound **4i** was isolated as white solid with melting point as >300 °C. For instance, the IR spectrum of the product **4i** showed characteristic bands at 3446 cm^{-1} corresponding to the NH_2 group and broad peaks in the region of 3221-3274 cm^{-1} due to two –NH groups present in the cyclic ring system. In the IR spectrum, cyano group in the compound appeared as sharp signal at 2187 cm^{-1} further proved the formation of spiro[indoline-3,4′-pyrazolo[3,4-*b*]pyridine] derivative. The mass spectrum of **4i** showed a molecular ion peak at 382.0 $(M)^+$.

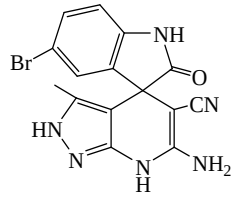
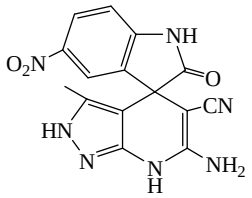
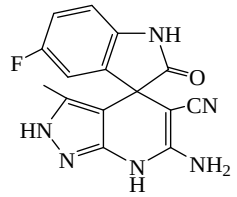
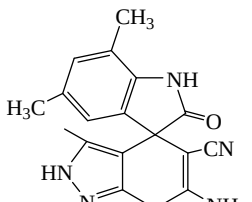
6.3.2 ^{1}H NMR and ^{13}C NMR spectral studies

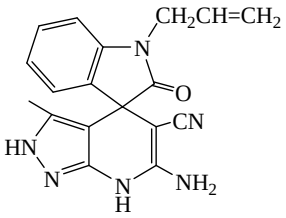
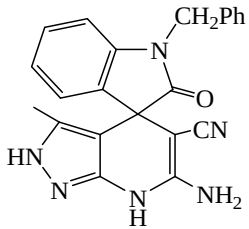
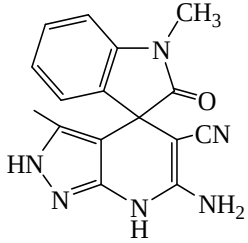
^{1}H NMR and ^{13}C NMR spectra of the compounds synthesized were recorded in DMSO-d_6 and $CDCl_3$ using TMS as an internal standard on a Bruker spectrophotometer at 400 and 100 MHz respectively.

In the ^{1}H NMR spectrum of **4i**, two -NH hydrogen atoms with chemical shifts 11.87 and 9.25 ppm were observable. Total nine protons of the final structure resonated in the region of 6.94-7.41 ppm with the characteristic signals and coupling constants. A sharp singlet at 5.72 ppm confirms the presence of $–NH_2$ group in the compound. The ^{13}C NMR spectrum of **4i** demonstrated signals at δ 177.8 ppm due to C=O group in the system. The aromatic carbon atoms resonated in the region of 121.0-146.5 ppm and the signal at 97.9 ppm confirmed the presence of the spiro carbon atom.

Table 6.3: IR, Mass, ^{1}H NMR and ^{13}C NMR spectral data of the compounds synthesized **(4a-j)**

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
1	 **4a**	3364, 3215-3245, 2382, 1712 (C=O)	292 m/z as $[M]^+$ for $C_{15}H_{12}N_6O$	1.46 (s, 3H, CH_3), 5.65 (s, 2H, NH_2), 6.86 (d, 1H, *J* = 7.2 Hz), 6.96-7.04 (m, 2H), 7.20 (t, 1H), 9.17 (s, 1H, NH), 10.35 (s, 1H, NH), 11.84 (s, 1H, NH)	8.8, 53.0, 56.0, 98.3, 109.4, 121.0, 122.1, 124.9 128.9, 133.8, 134.96, 141.2, 146.5, 154.1, 179.6 (C=O)
2	 **4b**	3353, 3210-3239, 2278, 1709 (C=O)	306 m/z as $[M]^+$ for $C_{16}H_{14}N_6O$	1.53 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 5.47 (s, 2H, NH_2), 6.72 (d, 1H, *J* = 7.6 Hz), 6.81 (s, 1H), 6.93–6.96 (m, 1H), 9.07 (s, 1H, NH), 10.12 (s, 1H, NH), 11.66 (s, 1H, NH)	8.7, 20.8, 48.2, 53.5, 97.4, 108.6, 121.0, 123.4, 125.0, 128.5, 130.8, 132.7, 135.0, 138.4, 154.3, 179.5 (C=O)
3	 **4c**	3368, 3214-3247, 2268, 1710 (C=O)	326 m/z as $[M]^+$ for $C_{15}H_{11}ClN_6O$	1.61 (s, 3H, CH_3), 5.71 (s, 2H, NH_2), 6.89 (d, 1H, *J* = 7.2 Hz), 6.92 (s, 1H), 6.96–7.02 (m, 1H), 9.11 (s, 1H, NH), 10.26 (s, 1H, NH), 11.78 (s, 1H, NH)	8.9, 48.1, 54.1, 98.0, 109.2, 121.4, 122.3, 125.0, 128.7, 129.6, 131.1, 135.4, 141.5, 154.9, 179.4 (C=O)

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
4	 **4d**	3342, 3221-3238, 2274, 1715 (C=O)	371 m/z as $[M]^+$ for $C_{15}H_{11}BrN_6O$	1.52 (s, 3H, CH_3), 5.72 (s, 2H, NH_2), 6.84 (d, 1H, *J* = 7.6 Hz), 7.07 (s, 1H), 7.35–7.38 (m, 1H), 9.23 (s, 1H, NH), 10.53 (s, 1H, NH), 11.92 (s, 1H, NH)	8.4, 49.2, 55.4, 98.2, 108.9, 121.0, 124.3, 125.9, 129.4, 132.3, 133.9, 141.7, 145.0, 154.8, 179.1 (C=O)
5	 **4e**	3337, 3218-3235, 2312, 1706 (C=O)	337 m/z as $[M]^+$ for $C_{15}H_{11}N_7O_3$	1.61 (s, 3H, CH_3), 5.72 (s, 2H, NH_2), 6.88 (d, 1H, *J* = 7.2 Hz), 7.09 (s, 1H), 7.04–7.21 (m, 1H), 9.32 (s, 1H, NH), 10.76 (s, 1H, NH), 12.08 (s, 1H, NH)	8.6, 49.8, 56.1, 99.2, 110.9, 122.3, 123.4, 127.2, 129.1, 130.7, 132.2, 141.3, 144.4, 154.3, 180.1 (C=O)
6	 **4f**	3341, 3219-3226, 2274, 1718 (C=O)	310 m/z as $[M]^+$ for $C_{15}H_{11}FN_6O$	1.28 (s, 3H, CH_3), 5.69 (s, 2H, NH_2), 6.78 (d, 1H, *J* = 7.6 Hz), 6.95 (s, 1H), 6.99–7.11 (m, 1H), 9.12 (s, 1H, NH), 10.24 (s, 1H, NH), 11.85 (s, 1H, NH)	8.9, 48.6, 54.0, 98.2, 109.6, 121.5, 122.2, 125.0, 128.3, 129.4, 131.8, 135.2, 141.0, 154.5, 179.7 (C=O)
7	 **4g**	3356, 3227-3238, 2319, 1714 (C=O)	320 m/z as $[M]^+$ for $C_{17}H_{16}N_6O$	1.36 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 5.65 (s, 2H, NH_2), 6.68 (d, 1H, *J* = 7.6 Hz), 6.91 (s, 1H), 6.92–6.98 (m, 1H), 9.23 (s, 1H, NH), 10.19 (s, 1H, NH), 11.42 (s, 1H, NH)	8.8, 20.7, 48.4, 55.3, 98.4, 108.6, 121.0, 122.7, 125.0, 128.5, 130.4, 132.8, 134.0, 137.3, 152.3, 174.5 (C=O)

Entry	Structure of the product	IR spectra (KBr, ν, cm^{-1})	Mass spectra (m/z)	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)
8	 **4h**	3341, 3219-3225, 2289, 1708 (C=O)	332 m/z as $[M]^+$ for $C_{18}H_{16}N_6O$	1.21 (d, 3H, CH_3, J = 7.6 Hz), 4.74-4.82 (dd, 2H, CH_2, J_1, J_2 = 15.2 Hz), 5.69 (s, 2H, NH_2), 7.00-7.18 (m, 3H), 7.20-7.35 (m, 4H), 9.19 (s, 1H, NH), 11.63 (s, 1H, NH)	8.8, 42.3, 48.2, 53.4, 97.6, 108.0, 121.2, 122.7, 123.8, 124.1, 127.5, 127.8, 128.1, 128.6, 134.2, 134.8, 176.3 (C=O)
9	 **4i**	3446, 3221-3274, 2187, 1715 (C=O)	382 m/z as $[M]^+$ for $C_{22}H_{18}N_6O$	1.26 (d, 3H, CH_3, J = 7.6 Hz), 4.84-4.99 (dd, 2H, CH_2, J_1, J_2 = 15.6 Hz), 5.72 (s, 2H, NH_2), 6.95 (d, 1H, J = 7.6 Hz), 7.00-7.07 (m, 2H), 7.18-7.23 (m, 1H) 7.24-7.33 (m, 3H), 7.40 (d, 2H, J = 7.6 Hz), 9.25 (s, 1H, NH), 11.87 (s, 1H, NH)	8.5, 42.9, 48.3, 53.3, 97.9, 108.9, 109.2, 121.0, 122.1, 123.0, 124.4, 127.4, 127.4, 128.2, 128.4, 134.0, 134.1, 136.3, 141.7, 146.5, 154.7, 177.8 (C=O)
10	 **4j**	3390, 3216-3231, 2289, 1710 (C=O)	306 m/z as $[M]^+$ for $C_{16}H_{14}N_6O$	3.34 (s, 3H, CH_3), 1.25 (d, 3H, CH_3, J = 7.2 Hz), 5.68 (s, 2H, NH_2), 7.04-7.26 (m, 4H), 9.66 (s, 1H, NH), 10.46 (s, 1H, NH)	8.9, 35.0, 96.2, 108.1, 121.3, 122.5, 123.9, 124.4, 126.8, 127.3, 128.0, 128.5, 134.2, 134.7, 177.2 (C=O)

6.4 RESULTS AND DISCUSSION

6.4.1 Comparison of the reaction under different reaction conditions

To examine the effect of microwave irradiation and the solvent, we have chosen the isatin, malononitrile and 5-amino-3-methylpyrazole as the model substrates for the synthesis of 6′-amino-3′-methyl-2-oxo-2′,7′-dihydrospiro [indoline-3,4′-pyrazolo[3,4-*b*]pyridine]-5′-carbonitrile derivative **4a** using 2,2,2-trifluoroethanol under microwave irradiation. The model reaction was studied under conventional and microwave conditions. For this purpose, the temperature of the reaction mixture was varied from 80-150 °C and time of the reaction was varied from 8 to 30 min. and 30 min. to 4 h under the microwave and conventional conditions respectively. Percentage (%) isolated yield was observed in each case and the obsrvations are summarized in **Table 6.4**.

Table 6.4: Dependency of the process under varying conventional and microwave conditions

Temperature (^{o}C)	**Conventional**					**Microwave**				
	Time (h)					**Time (min.)**				
	0.5	**1.0**	**2.0**	**3.0**	**4.0**	**8**	**10**	**15**	**20**	**30**
80	15	21	30	32	32	22	28	56	75	75
100	20	38	50	60	62	44	57	71	80	80
120	45	54	65	70	71	48	62	78	84	85
150	58	70	72	73	73	77	83	88	92	92

From the Table **6.4** clearly shows that the yield of spiro[indoline-3,4′-pyrazolo[3,4-*b*]pyridine] derivatives increased with the increasing reaction temperature and time under both processes. Further, the yield of spiro[indoline-3,4′-pyrazolo[3,4-*b*]pyridine] derivatives was higher (up to ~30-40%) in all reaction-time ranges under the microwave process than under the conventional thermal process. Lower temperature and shorter time were required under the microwave process, in comparison to that required for the conventional thermal process. High yield, reduced reaction temperature and time exhibited under microwave process can be attributed to a significant increase in the rate of reaction of reactive species.

In order to develop a viable approach, the model reaction was investigated using different solvents under microwave irradiation and the observations are listed in Table **6.5**. Under microwave irradiation, 2,2,2-trifluoroethanol was found to be the best as compared to other solvents such as acetonitrile, THF, benzene, DMF, methanol and ethanol.

Table 6.5: Optimization of reaction conditions for the synthesis of **4a** under microwave irradiation

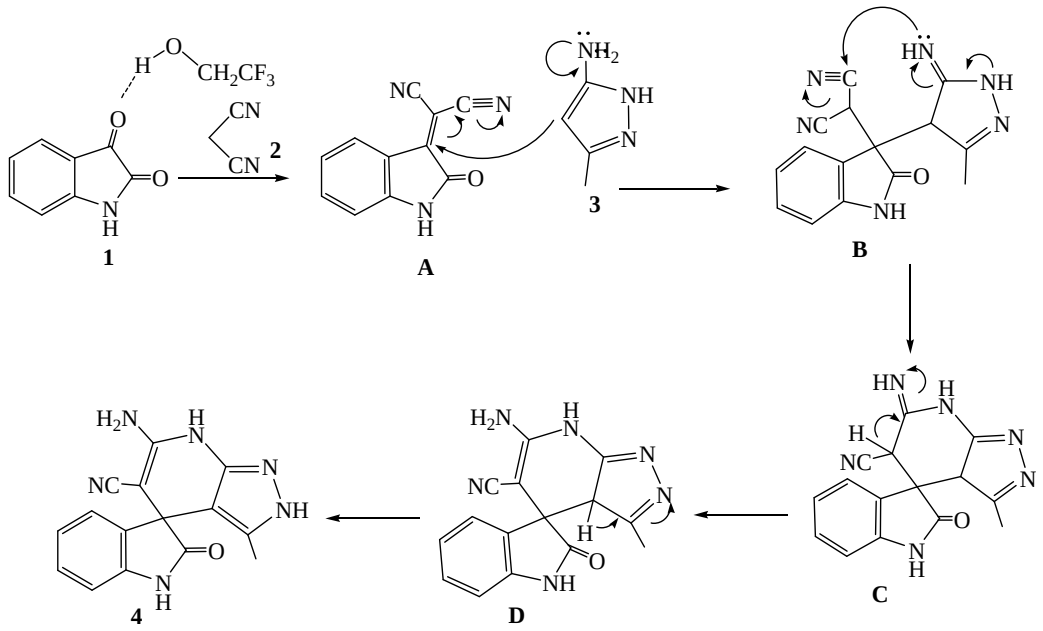
Entry	**Solvent**	**Microwave**			
		Temp (^{o}C)	**Power (Watt)**	**Time (min.)**	**Yield* (%)**
1	Acetonitrile	150	250	70	48
2	THF	150	250	70	42
3	Benzene	150	250	60	45
4	DMF	150	250	70	50
5	Methanol	150	250	45	70
6	Ethanol	150	250	40	80
7	**TFE**	**150**	**250**	**20**	**92**

* = Isolated Yield

The model reaction was also studied by varying microwave power (150, 180, 250 and 300 W) and it was concluded that 250 W power output at 150 ^{o}C was required to accomplish maximum conversion to product **4a**.

6.4.2 Mechanism

The plausible mechanism for the synthesis of products **4a-j** is shown in Scheme **6.2**. It is believed that the electrophilic character of the carbonyl group is activated by the high hydrogen bond donating ability of CF_3CH_2OH which facilitates the *in situ* generation of intermediate **A** by the knoevenagel condensation of isatin **1** and malononitrile **2**. Subsequent Michael addition of the electron-rich heterocyclic amine **3** to intermediate **A** ocurrs to provide another intermediate **B**, which then undergoes intramolecular cyclisation resulting in spiro ring systems **C** and **D**. At last, **D** undergoes tautomerism to produce the final product **4**.



Scheme 6.2: Possible mechanism for the synthesis of spiro[indoline-3,4´-pyrazolo[3,4-*b*]pyridine] derivatives

6.5 CONCLUSION

In conclusion, we have successfully developed a simple, green and efficient one-pot multi-component synthesis of spiro[indoline-3,4´-pyrazolo[3,4-*b*]pyridine] derivatives from easily available starting materials using 2,2,2-trifluoroethanol as a solvent under microwave irradiation. This protocol is greener synthetic strategy as it can be easily operated under catalyst-free conditions and quite useful in terms of atom economy, shortened reaction time and reusability of the solvent.

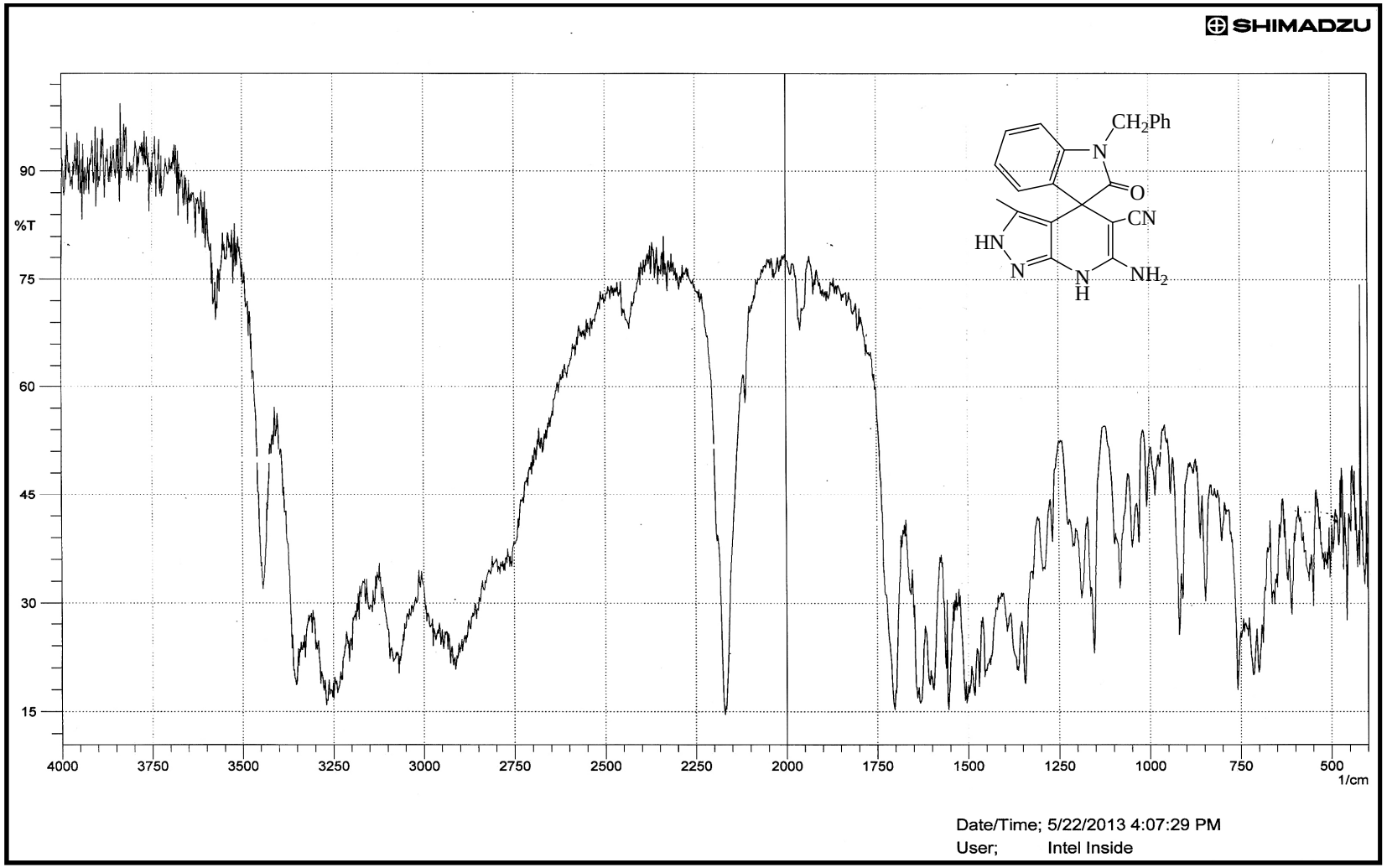


Figure 6.1: FT-IR spectrum of **6′-amino-7′-benzyl-3′-methyl-2-oxo-2′-hydrospiro[indoline-3,4′-pyrazolo[3,4-*b*]pyridine]-5′-carbonitrile derivative (4i)**

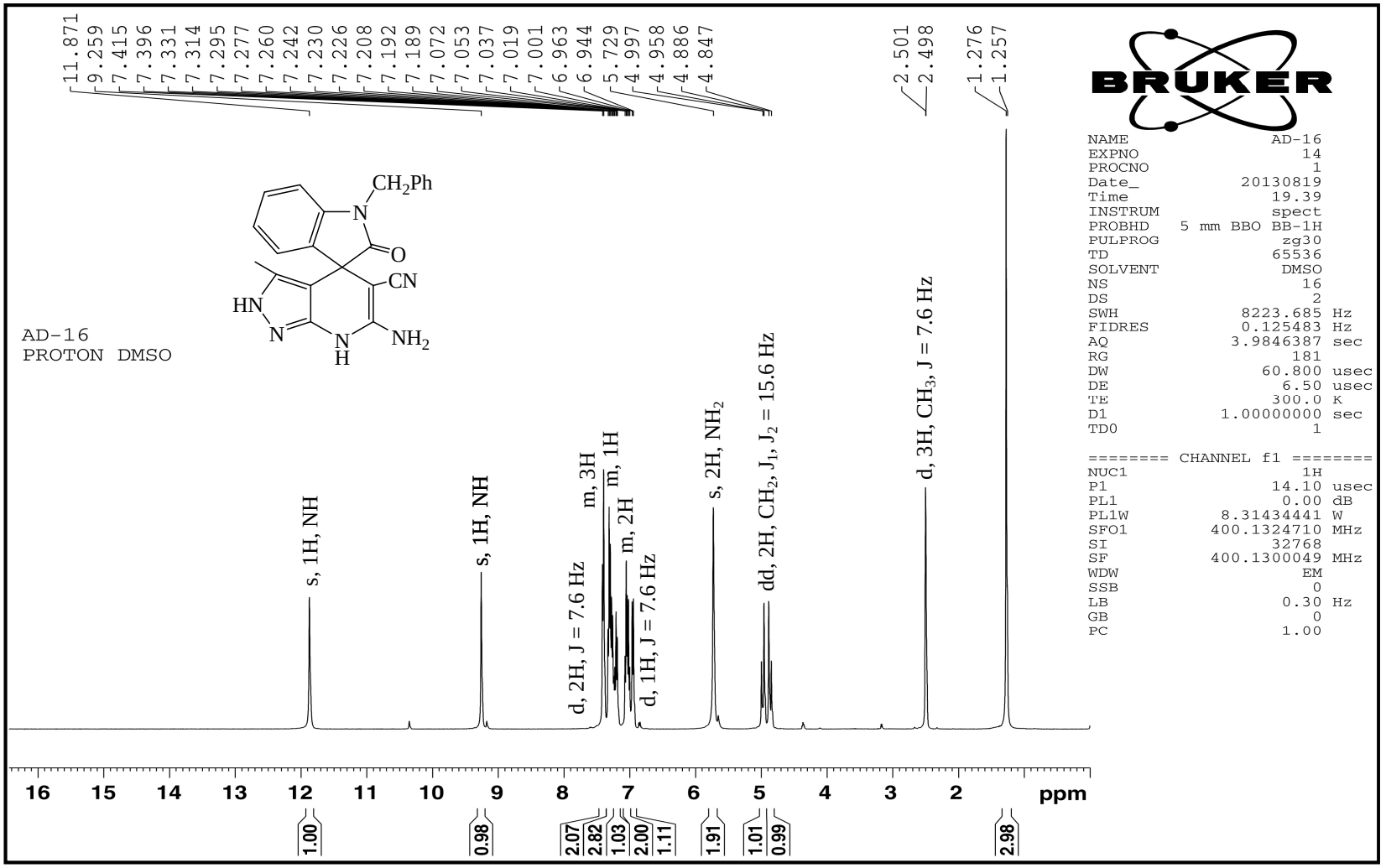


Figure 6.2: ^{1}H NMR spectrum of **6′-amino-7′-benzyl-3′-methyl-2-oxo-2′-hydrospiro[indoline-3,4′-pyrazolo [3,4-*b*]pyridine]-5′-carbonitrile derivative (4i)**

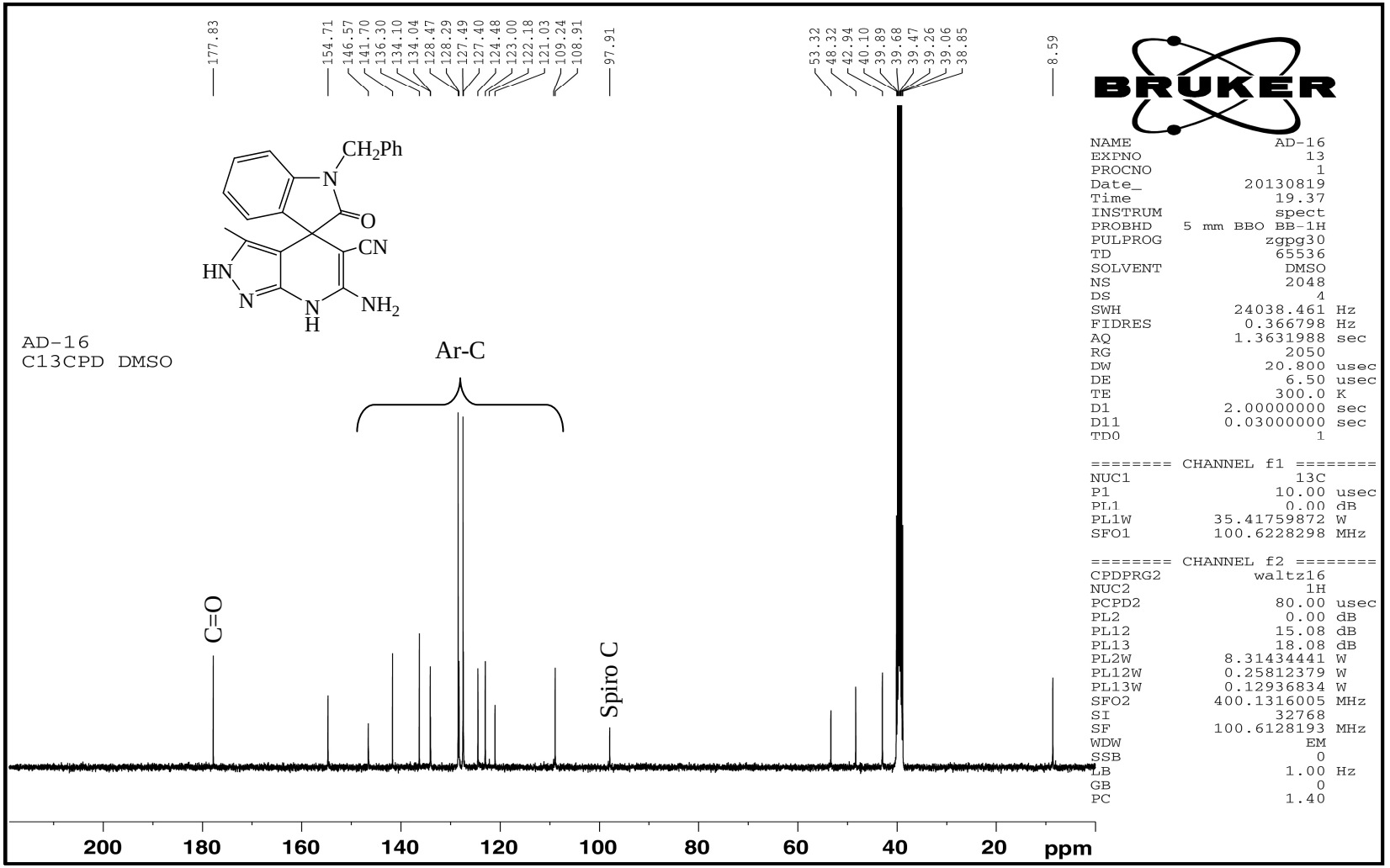


Figure 6.3: ^{13}C NMR spectrum of **6′-amino-7′-benzyl-3′-methyl-2-oxo-2′-hydrospiro[indoline-3,4′-pyrazolo [3,4-*b*]pyridine]-5′-carbonitrile derivative (4i)**

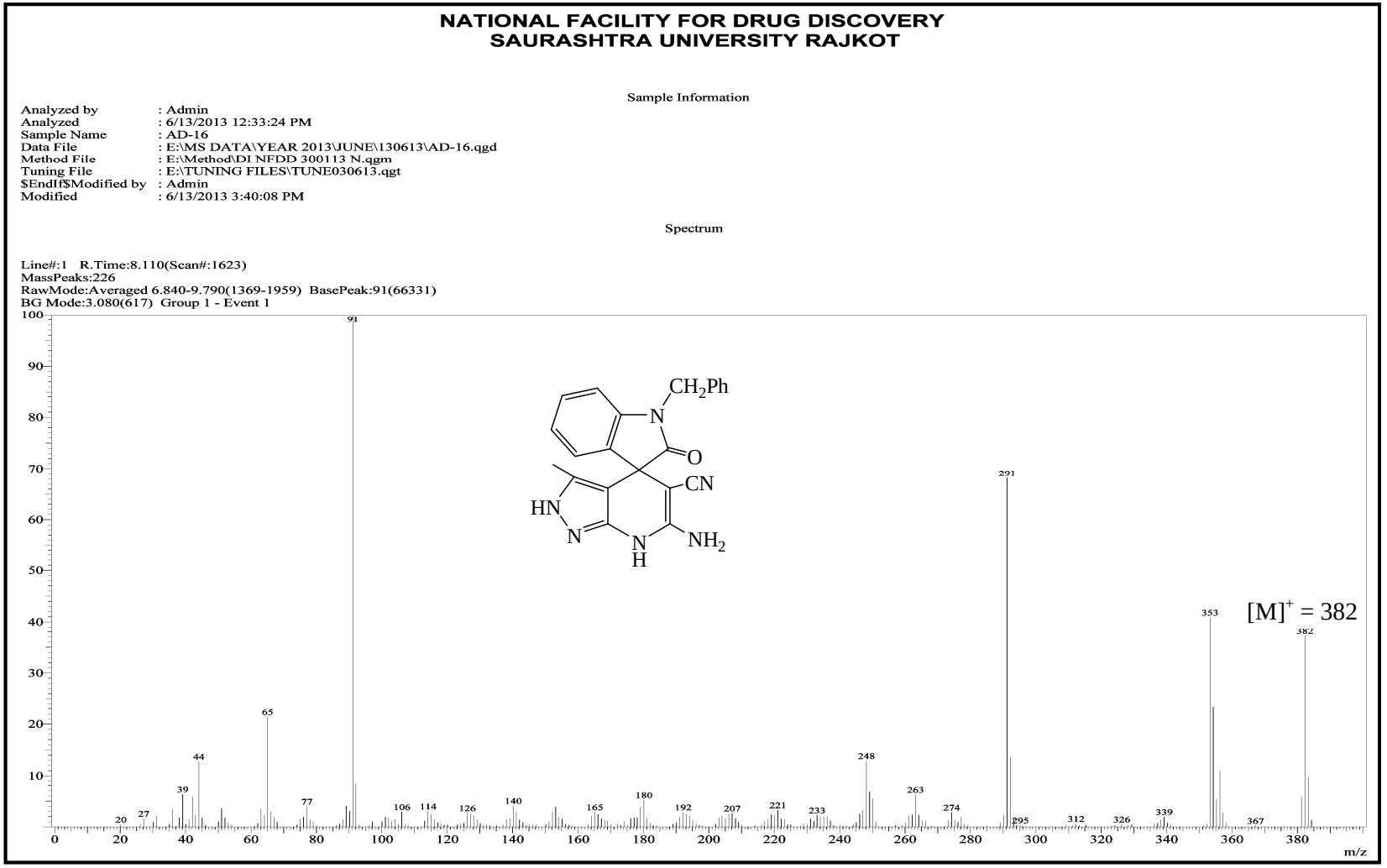


Figure 6.4: Mass spectrum of **6′-amino-7′-benzyl-3′-methyl-2-oxo-2′-hydrospiro[indoline-3,4′-pyrazolo [3,4-*b*]pyridine]-5′-carbonitrile derivative (4i)**

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CHAPTER 7

Biological Studies

7.1 INTRODUCTION

Heterocyclic compounds hold a special place in biological processes and are widely spread as natural products. They are extensively found in nature in a variety of forms, particularly in nucleic acids, plant alkaloids, as well as in haem and chlorophyll. In addition, some vitamins, proteins, hormones also contain aromatic heterocyclic framework. Synthetically produced heterocycles intended by organic chemists are used for instance as agrochemicals and pharmaceuticals which play a key role in human life. Heterocycles have a huge potential as the most promising molecules as lead structures for the design of new drugs.[1-3] The remarkable ability of heterocyclic nuclei serves both as biomimetics and reactive pharmacophores which has largely contributed to their value as traditional key elements of numerous drugs. Because of this, the synthesized heterocycles have received special attention within combinatorial chemistry, which has matured to become a significant tool in many aspects of chemistry.[4,5]

In our laboratory, we have synthesized a large variety of heterocyclic compounds which demonstrated interesting pharmacological and biological activities. Representative number of compounds has been screened for α-amylase inhibition assay, anti-oxidant, antimicrobial, antimycobacterial and antimalarial activities. All the tables of biological activity are in accordance with the table numbers and compound numbers mentioned in the chapters.

The said activities were carried out at various institutes including Biogenics, Dharwad, Karnataka and Microcare Laboratory, Surat, Gujarat.

7.1.1 α–Amylase inhibition assay

α-Amylase is an important enzyme present in the body which is responsible for the hydrolysis of polysaccharides, such as dietary starch. Inhibition of this enzyme might be extremely advantageous for diabetic patients because it could lower the glucose levels in the blood. One of the therapeutic approaches adopted so far to ameliorate post-prandial hyperglycemia involves the retardation of glucose absorption *via* the inhibition of carbohydrate hydrolyzing enzymes *viz*., α-glycosidase and α-amylase in the digestive organs.[6] Therefore, there is a

requirement to discover and develop new therapeutic agents to avoid the emergence of disease.

7.1.1.1 Method for α–Amylase inhibition assay

The chromogenic DNSA method had been used to perform the α–amylase inhibition assay.[7] The total assay mixture composed of 1400 µl of 0.05 M sodium phosphate buffer (pH 6.9), 50 µl of amylase (Diastase procured from HiMedia, Mumbai, Cat No. RM 638) and extracts at concentration 100, 250 and 500 µg were incubated at 37°C for 10 min. After pre-incubation, 500 µl of 1% (w/v) starch solution in the above buffer was added to each tube and incubated at 37°C for 15 min. The reaction was terminated with 1.0 ml DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature and the absorbance measured at 540 nm. The control amylase represented 100% enzyme activity and did not contain any sample of analysis. To eliminate the absorbance produced by sample, appropriate extract controls with the extract in the reaction mixture in which the enzyme was added after adding DNS. The maltose liberated was determined by the help of standard maltose curve and activities were calculated according to the following formula:

$$\text{Activity} = \frac{\text{Conc. of maltose liberated X mL of enzyme used}}{\text{Mol.wt. of maltose X incubation time (min.)}} \text{ X dilution factor}$$

One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per min under the assay conditions. The inhibitory/induction property shown by the sample was compared with that of control and expressed as percent induction/inhibition. This was calculated according to the following formula:

$$\%\ \text{inhibition/induction} = \frac{\text{Activity in presence of compound}}{\text{Control Activity}} \text{ X } 100$$

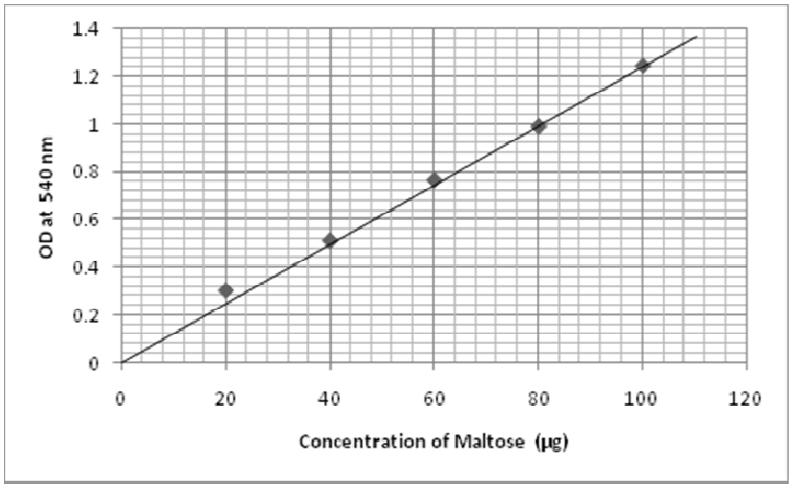


Figure 7.1: Standard Maltose Curve

7.1.2 Anti-oxidant activity

Antioxidants are a class of diverse chemical structures that are capable of decreasing or preventing oxidation of the sensitive molecules through different mechanisms like chelation of active metal ions, free radicals scavenging or inhibition of pro-oxidant enzymes.[8] The oxidative damage to proteins has been found to be high in specific brain regions and are elevated during aging and in some types of neurodegenerative disorders.[9,10]

7.1.2.1 Method

The compounds were also tested for antioxidant property by DPPH method.[11]

Reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (DPPH method):

The nitrogen centred stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at $\lambda = 517$ nm, which is purple in colour. This property makes it suitable for spectrophotometric studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts into 1,1-diphenyl-2-picrylhydrazine. The resulting decolourization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties.

Different concentrations (10µg, 50µg and 100µg) of samples as well as the standard compound Butylated hydroxy anisole (BHA) were taken in different test tubes and the volume was made uniform to 500µl by adding methanol. Five

milliliters of a 0.1 mM methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH) was added to these tubes and shaken vigorously. A control test tube without the test compound, but with an equivalent amount of methanol was also maintained. Afterwards, the tubes were allowed to stand at room temperature for 20 min. and the absorbance of the samples was measured at 517 nm. The difference between the sample and the control experiments was taken.

Radical scavenging activity was expressed as the percentage inhibition and calculated using the following formula:

$$\% \text{ free radical scavenging activity} = \frac{\text{Absorption of control - Absorption of sample}}{\text{Absorption of control}} \text{ X } 100$$

7.1.3 Antimicrobial activity

The global concern has been on the rise over the development of multi-drug-resistant pathogens which is causing a rigorous problem in the treatment of diseases.[12,13] To deal with this issue, continuous efforts have been done in the direction to discover some solution to fight against these resistant pathogens. One of the solutions to conquer this problem is to find some new structural moieties which could act as a new drug against the resistant pathogens.[14]

7.1.3.1 Strains of bacteria

Antibacterial activity was screened against gram positive (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 443) and gram negative (*Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441) bacteria by using Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin as standard antibacterial agents. The selected bacteria are known to cause human diseases whereas *Staphylococcus aureus* causes staph-related illness, internal abscess and food poisoning. *Streptococcus pyogenes* causes scarlet fever and strep throat, while *Escherichia coli* causes bloody diarrhoea and *Pseudomonas aeruginosa* causes respiratory system infections, dermatitis, soft tissue infections, bacteremia and urinary tract infections. Therefore, we have selected these bacteria for the screening process.

Escherichia coli

Escherichia coli, one of the most extensively studied organisms, is a gram-negative bacterium which abundantly occurs in the lower intestine of mammals. *E.*

coli is of major public health concern due to its high resistance, low infectious dose, and various other disease symptoms, ranging from mild diarrhoea to hemorrhagic colitis and the hemolytic-uremic syndrome.[15,16]

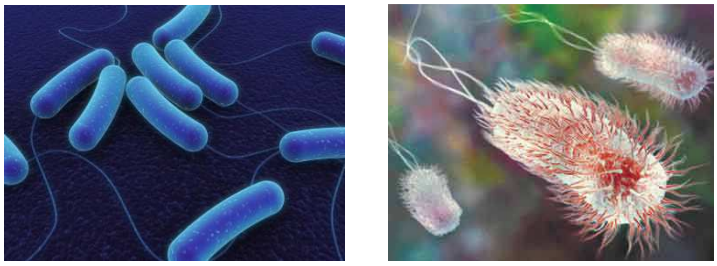


Figure 7.2: *Escherichia coli*

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative, opportunistic bacterium which is found everywhere in nature. It is quite able to infect plants, nematodes, insects and mammals. While it rarely infects healthy individuals, this bacterium is responsible for life-threatening infections in the critically ill persons.[17,18] It is known to be one of the most important causes of hospital-acquired infections, having a particularly high mortality rate among people with compromised immune systems.[19,20]

Figure 7.3: *Pseudomonas aeruginosa*

Staphylococcus aureus

Staphylococcus aureus is a gram-positive bacterium which silently stays as our natural flora, and threatens our life as a persistent pathogen. It is gradually becoming a health risk for both humans and animals. In addition to its ability to take in our immune system, its multi-drug opposition phenotype makes it one of the most continual pathogenic bacteria in the history of antibiotic chemotherapy.[21,22]

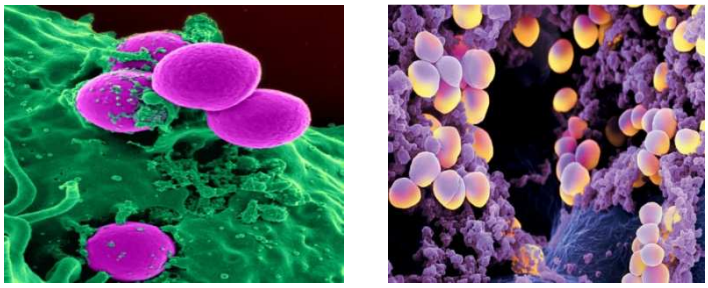


Figure 7.4: *Staphylococcus aureus*

Streptococcus pyogenes

Streptococcus pyogenes is a gram-positive bacterial pathogen which is non-motile and spheri-cal in shape. It is responsible for a range of human diseases, from trivial to lethal.[23,24] It is the major cause of pharyngitis accompanied by throat infections in humans and is the major causative agent for tonsillitis and may also cause other invasive infections such as meningitis, and necrotizing fasciitis, etc.[25]

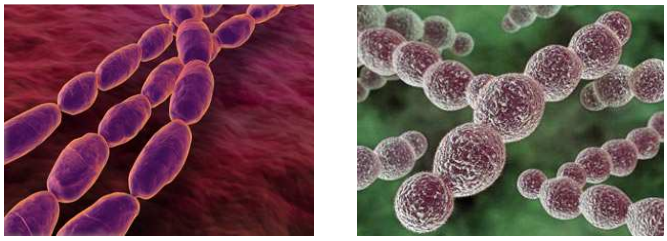


Figure 7.5: *Streptococcus pyogenes*

7.1.3.2 Strains of fungi

Antifungal activity was screened against three fungal species *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323 fungi by using Nystatin and Greseofulvin as a standard antifungal agents. The selected fungal species are known to cause human diseases whereas *Candida albicans* causes pathological lesion of the skin. *Aspergillus niger* causes black mold of onions, while *Aspergillus clavatus* causes occupational hypersensitivity pneumonitis. Therefore, we have selected these fungal species for the screening process.

Candida albicans

Candida albicans, the most virulent Candida species, is an opportunistic fungus which embodies an important public health confront with a high economic and medical significance due to the increased costs of cure, time of hospitalization and high morbidity and mortality rates, especially on immuno-compromised human beings.[26-29]

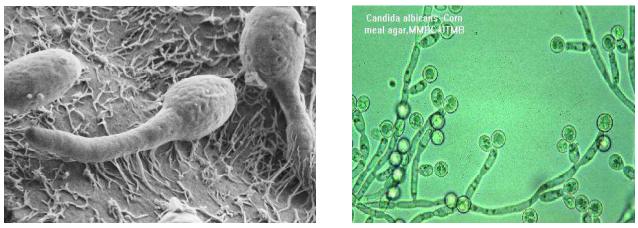


Figure 7.6: *Candida albicans*

Aspergillus niger

Aspergillus niger is a xerophilic, filamentous and a widespread aerobic fungus which grows on a large variety of substrates,[30] degrades celluloses and hemicelluloses and causes biodeterioration of oil-derived lubricants, polyvinyl chloride, and starch/polyethylene plastics.[31]

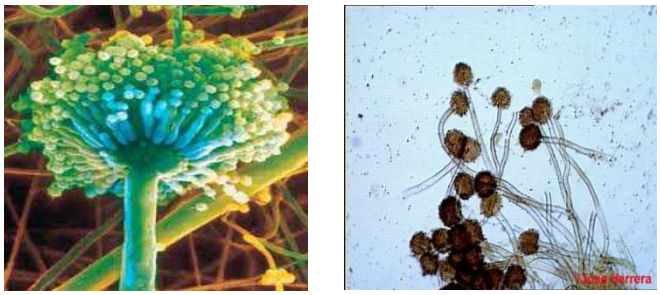


Figure 7.7: *Aspergillus niger*

Aspergillus clavatus

Aspergillus clavatus, a filamentous fungus, is a potent lung allergen, but it seldom causes invasive disease.[32] It may grow as blue-green mats on the germinating malting floors[33] and causes extrinsic allergic alveolitis called Malt-workers lung.[34]

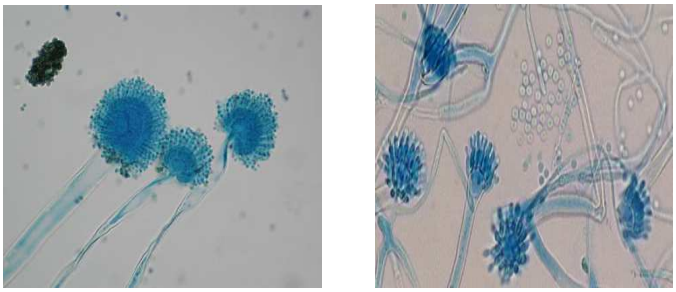


Figure 7.8: *Aspergillus clavatus*

The broth microdilution method as described by Rattan was used to carry out the minimum inhibitory concentrations (MICs) of the synthesized compounds.[35] All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and tested against above mentioned known drugs. Mueller Hinton broth method was used as nutrient medium to grow bacteria and fungus. Inoculum size for test strain was adjusted to 10^8 CFU (Colony Forming Unit) per millilitre by comparing the turbidity.

Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 ^oC for bacteria and 22 ^oC for fungus. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. Each synthesized drug was diluted obtaining 2000 mg/ml concentration, as a stock solution. In primary screening 500, 250 and 125 mg/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5, 6.250, 3.125 and 1.5625 mg/ml concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

7.1.3.3 Methods for antimicrobial assay

7.1.3.3a Method for Antibacterial assay[36,37]

Mueller Hinton agar medium (HiMedia, India) was used as the bacteriological medium. The compounds were diluted in 100% dimethylsulphoxide

(DMSO) at the concentrations of 5 mg/mL. The Mueller Hinton agar was heated to melt followed by cooling upto 48-50 ºC. To the molten agar, a standardized inoculum (1.5×10^8 CFU/mL, 0.5 McFarland) was then added aseptically and the entire mixture was poured into petri dishes which were earlier made sterile to give a solid plate. The wells which were prepared in the seeded agar plates were introduced in the well (the wells were made through cork borer vertically to petriplate upto 10 mm). The incubation of the plates was done by keeping them overnight at 37 ºC. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, Streptomycin. For each bacterial strain, controls were maintained where pure solvents were used instead of the synthetic compound. For measurement of the diameter of resulting zones, subtraction of the control zones from the test zones were carried out (the diameter is taken through the centre point of zone of inhibition and a zone of inhibition was calculated after adding the sample which can be visualized on the surface of the plate inoculated with bacteria and fungi). To minimize the error, the experiment was repeated three to four times and the mean values were presented.

7.1.3.3b Method for Antifungal assay[38]

Fungus colonies were subcultured onto Sabouraud's dextrose agar, SDA (Merck, Germany) and respectively incubated at 37 °C for 24 h and 25°C for 2-5 days. Suspensions of fungal spores were prepared in sterile PBS and adjusted to a concentration of 106 cells/mL. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 mL of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37 °C. After incubation of 24 h, bioactivities were determined by measuring the diameter of inhibition zone in mm. All experiments were made in triplicate and means were calculated.

7.1.4 Anti-mycobacterial activity

Tuberculosis (TB) is a disease which is caused by *Mycobacterium tuberculosis* (Mtb), still remains a global health crisis which is the cause of higher

mortality rate worldwide. The World Health Organization estimates that due to Tuberculosis, approximately two million people die every year.[39] Overcrowding of poor people living in huge cities also leads to a high occurrence of the disease.[40] This situation contributes to the greater incidence of TB at the accelerated speed.[41] Thus, there is a pressing need for new chemotherapeutic agents to combat the emergence of drugs resistance and to shorten the duration of treatment of Tuberculosis to improve the patient compliances.

7.1.4.1 Strain of bacterium

Mycobacterium tuberculosis (M. tuberculosis)

Mycobacterium tuberculosis is a bacterial pathogen from the family Mycobacteriaceae and is the causative agent of tuberculosis. It is aerobic in nature and requires high level of oxygen. It is primarily a pathogen of the mammalian respiratory system and infects the lungs.[42,43]

Figure 7.9: *Mycobacterium tuberculosis*

7.1.4.2 Method for antimycobacterial assay

MIC of compounds was determined against *M. tuberculosis* H37Rv strain by using Lowenstein-Jensen medium.[44] Determination of MIC of the test compounds against *M. tuberculosis* H37Rv were performed by L. J. agar (MIC) method where primary 1000, 500 and 250 mg/ml and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25 and 3.25 mg/ml dilutions of each test compound were added liquid L. J. Medium and then media were sterilized by inspissations method. A culture of *M. tuberculosis* H37Rv growing on L. J. medium was harvested in 0.85% saline in bijou bottles. All test compounds make first stock solution of 2000 mg/ml concentration of compounds was prepared in DMSO. These tubes were then incubated at 37^{o}C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5 x 10^4 bacilli per ml). These tubes were then incubated at 37^{o}C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. Tubes having the compounds were compared with

control tubes where medium alone was incubated with *M. tuberculosis* H37Rv. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The standard strain *M. tuberculosis* H37Rv was tested with known drug isoniazid.

7.1.5 Anti-malarial activity

Malaria is a mosquito-borne infectious disease causing the greatest mortality worldwide.[45] It is still one of the most severe parasitic disease caused by the multiplication of the protozoan parasite *Plasmodium falciparum* in erythrocytes.[46] Estimated worldwide deaths due to malaria was recorded 0.881 million annually, out of which 90% of them were from the African region.[47] Hence, there is a growing need to discover important lead compounds for the discovery of more selective and effectual antimalarial agents for both treatment and prophylaxis.[48,49]

7.1.5.1 Strain for malaria

***Plasmodium falciparum* (*P. falciparum*)**

Plasmodium falciparum is a protozoan parasite of the species of *Plasmodium* which causes malaria in human beings. Female Anopheles mosquito is the carrier of this parasite. In tropical areas, multi-drug resistant *Plasmodium falciparum* is the major health problem.[50]

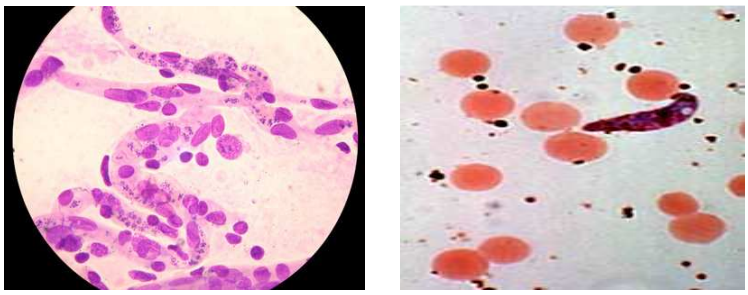


Figure 7.10: *Plasmodium falciparum*

7.1.5.2 Method for antimalarial assay

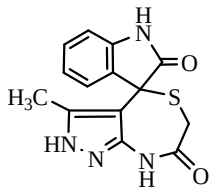
The *in vitro* antimalarial assay was carried out in 96 well microtitre plates according to the micro assay protocol of Rieckmann[51] and co-workers with minor modifications. The cultures of *P. falciparum* strain were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the

ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µl of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and uniformly maintained with 50% RBCs (O^+).

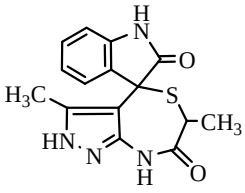
A stock solution of 5mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20 µl volume were added to the test wells so as to obtain final concentrations (at five fold dilutions) ranging between 0.4 µg/ml to 100 µg/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37°C in a candle jar. After 36 to 40 h incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Chloroquine and Quinine was used as the reference drug. The mean number of rings, trophozoites and schizonts were recorded per 100 parasites from duplicate wells after incubation for 38 hours, and percent maturation inhibition with respect to control group.

7.2 Results of biological screening

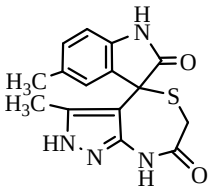
7.2.1 Chapter 2: *α-Amylase inhibition assay and anti-oxidant activities of pyrazolo[3,4-e][1,4]thiazepinone derivatives*



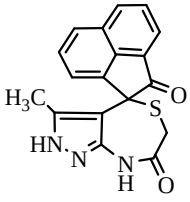
4a (ARS-41)



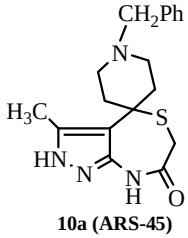
4b (ARS-42)



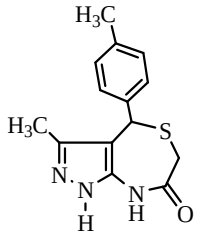
4c (ARS-43)



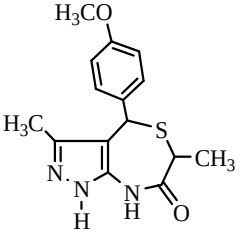
8a (ARS-44)



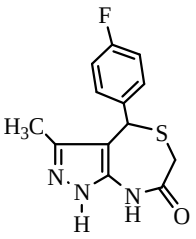
10a (ARS-45)



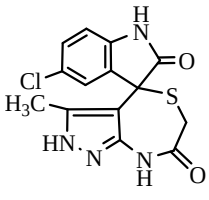
12a (ARS-46)



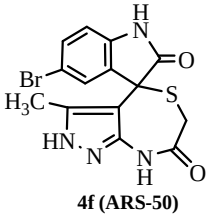
12d (ARS-47)



12e (ARS-48)



4e (ARS-49)



4f (ARS-50)

α-Amylase inhibition assay

The α-amylase inhibition assay of compounds (coded **ARS-41**, **ARS-42**, **ARS-43**, **ARS-44** and **ARS-45**) was determined using the method described in the introduction of the chapter. The screening data are shown in **Figure 7.11**.

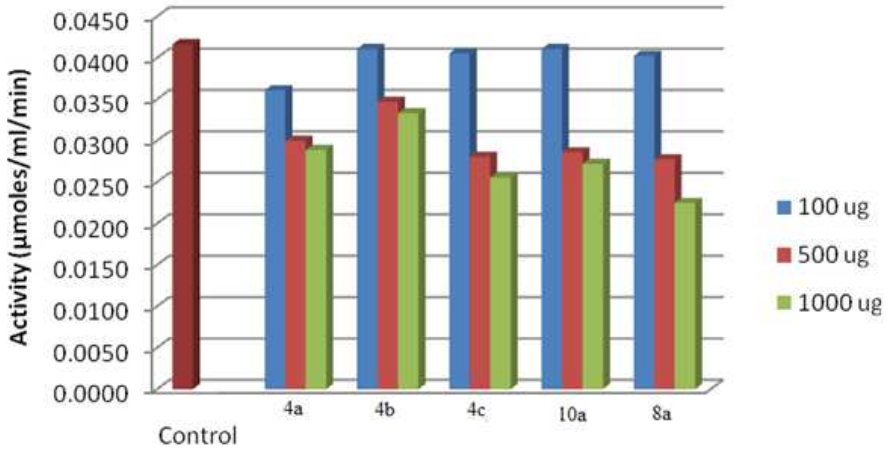


Figure 7.11: Graph showing the comparative analysis of α–Amylase inhibition assay

Table 7.1: Table showing the assay data

Sample	OD at 540 nm	Concentration of Maltose liberated (µg)	Activity (µmoles/ml/min)	% activity	% inhibition
Control	1.87	150	0.0416	100.00	0.00
4a (100 µg)	1.62	130	0.0361	86.73	13.27
4a(500 µg)	1.35	108	0.0300	72.05	27.95
4a (1000 µg)	1.3	104	0.0289	69.38	30.62
4b (100 µg)	1.85	148	0.0411	98.74	1.26
4b (500 µg)	1.56	125	0.0347	83.40	16.60
4b (1000 µg)	1.5	120	0.0333	80.06	19.94
4c (100 µg)	1.82	146	0.0405	97.41	2.59
4c (500 µg)	1.26	101	0.0280	67.38	32.62
4c (1000 µg)	1.14	92	0.0255	61.38	38.62
10a (100 µg)	1.85	148	0.0411	98.74	1.26
10a (500 µg)	1.28	103	0.0286	68.72	31.28
10a (1000 µg)	1.23	98	0.0272	65.38	34.62
8a (100 µg)	1.81	145	0.0402	96.74	3.26
8a (500 µg)	1.25	100	0.0278	66.72	33.28
8a (1000 µg)	1	81	0.0225	54.04	45.96

Results and inference:

From the **Table 7.1**, it is quite clear that among all the samples, **8a** substituted with acenaphthaquinone group displayed better inhibition at 1000 µg concentration. **4c** having methyl group on the indole ring and **10a** substituted with N-benzyl group have performed almost similarly, though a better inhibition was displayed by **4c** at 1000 µg concentration. And, the compound **4b** having methyl group on the thiazepine ring has poorly inhibited enzyme activity at all concentrations.

Anti-oxidant activity

The anti-oxidant activity of compounds (coded **ARS-46**, **ARS-47**, **ARS-48**, **ARS-49** and **ARS-50**) was determined using the DPPH method which is described in the introduction of the chapter. The screening data are shown in **Table 7.2**.

Table 7.2: Results of anti-oxidant activity

µg of sample/ Samples	ARS-46 (12a)	ARS-47 (12d)	ARS-48 (12e)	ARS-49 (4e)	ARS-50 (4f)	BHA
10	1.30	2.26	1.65	1.04	1.83	30.96
50	4.35	11.30	4.17	1.74	2.43	69.39
100	6.96	19.22	14.43	2.09	2.70	84.70

Results and inference:

All the screened compounds showed poor anti-oxidant activity as compared to the standard, BHA.

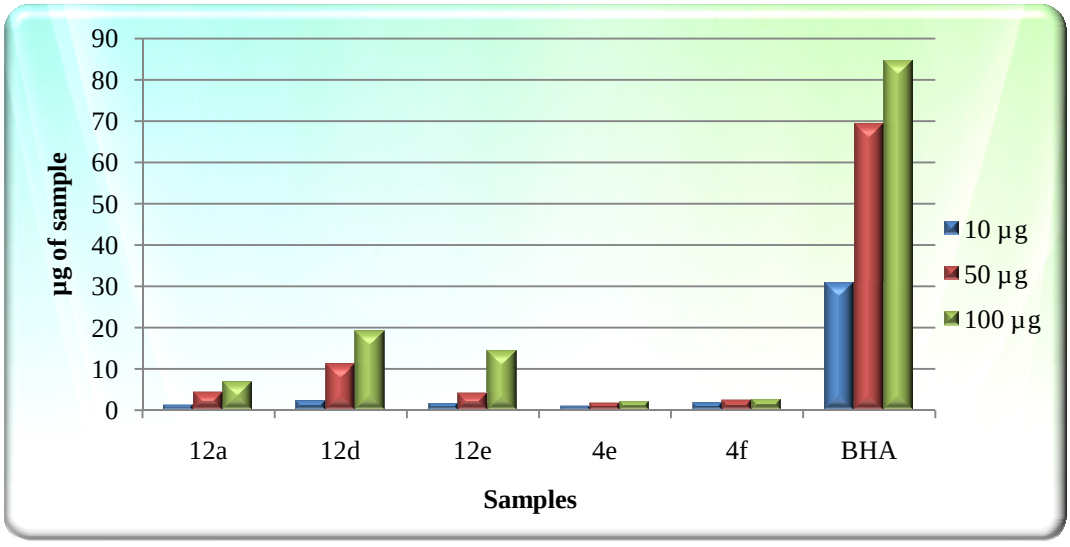
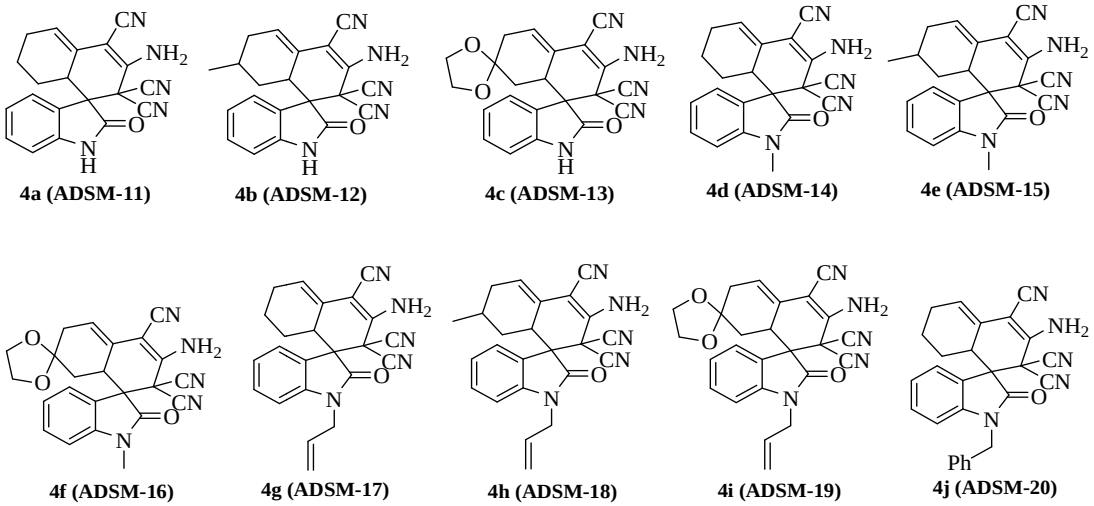


Figure 7.12: Results of anti-oxidant activity

7.2.2 Chapter 5: *Antimicrobial, antimycobacterial and antimalarial activities of spirooxindole derivatives*



The antimicrobial activity of representative compounds was determined using broth micro dilution method, which is described in introduction of the chapter. The antimicrobial screening data are shown in **Figure 7.13** and **7.14**.

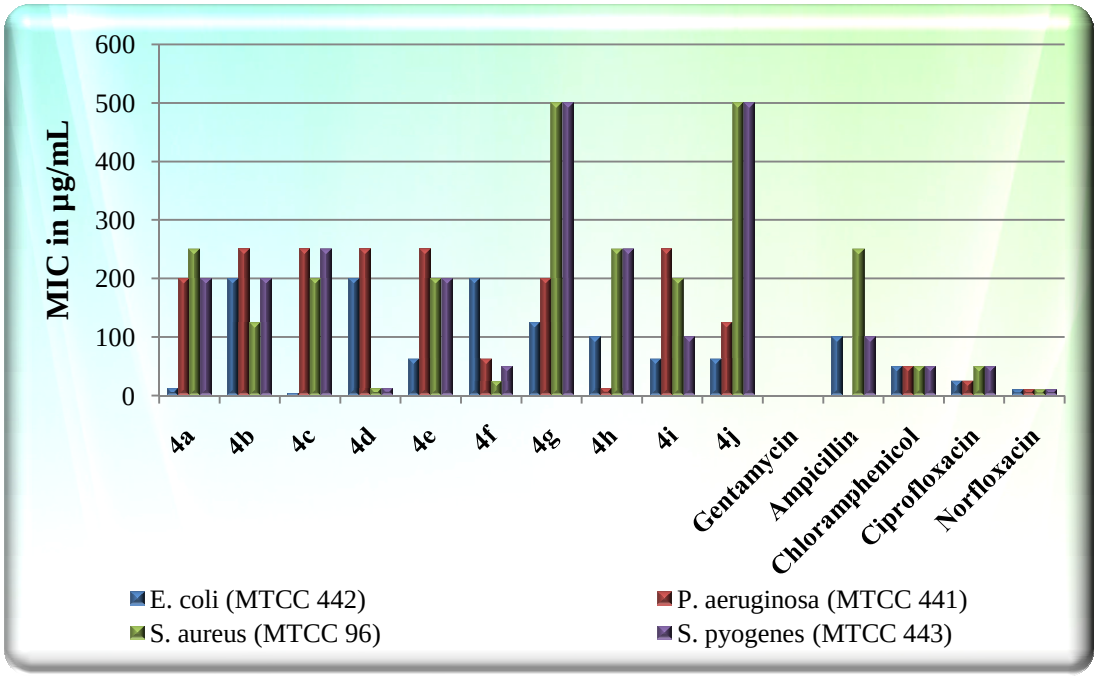


Figure 7.13: Antibacterial activity of the tested compounds (MIC in µg/mL)

Table 7.3: Antibacterial activity of tested compounds and antibiotics (MIC[a] µg/mL)

Compound	*E. coli* (MTCC 442)	*P. aeruginosa* (MTCC 441)	*S. aureus* (MTCC 96)	*S. pyogenes* (MTCC 443)
4a	12.5	200	250	200
4b	200	250	125	200
4c	3.25	250	200	250
4d	200	250	12.5	12.5
4e	62.5	250	200	200
4f	200	62.5	25	50
4g	125	200	500	500
4h	100	12.5	250	250
4i	62.5	250	200	100
4j	62.5	125	500	500
Gentamycin[b]	0.05	1	0.25	0.5
Ampicillin[b]	100	--	250	100
Chloramphenicol[b]	50	50	50	50
Ciprofloxacin[b]	25	25	50	50
Norfloxacin[b]	10	10	10	10

[a] MIC = Minimum inhibitory concentration, the lowest concentration of the compound which inhibits the growth of the bacterium by at least 99 %.
[b] = Standard drug.

Results and inference:

As shown in **Table 7.3**, one of the synthesized compounds **4c** having dioxamethylene group on the naphthalene ring showed the highest anti-bacterial inhibitory activity (MIC = 3.25 µg/mL) against the tested microbial strain, *E. coli*. Other compounds showed excellent activity (MIC = 12.5 µg/mL) against the tested microbial strains, i.e., **4a** against *E. coli*; compound **4d** substituted with methyl group at the nitrogen atom of the indole ring against *S. aureus* and *S. pyogenus* and compound **4h** having allyl group at the nitrogen atom of the indole ring against *P. aeruginosa*. Compound **4f** substituted with methyl group at the nitrogen atom of the indole ring and dioxamethylene group on the naphthalene ring showed good activity (MIC = 25 and 50 µg/mL) against *S. aureus* and *S. pyogenus* respectively. The significant results (MIC = 62.5 µg/mL) were obtained in case of **4e** substituted with methyl group at the nitrogen atom of the indole ring and on the naphthalene ring**, 4i** having allyl group at the nitrogen atom of the indole ring and dioxamethylene group on the naphthalene ring and **4j** substituted with benzyl group at the nitrogen atom of

the indole ring against *E. coli* and compound **4f** against *P. aeruginosa*. Rest of the compounds exhibited moderate to poor inhibitory activities against the tested microbial strains.

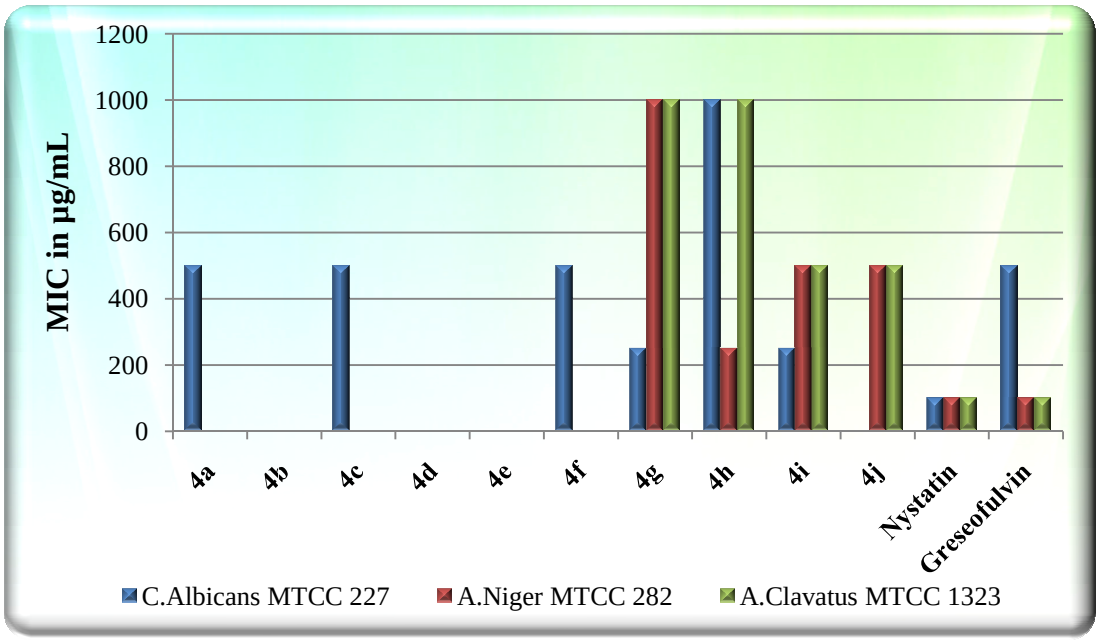


Figure 7.14: Antifungal activity of the tested compounds (MIC in µg/mL)

Table 7.4: Antifungal activity of the tested compounds (MIC in µg/mL)

Compound	MIC[a] (µg/mL)		
	C. albicans **MTCC 227**	***A. niger*** **MTCC 282**	***A. clavatus*** **MTCC 1323**
4a	500	>1000	>1000
4b	>1000	>1000	>1000
4c	500	>1000	>1000
4d	>1000	>1000	>1000
4e	>1000	>1000	>1000
4f	500	>1000	>1000
4g	250	1000	1000
4h	1000	250	1000
4i	250	500	500
4j	>1000	500	500
Nystatin[b]	100	100	100
Greseofulvin[b]	500	100	100

[a] MIC = Minimum inhibitory concentration, the lowest concentration of the compound which inhibits the growth of the fungus by at least 99 %.
[b] = Standard drug.

Results and inference:

As shown in **Table 7.4**, compound **4g** having allyl group at the nitrogen atom of the indole ring, **4i** substituted with allyl group at the nitrogen atom of the indole ring and dioxamethylene group on the naphthalene ring and **4h** having allyl group at the nitrogen atom of the indole ring and methyl group on the naphthalene ring exhibited the inhibitory activity (MIC = 250 µg/mL) against the tested fungal strain, *C. albicans* and *A. niger* respectively. The compounds **4a**, **4c** having dioxamethylene group on the naphthalene ring and **4f** substituted with methyl group at the nitrogen atom of the indole ring and dioxamethylene group on the naphthalene ring exhibited the inhibitory activity (MIC = 500 µg/mL) against the fungal strain, *C. albicans*. Compounds **4i** and **4j** substituted with benzyl group at the nitrogen atom of the indole ring exhibited the inhibitory activity (MIC = 500 µg/mL) against the tested fungal strains, *A. niger* and *A. clavatus*. Rest of the compounds showed poor inhibitory activity against the tested fungal strains.

Antimycobacterial activity

MIC of compounds was determined against *M. tuberculosis* H37Rv strain by using Lowenstein-Jensen medium (conventional method), which is described in the introduction of chapter. The antimycobacterial screening data are shown in **Figure 7.15**.

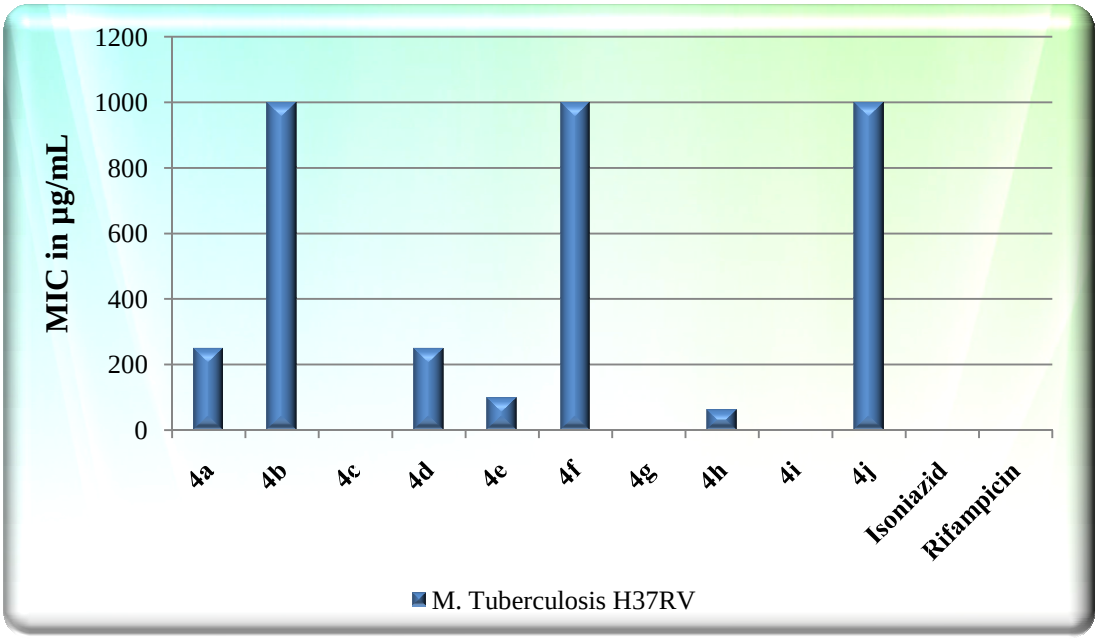


Figure 7.15: Antimycobacterial activity of the tested compounds (MIC in µg/ml)

Table 7.5: Antimycobacterial activity of tested compounds against *Mycobacterium tuberculosis* $H_{37}Rv$ microbial strain

Compound	MIC[a] (µg/mL)
4a	250
4b	1000
4c	0.52
4d	250
4e	100
4f	1000
4g	3.25
4h	62.5
4i	1
4j	1000
Isoniazid[b]	0.20
Rifampicin[b]	0.25

[a] MIC = Minimum inhibitory concentration, the lowest concentration of the compound which inhibits the growth of the mycobacterium by at least 99 %.

[b] = Standard drug.

Results and inference:

From **Table 7.5**, it was clearly concluded that compound **4c** having dioxamethylene group on the naphthalene ring showed highest inhibitory activity (MIC = 0.52 µg/mL) against *Mycobacterium tuberculosis* while compound **4i** substituted with allyl group at the nitrogen atom of the indole ring and dioxamethylene group on the naphthalene ring showed good inhibitory activity (MIC = 1 µg/mL). On the other hand, compound **4g** having allyl group at the nitrogen atom of the indole ring exhibited significant inhibitory activity (MIC = 3.25 µg/mL). Rest of the compounds showed a poor inhibitory activity.

Antimalarial Activity

Mean IC50 value of compounds was determined against *Plasmodium falciparum* strain by using RPMI 1640 medium (Rieckmann method), which is described in introduction of chapter. The antimycobacterial screening data are shown in **Figure 7.16**.

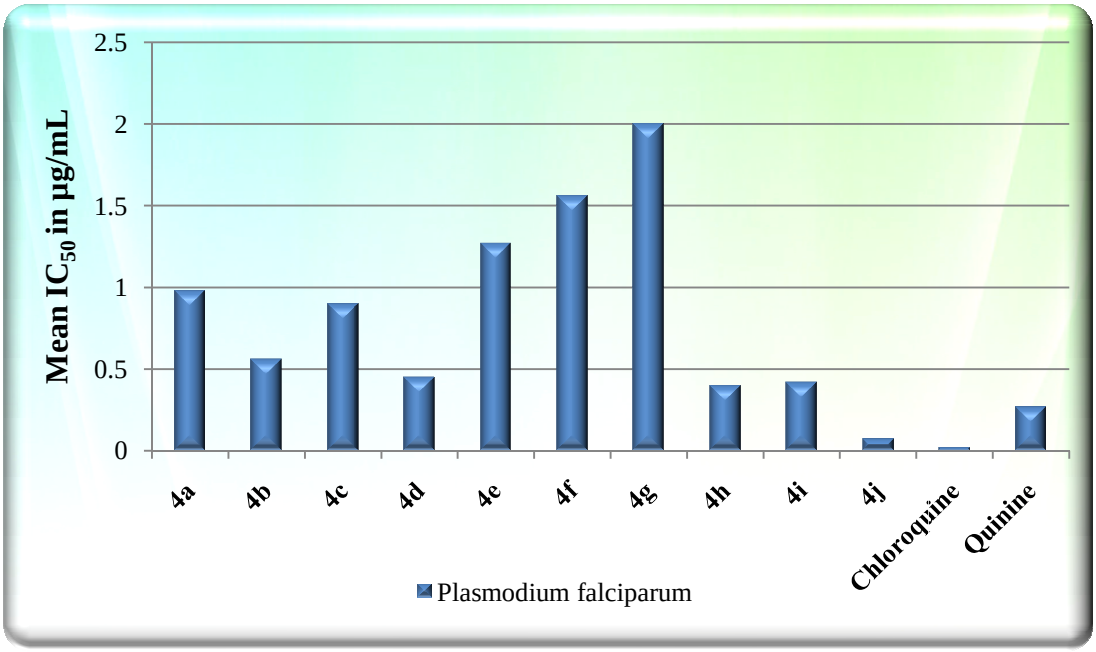


Figure 7.16: Antimalarial activity of the tested compounds (Mean IC50 in µg/ml)

Table 7.6: Antimalarial activity of tested compounds against *Plasmodium falciparum* strain

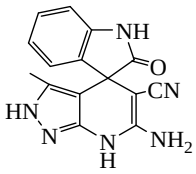
Compound	Mean IC_{50} values (µg/mL)
4a	0.98
4b	0.56
4c	0.90
4d	0.45
4e	1.27
4f	1.56
4g	2.00
4h	0.40
4i	0.42
4j	0.073
Chloroquine[b]	0.020
Quinine[b]	0.268

[a]MIC = Minimum inhibitory concentration, the lowest concentration of the compound which inhibits the growth of the *Plasmodium falciparum* by at least 99%.
[b] = Standard drug

Results and inference:

From **Table 7.6**, it was concluded that the tested compounds were found to exhibit good to moderate inhibitory activity against *Plasmodium falciparum*. Compound **4j** showed excellent activity (MIC = 0.073 µg/mL) against *P. falciparum*. Compounds **4h** substituted with allyl group at the nitrogen atom of the indole ring and methyl group on the naphthalene ring (MIC = 0.40 µg/mL), **4i** having allyl group at the nitrogen atom of the indole ring and dioxamethylene group on the naphthalene ring (MIC = 0.42 µg/mL), and **4d** substituted with methyl group at the nitrogen atom of the indole ring (MIC = 0.45 µg/mL) showed good activity. Rest of the compounds showed a moderate to poor inhibitory activity against *P. falciparum*.

7.2.3 Chapter 6: *Antimicrobial, antimycobacterial and antimalarial activities of spiro [indoline-3,4′-pyrazolo[3,4-b]pyridine derivatives*



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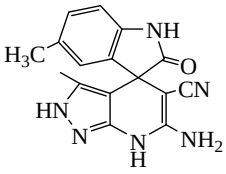
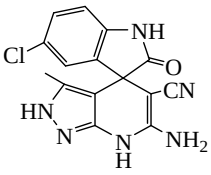
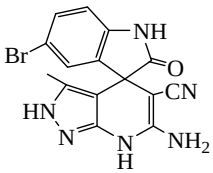


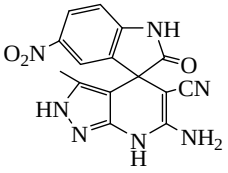
4b (ADP-41)



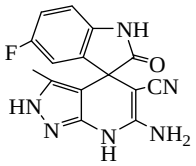
4c (ADP-42)



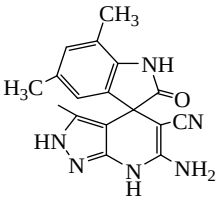
4d (ADP-43)



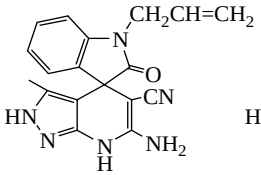
4e (ADP-44)



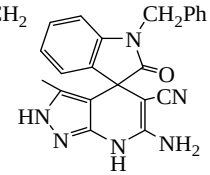
4f (ADP-45)



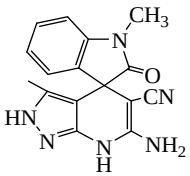
4g (ADP-46)



4h (ADP-47)



4i (ADP-48)



4j (ADP-49)

Antimicrobial activity

The antimicrobial activity of representative compounds was determined using broth micro dilution method, which is described in introduction of the chapter. The antimicrobial screening data are shown in **Figure 7.17**.

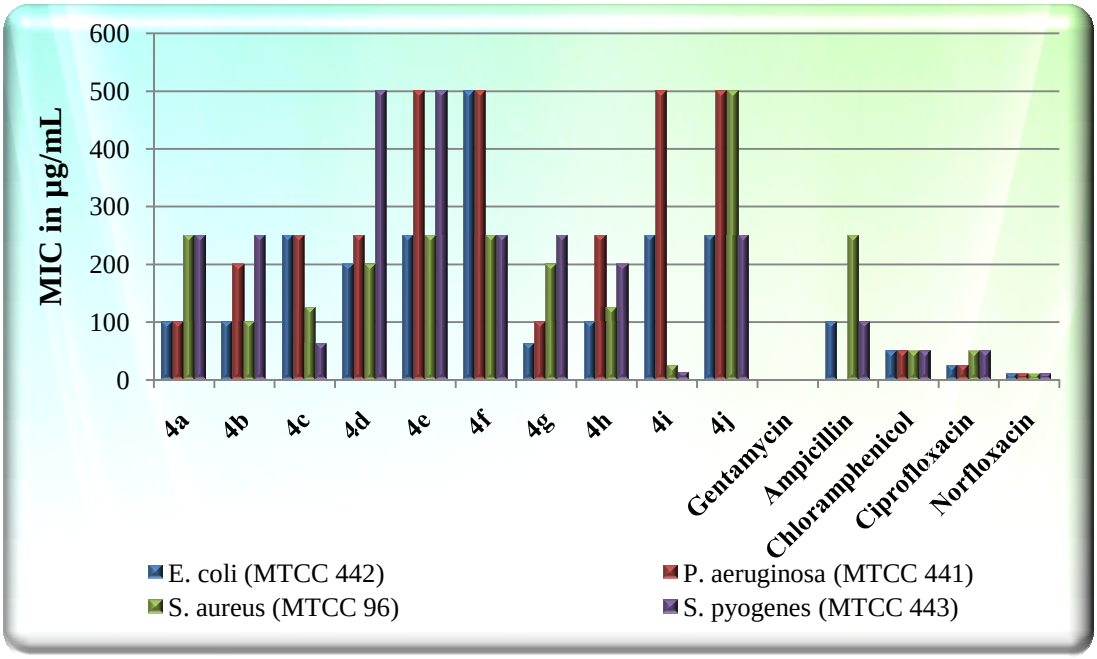


Figure 7.17: Antibacterial activity of the tested compounds (MIC in µg/mL)

Table 7.7: Antibacterial activity of tested compounds and antibiotics (MIC in µg/mL)

Compound	MIC[a] (µg/mL)			
	E. coli **MTCC 443**	***P. aeruginosa*** **MTCC 441**	***S. aureus*** **MTCC 96**	***S. pyogenus*** **MTCC 442**
4a	100	100	250	250
4b	100	200	100	250
4c	250	250	125	62.5
4d	200	250	200	500
4e	250	500	250	500
4f	500	500	250	250
4g	62.5	100	200	250
4h	100	250	125	200
4i	250	500	25	12.5
4j	250	500	500	250
Gentamycin[b]	0.05	1	0.25	0.5
Ampicillin[b]	100	--	250	100
Chloramphenicol[b]	50	50	50	50
Ciprofloxacin[b]	25	25	50	50
Norfloxacin[b]	10	10	10	10

[a] MIC = Minimum inhibitory concentration, the lowest concentration of the compound which inhibits the growth of the bacterium by at least 99 %.
[b] = Standard drug.

Results and inference:

As shown in **Table 7.7**, some of the synthesized compounds showed excellent activity (MIC = 12.5 µg/mL) against the tested microbial strains, i.e., **4i** substituted with benzyl group at the nitrogen atom of the indole ring against *S. pyogenus* and good inhibitory activity (MIC = 25 µg/mL) against *S. aureus*. Compounds **4c** having chloro group at indole ring and **4g** substituted with two methyl groups at indole ring showed moderate activity (MIC = 62.5 µg/mL) against *S. pyogenus* and *E. coli* respectively. The significant results (MIC = 100 µg/mL) were obtained in case of **4a**, **4b** having methyl group at indole ring and **4h** substituted with allyl group at the nitrogen atom of the indole ring against *E. coli*; **4b** against *S. aureus* and compounds **4a** and **4g** against *P. aeruginosa* respectively. Moderate inhibitory activity was shown by compounds **4c** and **4h** against *S. aureus*. All the synthesized compounds showed poor inhibitory anti-bacterial activity against the tested bacterial strains.

Anti-fungal activity

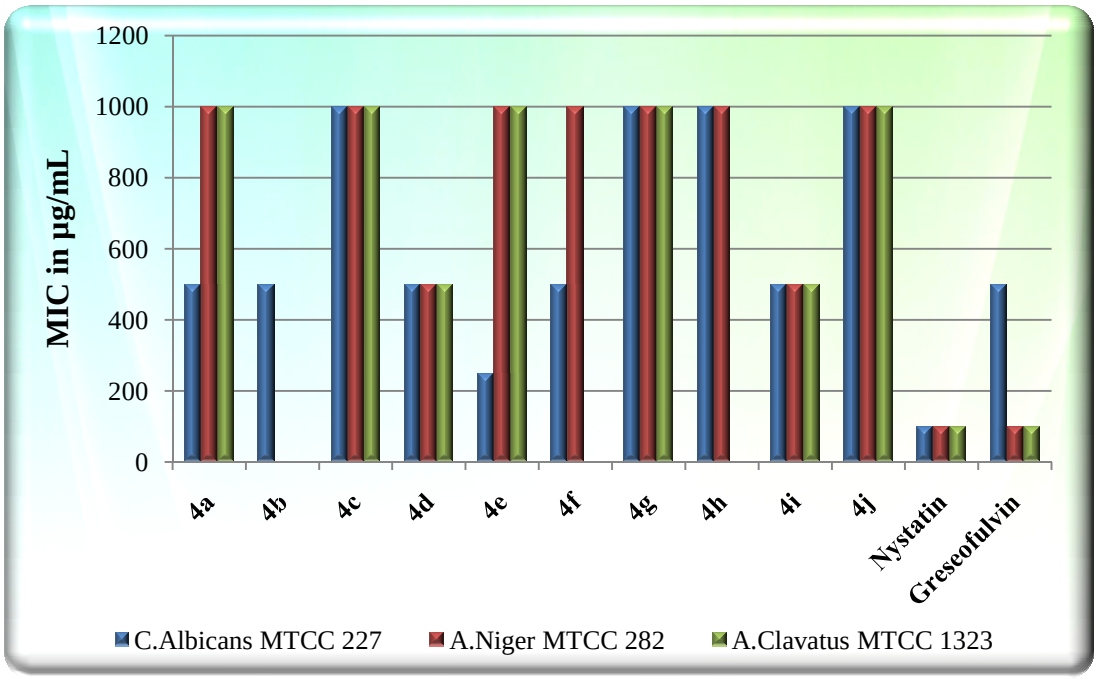


Figure 7.18: Antifungal activity of representative compounds (MIC in µg/mL)

Table 7.8: Antifungal activity of representative compounds (MIC in µg/mL)

Compound	MIC[a] (µg/mL)		
	C. albicans **MTCC 227**	***A. niger*** **MTCC 282**	***A. clavatus*** **MTCC 1323**
4a	500	1000	1000
4b	500	>1000	>1000
4c	1000	1000	1000
4d	500	500	500
4e	250	1000	1000
4f	500	1000	>1000
4g	1000	1000	1000
4h	1000	1000	>1000
4i	500	500	500
4j	1000	1000	1000
Nystatin[b]	100	100	100
Greseofulvin[b]	500	100	100

[a] MIC = Minimum inhibitory concentration, the lowest concentration of the compound which inhibits the growth of the fungus by at least 99 %.
[b] = Standard drug.

Results and inference:

As shown in **Table 7.8**, compound **4e** having nitro group at indole ring exhibited the inhibitory activity (MIC = 250 µg/mL) against the tested fungal strain, *C. albicans*. The compounds **4a**, **4b** substituted with methyl group at indole ring, **4d** having bromo group at indole ring, **4f** substituted with fluoro group at indole ring and **4i** having benzyl group at the nitrogen atom of the indole ring exhibited the inhibitory activity (MIC = 500 µg/mL) against the fungal strain, *C. albicans*. Compounds **4d** and **4i** exhibited the inhibitory activity (MIC = 500 µg/mL) against the tested fungal strains, *A. niger* and *A. clavatus*. Rest of the compounds showed poor inhibitory activity against the tested fungal strains.

Antimycobacterial activity

MIC of compounds was determined against *M. tuberculosis* H37Rv strain by using Lowenstein-Jensen medium (conventional method), which is described in the introduction of chapter. The antimycobacterial screening data are shown in **Figure 7.19**.

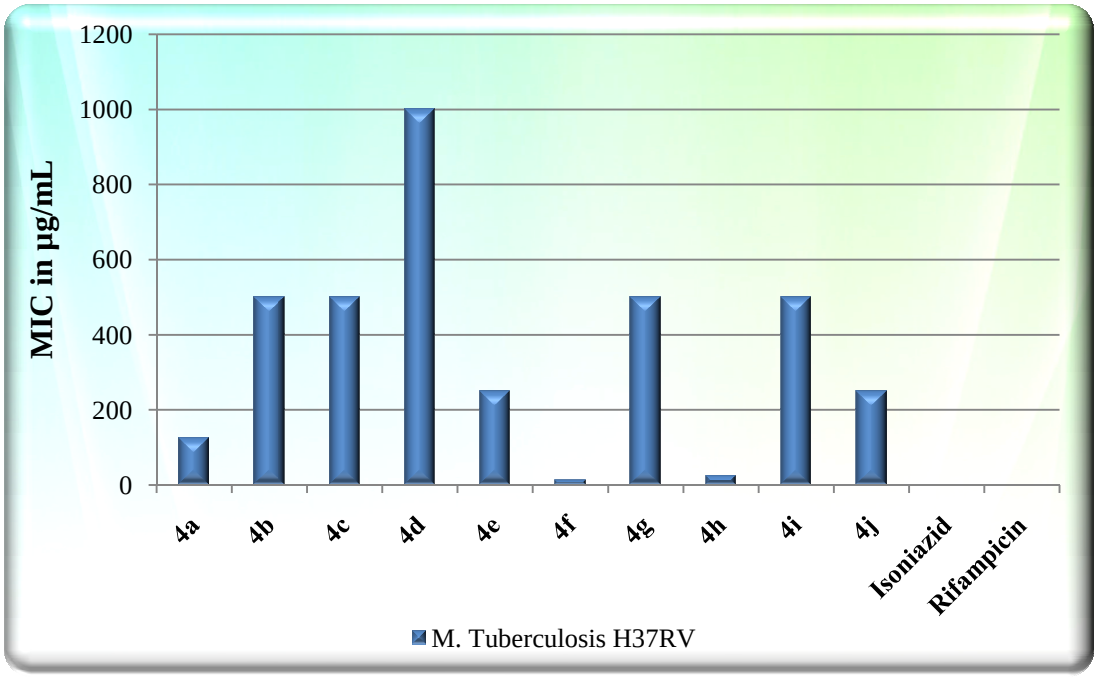


Figure 7.19: Antimycobacterial activity of the tested compounds (MIC in µg/ml)

Table 7.9: Antimycobacterial activity of tested compounds against *Mycobacterium tuberculosis* H_{37}Rv microbial strain

Compound	MIC[a] (µg/mL)
4a	125
4b	500
4c	500
4d	1000
4e	250
4f	12.5
4g	500
4h	25
4i	500
4j	250
Isoniazid[b]	0.20
Rifampicin[b]	0.25

[a] MIC = Minimum inhibitory concentration, the lowest concentration of the compound which inhibits the growth of the mycobacterium by at least 99 %.
[b] = Standard drug.

Results and inference:

From **Table 7.9**, it was clearly concluded that compound **4f** substituted with fluoro group at indole ring showed highest inhibitory activity (MIC = 12.5 µg/mL) against *Mycobacterium tuberculosis* while compound **4h** having allyl group at the nitrogen atom of the indole ring showed good inhibitory activity (MIC = 25 µg/mL). And, compound **4a** exhibited a significant inhibitory activity (MIC = 125 µg/mL). Compounds **4e** substituted with nitro group at indole ring and **4j** having methyl group at the nitrogen atom of the indole ring exhibited a moderate inhibitory activity (MIC = 250 µg/mL). Rest of the compounds showed poor inhibitory activity against the tested strain.

Anti-malarial activity

Mean IC50 value of compounds was determined against *Plasmodium falciparum* strain by using RPMI 1640 medium (Rieckmann method), which described in introduction of chapter. The antimycobacterial screening data are shown in **Figure 7.20**.

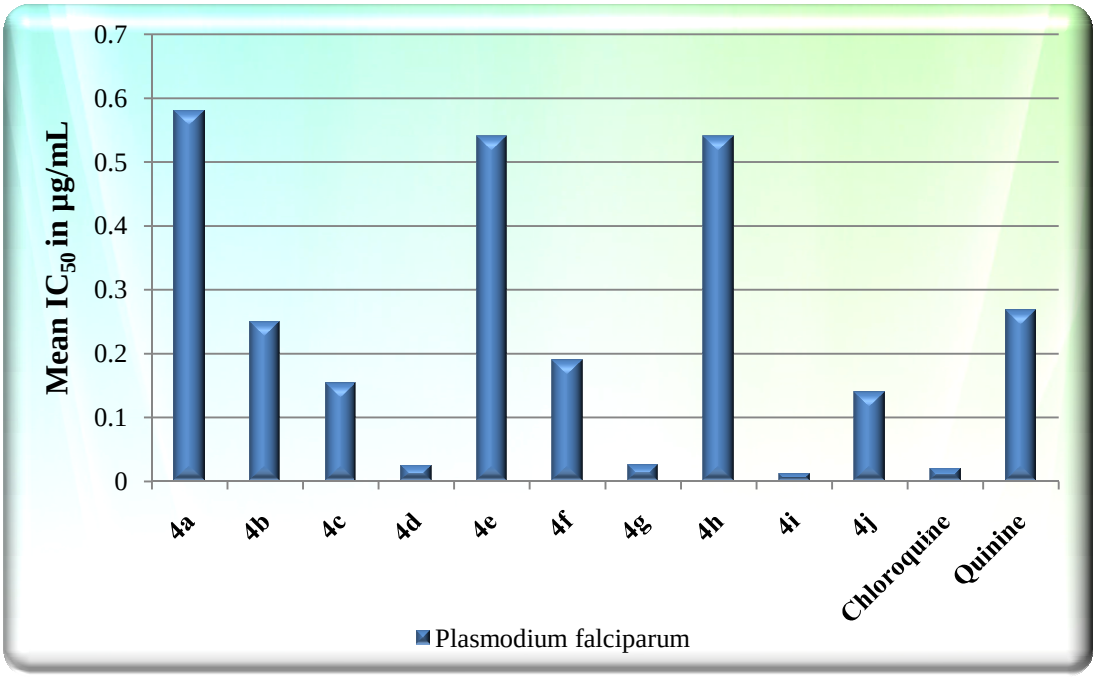


Figure 7.20: Antimalarial activity of the tested compounds (Mean IC50 in µg/ml)

Table 7.10: Antimalarial activity of tested compounds against *Plasmodium falciparum* strain

Compound	Mean IC_{50} values[a] (µg/mL)
4a	0.58
4b	0.25
4c	0.154
4d	0.025
4e	0.54
4f	0.19
4g	0.026
4h	0.54
4i	0.012
4j	0.14
Chloroquine[b]	0.020
Quinine[b]	0.268

[a]MIC = Minimum inhibitory concentration, the lowest concentration of the compound which inhibits the growth of the *Plasmodium falciparum* by at least 99%.
[b] = Standard drug

Results and inference:

From **Table 7.10**, it was concluded that the tested compounds were found to exhibit good to moderate activity against *Plasmodium falciparum*. Compound **4i** having benzyl group at the nitrogen atom of the indole ring showed excellent activity (MIC = 0.012 µg/mL) against *P. falciparum* as compared to *Chloroquine* and *Quinine*. Compounds **4d** substituted with bromo group at the indole ring (MIC = 0.025 µg/mL) and **4g** having two methyl groups at the indole ring (MIC = 0.026 µg/mL) were found to have comparable values as compared to *Quinine*. Compounds **4j** substituted with methyl group at the nitrogen atom of the indole ring (MIC = 0.14 µg/mL), **4c** having chloro group at the indole ring (MIC = 0.154 µg/mL), **4f** substituted with fluoro group at the indole ring (MIC = 0.19 µg/mL) and **4b** having methyl group at the indole ring (MIC = 0.25 µg/mL) showed good activity. Compounds **4h** substituted with allyl group at the nitrogen atom of the indole ring and **4e** having nitro group at the indole ring (MIC = 0.54 µg/mL) and **4a** (MIC = 0.58 µg/mL) showed moderate activity.

7.3 References:

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Appendix

LIST OF PUBLICATIONS

1. Anshu Dandia, Ruby Singh, Jyoti Joshi, **Shuchi Maheshwari** and Pragya Soni, "Ultrasound promoted catalyst-free and selective synthesis of spiro [indole-3,4'-pyrazolo[3,4-*e*][1,4]thiazepines] in aqueous media and evaluation of their anti-hyperglycemic activity", *RSC Advances*, **2013**, *3*, 18992–19001.

2. Anshu Dandia, Ruby Singh, Jyoti Joshi and **Shuchi Maheshwari**, "Magnetically separable $CuFe_2O_4$ nanoparticles: an efficient catalyst for the synthesis of quinoxaline derivatives in tap-water under sonication" *European Chemical Bulletin*, **2013**, *2(10)*, 825-829.

3. Anshu Dandia, Ruby Singh, Jyoti Joshi, and **Shuchi Maheshwari**, Green and chemoselective synthesis of pyrazolo[3,4-*e*][1,4]thiazepines and evaluation of their anti-infective activities, *Research on Chemical Intermediates*, **2014**, DOI 10.1007/s11164-013-1524-2.

LIST OF CONFERENCES/SEMINARS PRESENTED

1. "3rd International Conference on Heterocyclic Chemistry" held on December 10-13, **2011** in the Department of Chemistry, University of Rajasthan, **Jaipur**.
2. 19th ISCB International Conference (ISCBC-2013) titled "Recent Advances and Current Trends in Chemical and Biological sciences" held on March 2-5, **2013** jointly organized by Indian Society of Chemists and Biologists, Lucknow and Department of Chemistry, Mohanlal Sukhadia University, **Udaipur**.
3. National Seminar on "Chemistry for Economic Growth and Human Comforts" held on August 31, **2013** organized by the Department of Chemistry, University of Rajasthan, **Jaipur**.
4. National Seminar & Science Model Exhibition on "Innovations in Science and Technology for Inclusive Development" held on January 16-17, **2014** jointly organized by Dr. B. Lal Institute of Biotechnology and Indian Science Congress Association (Jaipur Chapter) in **Jaipur**.
5. National Conference on "Recent Trends in Research in Chemical Sciences" held on February 21-22, **2014** organized by the Department of Chemistry, Manipal University, **Jaipur**.
6. National Seminar on "Technological Advances and Their Impact on Environment" held on April 22, **2014** organized by the Department of Chemistry, Poornima College of Engineering, **Jaipur**.
7. Seminar on "Atomic Energy for Robust National Development" held on 5th September, **2014** organized by BARC and MNIT, **Jaipur**.

LIST OF CONFERENCES/SEMINARS ATTENDED

1. UGC and CSIR Sponsored 'National Symposium' on "Organic Synthesis" held on February 18-19, **2011** at ICG-The IIS University, **Jaipur**.
2. UGC Sponsored Symposium on "Celebrating International Year of Chemistry" held on September 17, **2011** at International College for Girls in **Jaipur**.

3. 'One Day National Seminar' on "Chemistry in our lives" held on December 12, **2011** organized by the Centre of Advanced Studies, Department of Chemistry, University of Rajasthan, **Jaipur**.
4. 'International Conference' on "New Emerging Trends in Chemistry" (A satellite conference of 3rd Indo-German Conference on Modelling Chemical and Biological (Re)activity) held on 3-4 March, **2013** organized by the Department of Chemistry, The IIS University, **Jaipur**.
5. Workshop on "Vision and contributions of Mahamana Pt. Madan Mohan Malaviya" held on 7-8 August, **2013** organized by Malaviya National Institute of Technology, **Jaipur**.

LIST OF COMMUNITY DEVELOPMENT PROGRAMMES ATTENDED

1. "Cancer Awareness Programme" held at Malaviya National Institute of Technology, **Jaipur** on 19th November, **2014**.
2. "HIV-AIDS Awareness Programme" held at Malaviya National Institute of Technology, **Jaipur** on 1st December, **2014**.

BRIEF BIO-DATA

Ms. Shuchi Maheshwari completed her school education in 2005. She has received her B.Sc. degree from Seth G. L. Bihani College, Sriganganagar in 2008. She has secured Master's degree from Malaviya National Institute of Technology, Jaipur in 2010. She has joined Ph.D. in July, 2010 under the supervision of Dr. Jyoti Joshi (Associate Professor), Department of Chemistry, Malaviya National Institute of Technology, Jaipur. During the course of her research work, three research papers have been published in peer reviewed international journals in the field of Chemistry which shows her interest in the field of research. During the whole tenure of her research work, she has been the recipient of several scholarship awards such as CSIR-JRF and CSIR-SRF, etc. She has attended various National and International seminars, workshops and conferences. Her research interests are focussed around the use of Green Chemistry, Heterocyclic chemistry and Organic synthesis. She has contributed as a volunteer in organising a National level workshop on Pandit Madan Mohan Malaviya and actively participated in various community development programmes organised by the Institute.