A Dissertation thesis entitled

"SYNTHESIS AND CHARACTERIZATION OF INDOLE DERIVATIVES: AN APPROACH TO INVESTIGATE THE ANTIMICROBIAL ACTIVITY"

Submitted in partial fulfillment of the requirements For the award of the degree of

Master of Science

IN

INDUSTRIAL CHEMISTRY

Submitted By

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2022-2023

Dedicated to

My Beloved Family

Without their love, support and constant encouragement,
this would not have been possible

DECLARATION

We undersigned, hereby declare that the work assimilated in the dissertation thesis entitled **"Synthesis and Characterization of Indole Derivatives: An approach to investigate the antimicrobial activity"** has been carried out by us at the Faculty of Science, Department of Industrial Chemistry, Atmiya University, Rajkot, Gujarat, India, under the supervision and Guidance of **Dr. Mehul L. Savaliya, Assistant Professor, Faculty of Science, Department of Industrial Chemistry, Atmiya University, Rajkot, Gujarat, India.**

To the best of our knowledge and belief, the work included in this thesis isquite original and has not been submitted to any other Institution or University for the award of any degree either in this or any other form.

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ABSTRACT

Indoles are a significant heterocyclic system in natural products and drugs. These are significant classes of chemicals and organic compounds that are key to cell biology. In recent years, there has been an increase in interest in the use of indole derivatives as biologically active molecules for the treatment of bacteria, cancer cells, and various forms of illnesses in the human body. In this review, we aim to highlight the construction of indoles as a moiety and the synthesis of derivatives from that moiety.

Keywords: indole, derivatives of indole.

1.0 INTRODUCTION

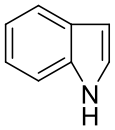
Indole is a heterocyclic aromatic compound that occurs naturally in many plants and animals, and is known to possess a range of biological activities. Its derivatives have been extensively studied and have shown potential in various medicinal and pharmaceutical applications, including anti-inflammatory, anticancer, and antiviral properties. One of the most promising applications of indole and its derivatives is their antimicrobial activity, which has become an area of increasing interest in the search for new and effective antimicrobial agents. The synthesis of indole derivatives has therefore become an area of increasing interest in organic chemistry research. Among the different approaches to synthesizing these derivatives, the use of various functional groups as starting materials and the modification of the indole structure have proven to be effective methods.

In this thesis, we focus on the synthesis of derivatives of indole using different chemical reactions and strategies. The objective of this work is to develop a practical and efficient method for the synthesis of indole derivatives that can be easily scaled up for industrial applications. The synthesis of indole derivatives has proven to be an effective strategy for discovering new antimicrobial agents with improved efficacy and reduced toxicity. Therefore, the development of practical and efficient methods for the synthesis of indole derivatives with potent antimicrobial activity has become a key area of research in organic chemistry.

To achieve this goal, we will explore various synthetic routes and reaction conditions. We will also investigate the effects of different functional groups on the reactivity and selectivity of these reactions.

Furthermore, we will analyze the structural and chemical properties of the synthesized indole derivatives using various spectroscopic and analytical techniques, including NMR, IR.

Overall, this thesis aims to contribute to the development of novel and efficient methods for the synthesis of indole derivatives, which may have important implications in the field of medicinal chemistry and drug discovery. and the development of new and effective indole-based antimicrobial agents that can be used in the treatment of various infectious diseases. By synthesizing and characterizing novel indole derivatives, we hope to provide valuable insights into the design and optimization of indole-based antimicrobial agents.

- Structure of indole: 

- Molecular Formula: C_8H_7N

- Molecular Weight: 117.15 gm/mol

2.0 LITERATURE REVIEW

2.0.1 SYNTHESIS

The Formylation of Indole and Some Reactions of 3-Formylindole.

This is the name of the paper that we take for the reference for make our basic moiety.

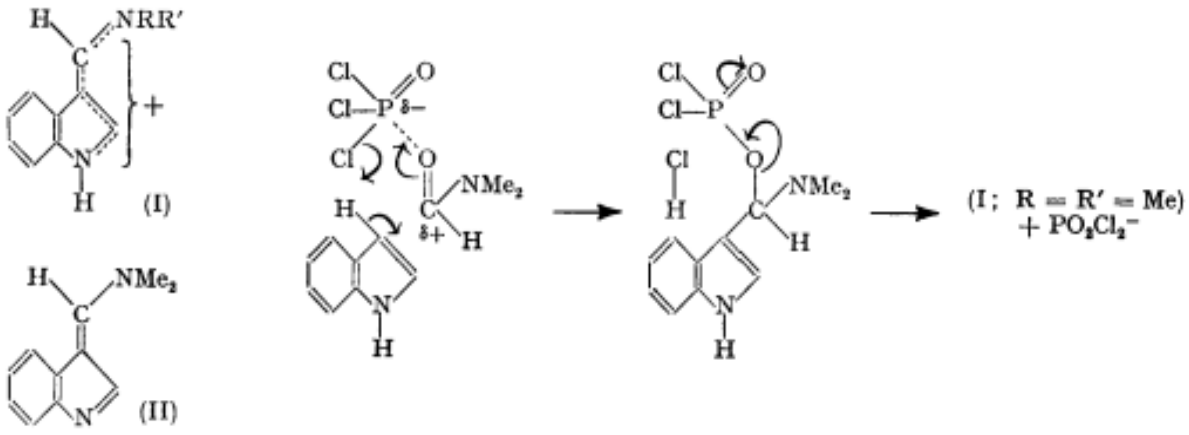


Figure.1 Reference reaction for basic moiety

- We used this reaction for the synthesis of our basic moiety as a reference
- This common reaction is known as a vilsmeier haack reaction, in this reaction Aldehyde group is attach at the top to the indole molecule.
- From this basic moiety we synthesis some derivatives of indole with the help of some molecule and some reactions.

An Efficient One-Pot, Three-Component Reaction: Synthesis of Complex-Annelated a-Carbolines via an Intramolecular [3+2]-Dipolar Cycloaddition Reaction.

In the second step of synthesis of basic moiety we used this paper and this reaction for our reference. In this reaction there are bromide chloride is attached at the bottom of the indole. In the space of bromide chloride we attached allyl chloride at the bottom of the indole.

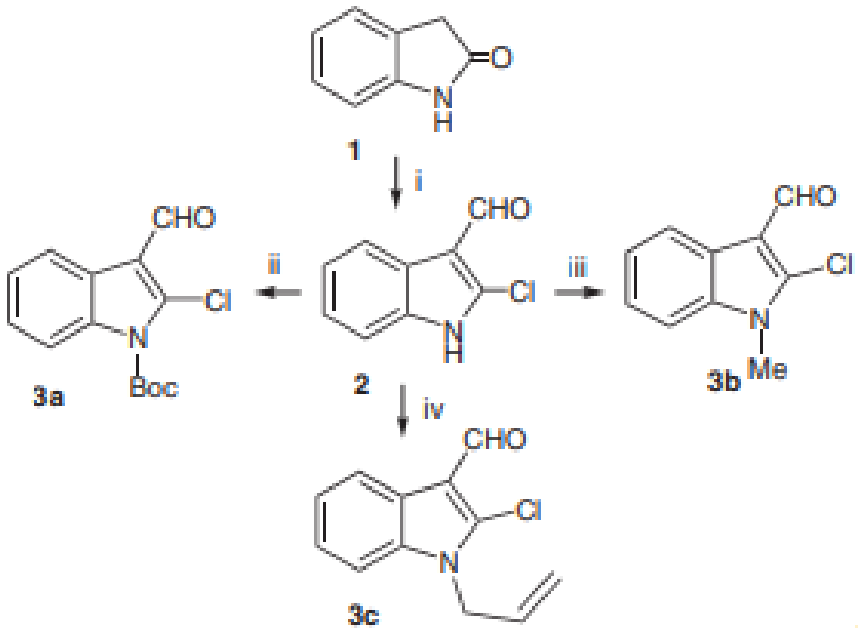


Figure.2 Reference reaction for basic moiety

From this some reference papers we made our basic moiety from the indole and from that basic moiety we made some different derivatives of indole with the help of some different molecules and some different reactions.

2.0.2 ADVANTAGES IN THE USE IF INDOLE DERIVATIVES AS A ANTIMICROBIAL AGENTS

Indole derivatives have been studied for their potential as antimicrobial agents, and there are several advantages associated with their use. Here are some of them:

- Broad spectrum of activity: Indole derivatives have been shown to exhibit antimicrobial activity against a wide range of microorganisms, including bacteria, fungi, and viruses.
- Low toxicity: Indole derivatives are generally considered to be safe and have low toxicity to humans and animals, making them a promising option for use as microbial agents.
- Mechanism of action: Indole derivatives have been shown to act on multiple targets in microorganisms, which can prevent the development of resistance to the agents.
- Stability: Indole derivatives are stable and can be formulated into various forms, including liquids, powders, and gels, which make them easy to store and use.
- Environmentally friendly: Indole derivatives are biodegradable and have low environmental impact, which makes them a promising option for use in environmentally sensitive areas.

2.0.3 DISADVANTAGES IN THE USE OF INDOLE DERIVATIVES AS ANTIMICROBIAL AGENTS

Indole derivatives have been used as antimicrobial agents due to their broad-spectrum activity against a range of microorganisms. However, there are also some disadvantages associated with their use. Here are some of them:

- Toxicity: Some indole derivatives have been found to be toxic to mammalian cells. This can limit their use as antimicrobial agents, especially in human medicine.

- Resistance: As with any antimicrobial agent, prolonged use of indole derivatives can lead to the development of resistance in microorganisms. This can reduce the effectiveness of the indole derivatives as antimicrobial agents over time.

- Limited efficacy: While indole derivatives have broad-spectrum activity, they may not be effective against all types of microorganisms. This can limit their use in certain clinical settings.

- Cost: The synthesis and purification of indole derivatives can be expensive, which may limit their use in resource-limited settings.

- Drug interactions: Indole derivatives can interact with other drugs, leading to potential side effects or reduced efficacy of either drug. This can complicate their use in combination therapy or in patients taking multiple medications.

2.0.4 CHARACTERIZATION OF INDOLE DERIVATIVES AS ANTIMICROBIAL AGENTS

Indole derivatives have been widely studied for their antimicrobial properties. They are a class of organic compounds that contain an indole ring, which is a bicyclic aromatic structure composed of a benzene ring fused to a pyrrole ring. Indole derivatives exhibit a broad range of antimicrobial activities against various microorganisms such as bacteria, fungi, and viruses.

Indole derivatives also exhibit antifungal activity against a variety of fungal pathogens, including Candida albicans, Aspergillus fumigatus, and Cryptococcus neoformans.

In addition to their antibacterial and antifungal properties, indole derivatives have also been shown to possess antiviral activity against several viruses.

Indole derivatives are a promising class of compounds with potent antimicrobial properties, making them potential candidates for the development of new antimicrobial agents.

2.1 MATERIALS AND METHOD

2.1.0 MATERIALS:

We have received our main molecule that is indole was obtained as an open-handed gift from our university. Our other materials include all chemicals, solvents, acids, and all apparatus are provided by our university**.**

2.1.1 METHODS:

- The FT-IR analysis of our sample products had been done on a FT-IR spectrophotometer.
- 1H-NMR analysis of our sample products was achieved on a FT-NMR spectrometer.

3.0 EXPERIMENTAL PROCEDURES

3.1.0: STEP:1 PREPARATION OF INDOLE-3-CARBALDEHYDE:

First, take 14.5 ml DMF in RBF and cool it to 0 degrees Celsius. Then add 4.37 ml $POCl_3$ dropwise by adding funnel maintaining temperature at 0 degrees Celsius. After completion of addition of $POCl_3$. Indole was soluble in DMF and added dropwise through addition funnel maintaining temperature at 0 degrees Celsius. After completion of addition of indole the reaction mixture was stirred at room temperature for 40 minutes. Then solution of NaOH was added dropwise. After the addition of NaOH reaction mixture was refluxed at 80-100 degrees Celsius overnight. Then workup was carried in water. Then filter the reaction mixture with the help of a funnel and filter paper.

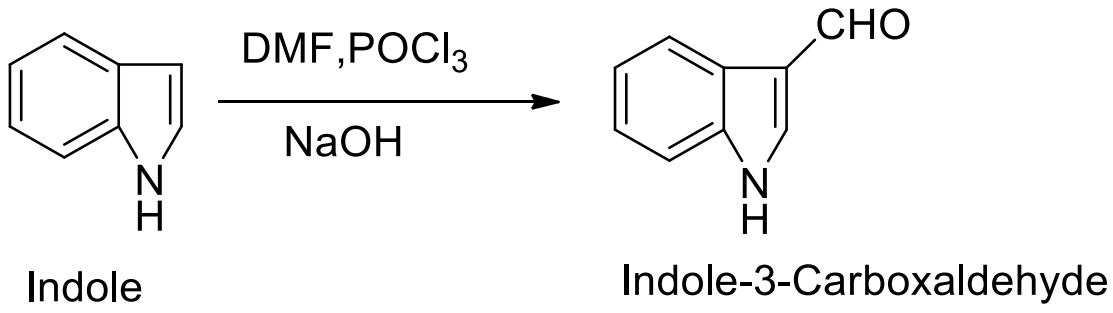


Figure.3 Preparation of indole-3-carboxaldehyde

Figure.4 Synthesis of Indole-3-carbaxaldehyde

3.1.1 STEP: 2 PREPARATION OF BASIC MOIETY

Take 1 equivalent and 10 gm of Indole-3-carboxaldehyde in RBF and soluble it in DMF. Then add 5 equivalent and 47.60 gm of K2CO3 and 1 equivalent and 11.43 gm of KI in this solution. Stir this reaction mixture for 30 minutes at room temperature. After 30 minutes add allyl chloride 1.2 equivalent and 6.73 ml and stir the reaction mixture overnight. Then the reaction mixture was workeuped in ice-cold water and the precipitate was formed. Than filter the reaction mixture with the help of a funnel and filter paper.

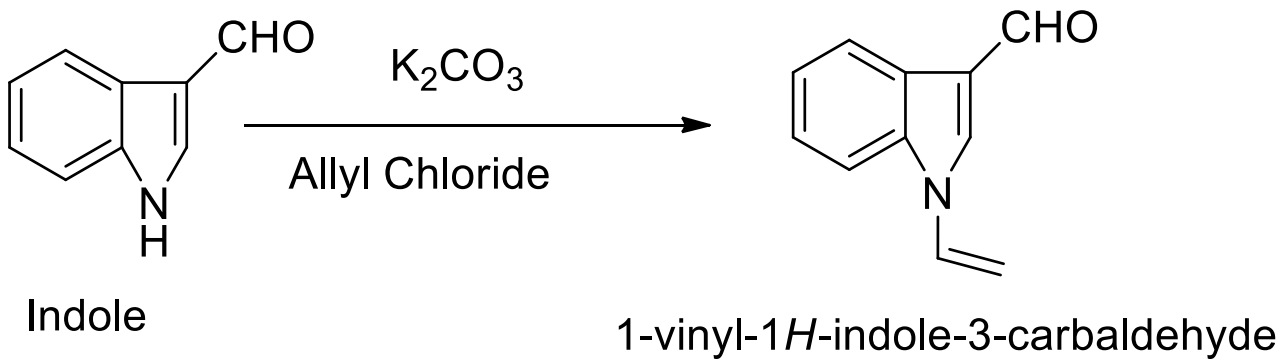


Figure.5 Reaction of indole-3-carboxaldehyde

Table.1 Calculation for Basic Moiety

	GRAM	MOLECULAR WEIGHT	EQUIVALENT WEIGHT
INDOLE	10	145.16	1
K2CO3	47.60	138.205	5
KI	6.73	76.52	1.

3.1.2 STEP: 3 PREPARATION OF INTERMEDIATE FOR SYNTHESIS OF DERIVATIVES

Take 1 equivalent and 5 ml of ethyl cyanoacetate and 1 equivalent and 1.41 ml of hydrazine hydrate (99%) and cool it in freezer for 30 minutes. The obtained product was washed with hexane & DCM.

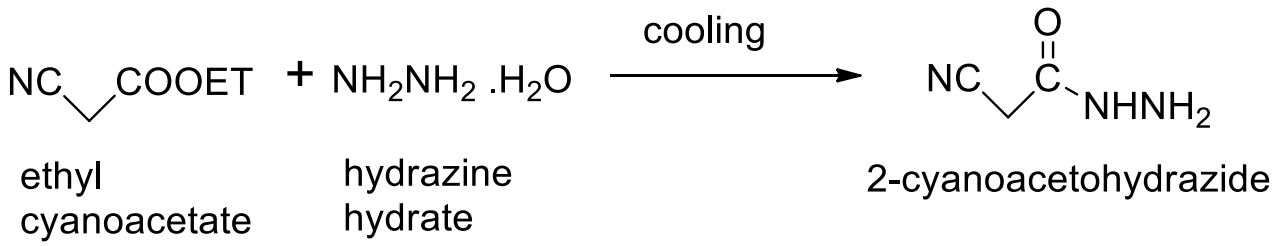


Figure.6 Reaction of Intermediate

Table.2 Calculation for Intermediate

	GRAM	MOLECULAR WEIGHT	EQUIVALENT WEIGHT
ETHYL CYANOACETATE	5 ml	113.11	1
HYDRAZINE HYDRATE	1.41 ml	32.0452	1

3.1.3 PREPARATION OF DERIVATIVE - 1

Check the solubility of 2-cyanoacetohydrazide in MeOH and DMF.
Take 1 equivalent and 5 grams of moiety (product-2) in MeOH and add a few drops of piperidine then add 1.2 equivalent and 2.89 grams of 2-cyanoacetohydrazide (product-3) and allow it to stir overnight. The obtained product was filtered with the help of a funnel and then washed with water.

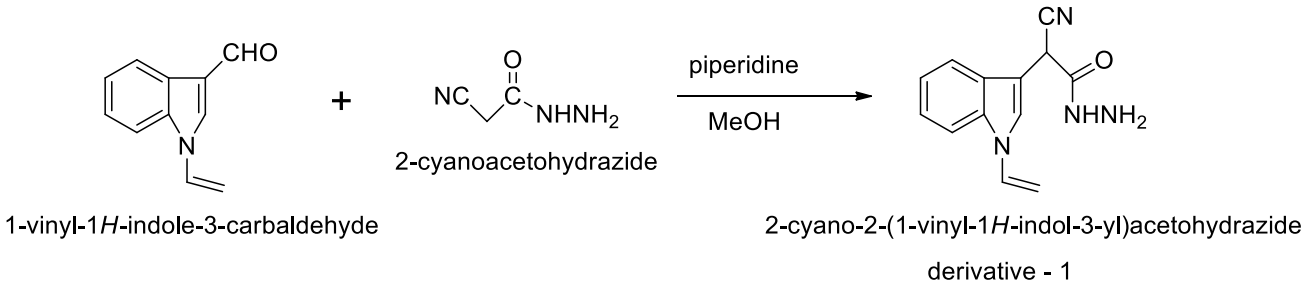


Figure.6 Preparation of derivative-1

Table.3 Calculation for Derivative-1

	GRAM	MOLECULAR WEIGHT	EQUIVALENT WEIGHT
PRODUCT-2	5 gm	171.20	1
PRODUCT-3	2.89 gm	99.09	1.2

3.1.4 PREPARATION OF INTERMEDIATE

Take 1 equivalent and 15 ml of ethyl cyanoacetate and 1 equivalent and 4.24 ml of hydrazine hydrate (99%) and cool it in freezer for 30 minutes. The obtained product was washed with hexane & DCM.

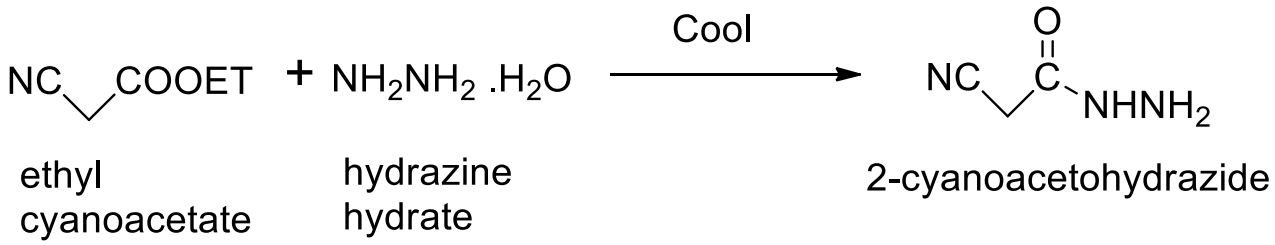


Figure.7 Reaction of Intermediate

Table.4 Calculation for Intermediate

	GRAM	MOLECULAR WEIGHT	EQUIVALENT WEIGHT
ETHYL CYANOACETATE	15 ml	113.11	1
HYDRAZINE HYDRATE	4.24 ml	32.0452	1

white product obtain is dissolved in water. Then add acetyl acetone dropwise. Then add 5-6 drops of concentrated HCl in this mixture. After sometime precipitates of (product-5) will obtain. Then filter the reaction mixture with the help of funnel and filter paper. If singlespot not obtain after filtration than again put it in water and stir because all reactant is water soluble.

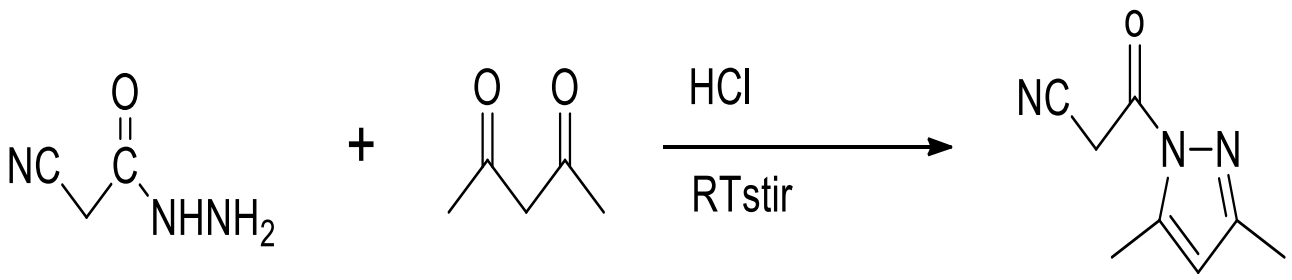


Figure.8 Reaction of Intermediate

Table.5 Calculation for Intermediate

	GRAM	**MOLECULAR WEIGHT**	**EQUIVALENT WEIGHT**
HYDRAZITE	7.21 gm	99.09	1
ACETYL ACETONE	7.42 ml	100.13	1

3.1.5 PREPARATION OF DERIVATIVE-2

Take 1 equivalent and 1.5 gm of product-5 and 1 equivalent and 0.973 gm of 4-bromoaniline in RBF and add in toluene as a solvent and reflux the reaction mixture overnight. The reaction mixture was workuped in ice-cold water and then the precipitates were formed. Then obtained product (product-6) was filtered out with the help of a simple funnel and filter paper. Checked product by TLC.

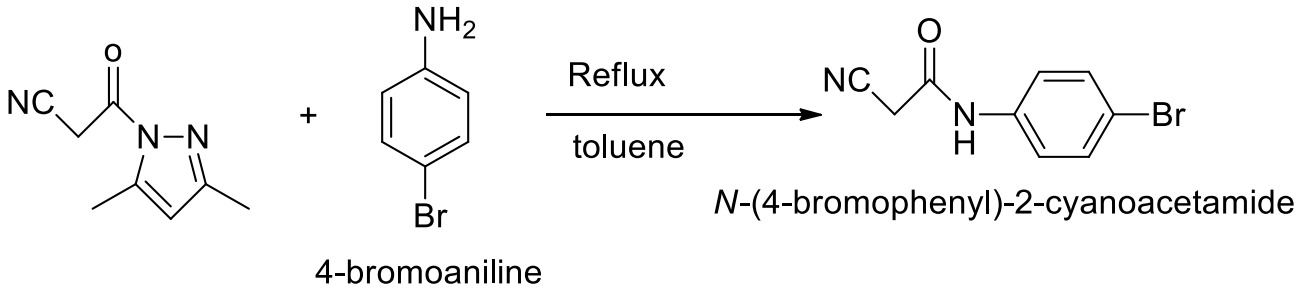


Figure.9 Reaction for derivative-2

Table.6 Calculation for Intermediate

	GRAM	MOLECULAR WEIGHT	EQUIVALENT WEIGHT
PRODUCT-5	1.5	163.18	1
4-BROMO ANILINE	0.973 gm	172.02	1

Check the solubility of 2-cyanoacetohydrazide in MeOH and DMF.
Take 1 equivalent and 1 grams of moiety (product-2) in MeOH and add a few drops of piperidine then add 1.2 equivalent and 1.675 grams of N-(4-bromophenyl)-2-cyanoacetamide (product-6) and allow it to stir overnight. The obtained product was filtered with the help of a funnel and then washed with water.

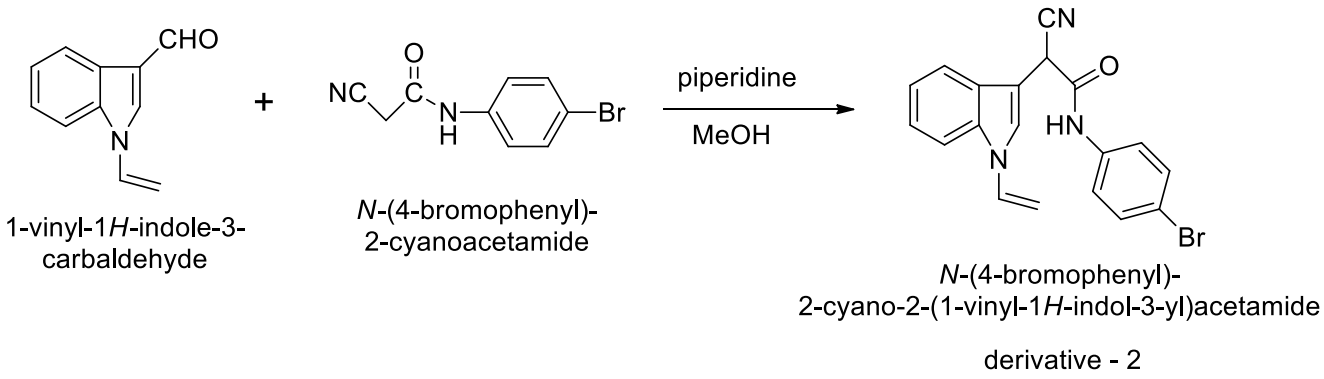


Figure.10 Preparation of derivative-2

Table.7 Calculation for derivative-2

	GRAM	MOLECULAR WEIGHT	EQUIVALENT WEIGHT
BASIC MOIETY	1 gm	171.20	1
PRODUCT-6	1.675 gm	239.07	1.2

3.1.6 PREPARATION OF DERIVATIVE-3

Take 1 equivalent and 1.5 gm of product-5 and 1 equivalent and 1.13 gm of anisidine in RBF and add in toluene as a solvent and reflux the reaction mixture overnight. The reaction mixture was workuped in ice-cold water and then the precipitates were formed. Then obtained product (product-7) was filtered out with the help of a simple funnel and filter paper. Checked product by TLC.

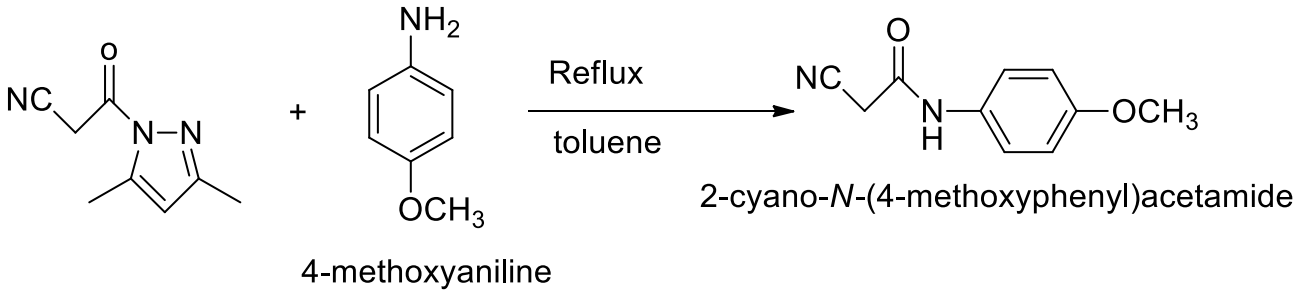


Figure.11 Reaction for derivative-3

Table.8 Calculation for intermediate

	GRAM	MOLECULAR WEIGHT	EQUIVALENT WEIGHT
PRODUCT-5	1.5 gm	163.18	1
ANISIDINE	1.13 gm	107.15	1

Check the solubility of 2-cyanoacetohydrazide in MeOH and DMF.
Take 1 equivalent and 0.13 grams of moiety (product-2) in MeOH and add a few drops of piperidine then add 1.2 equivalent and 0.15 grams of 2-cyano-N-(4-methoxyphenyl)acetamide (product-7) and allow it to stir overnight. The obtained product was filtered with the help of a funnel and then washed with water.

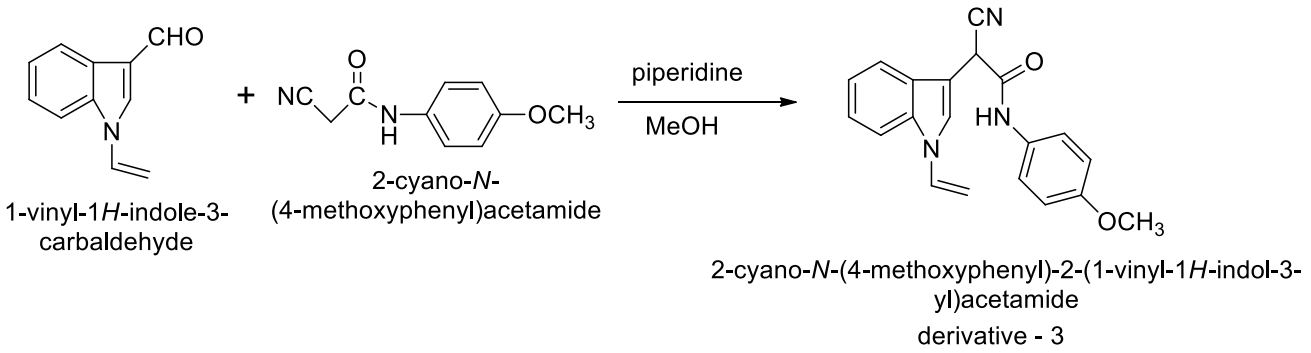


Figure.12 Preparation of derivative-3

Table.9 Calculation for derivative-3

	GRAM	MOLECULAR WEIGHT	EQUIVALENT WEIGHT
BASIC MOIETY	0.13 gm	171.20	1
PRODUCT-7	0.15 gm	239.07	1.2

3.1.7 PREPARATION OF DERIVATIVE-4

Take 1 equivalent and 1.5 gm of product-5 and 1 equivalent and 0.984 gm of toluidine in RBF and add in toluene as a solvent and reflux the reaction mixture overnight. The reaction mixture was workuped in ice-cold water and then the precipitates were formed. Then obtained product (product-8) was filtered out with the help of a simple funnel and filter paper. Checked product by TLC.

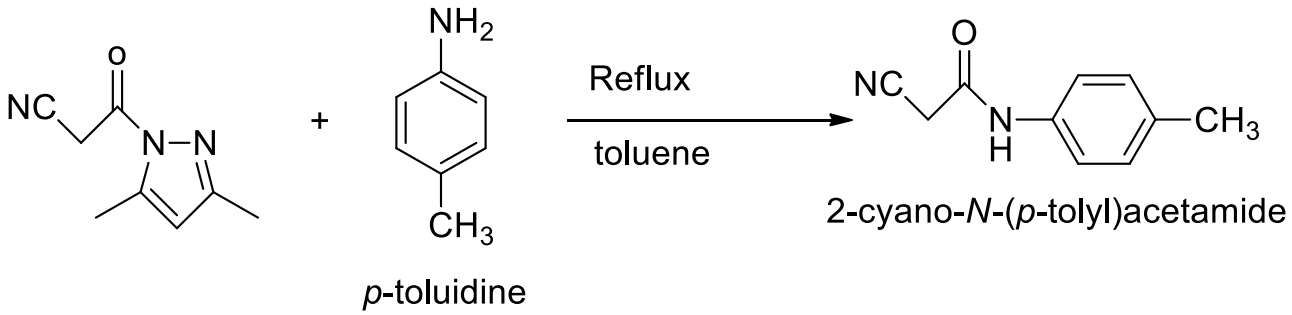


Figure.13 Reaction for derivative-4

Table.10 Calculation for intermediate

	GRAM	MOLECULAR WEIGHT	EQUIVALENT WEIGHT
PRODUCT-5	1.5 gm	163.18	1
TOLUIDINE	0.984 gm	107.15	1

Check the solubility of 2-cyanoacetohydrazide in MeOH and DMF.
Take 1 equivalent and 0.6 grams of moiety (product-2) in MeOH and add a few drops of piperidine then add 1.2 equivalent and 0.7 grams of 2-cyano-N-(p-tolyl)acetamide (product-8) and allow it to stir overnight. The obtained product was filtered with the help of a funnel and then washed with water.

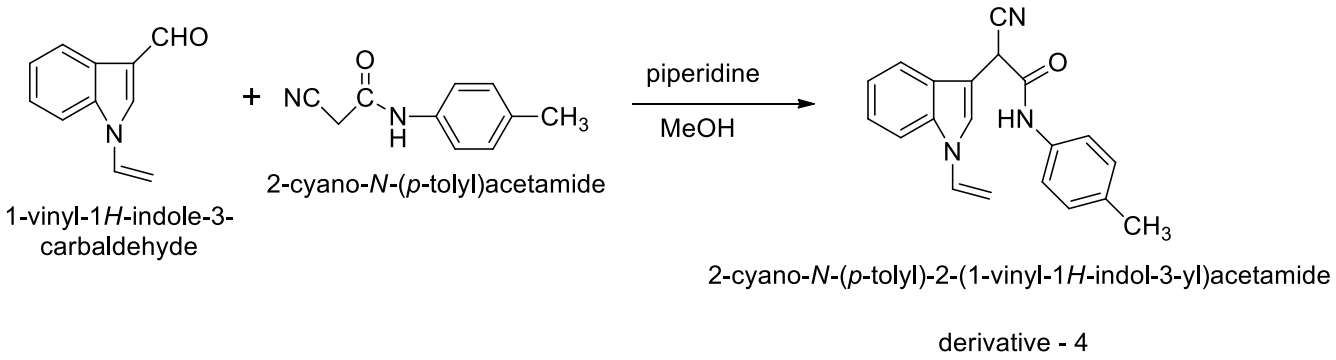


Figure.14 Preparation of derivative-4

Table.11 Calculation for derivative-4

	GRAM	MOLECULAR WEIGHT	EQUIVALENT WEIGHT
BASIC MOIETY	0.6 gm	171.20	1
PRODUCT-8	0.7 gm	239.07	1.2

3.1.8 PREPARATION OF DERIVATIVE-5

Take 1 equivalent and 1.5 gm of product-5 and 1 equivalent and 0.83 ml of toluidine in RBF and add in toluene as a solvent and reflux the reaction mixture overnight. The reaction mixture was workuped in ice-cold water and then the precipitates were formed. Then obtained product (product-9) was filtered out with the help of a simple funnel and filter paper. Checked product by TLC.

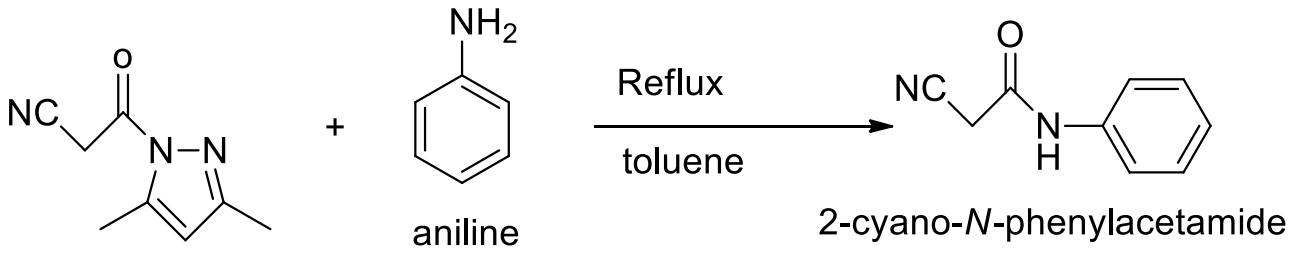


Figure.15 Reaction for derivative-5

Table.12 Calculation for intermediate

	GRAM	MOLECULAR WEIGHT	EQUIVALENT WEIGHT
PRODUCT-5	1.5 gm	163.18	1
ANILINE	0.83 ml	107.15	1

Check the solubility of 2-cyanoacetohydrazide in MeOH and DMF.
Take 1 equivalent and 0.07 grams of moiety (product-2) in MeOH and add a few drops of piperidine then add 1.2 equivalent and 0.07 grams of 2-cyano-N-phenylacetamide (product-9) and allow it to stir overnight. The obtained product was filtered with the help of a funnel and then washed with water.

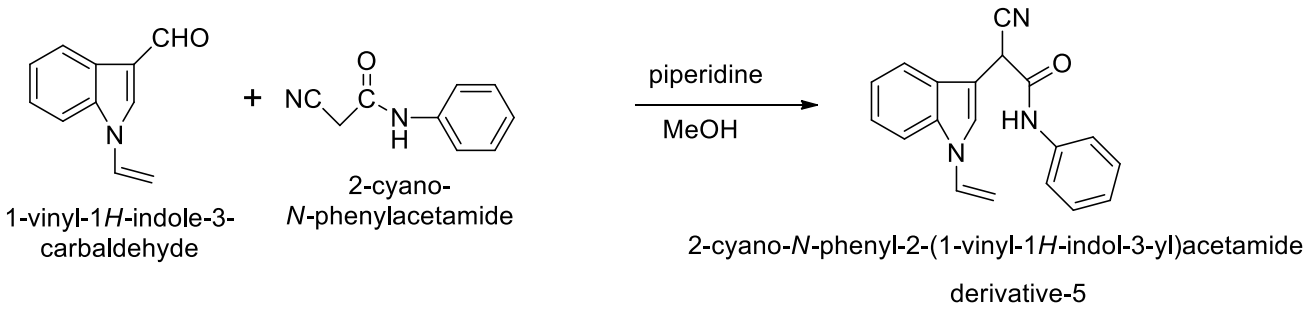


Figure.16 Preparation of derivative-5

Table.13 Calculation for derivative-5

	GRAM	MOLECULAR WEIGHT	EQUIVALENT WEIGHT
BASIC MOIETY	0.07 gm	171.20	1
PRODUCT-9	0.07 gm	239.07	1.2

4.0 PHYSICAL CHARACTERIZATION OF SYNTHESIZE COMPOUND

1. **2-cyano-2-(1-vinyl-1H-indole-3-yl)acetohydrazide**

M.W.	**M.F.**	**Color**	**% Yield**
240.26	$C_{13}H_{12}N_4O$	Dark Brown	43%

2. **N-(4-bromophenyl)-2-cyano-2-(1-vinyl-1H-indole-3-yl)acetamide**

M.W.	**M.F.**	**Color**	**% Yield**
380.24	$C_{19}H_{14}BrN_3O$	Dark Yellow	38%

3. **2-cyano-N-(4-methoxyphenyl)-2-(1-vinyl-1H-indole-3-yl)acetamide**

M.W.	**M.F.**	**Color**	**% Yield**
331.37	$C_{20}H_{17}N_3O_2$	Yellow	49%

4. **2-cyano-N-(p-tolyl)-2-(1-vinyl-1H-indole-3-yl)acetamide**

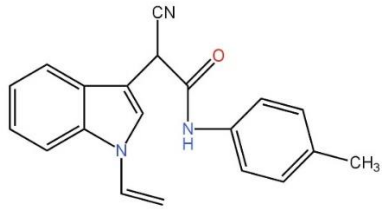
M.W.	**M.F.**	**Color**	**% Yield**
315.37	$C_{20}H_{17}N_3O$	Light Yellow	59%

5. **2-cyano-N-phenyl-2-(1-vinyl-1H-indole-3-yl-acetamide)**

M.W.	**M.F.**	**Color**	**% Yield**
301.34	$C_{19}H_{15}N_3O$	Yellow	55%

5.0 SPECTRAL CHARACTERIZATION

5.1.0 1H-NMR analysis of 2-cyano-N-(p-tolyl)-2-(1-vinyl-1H-indole-3-yl)acetamide



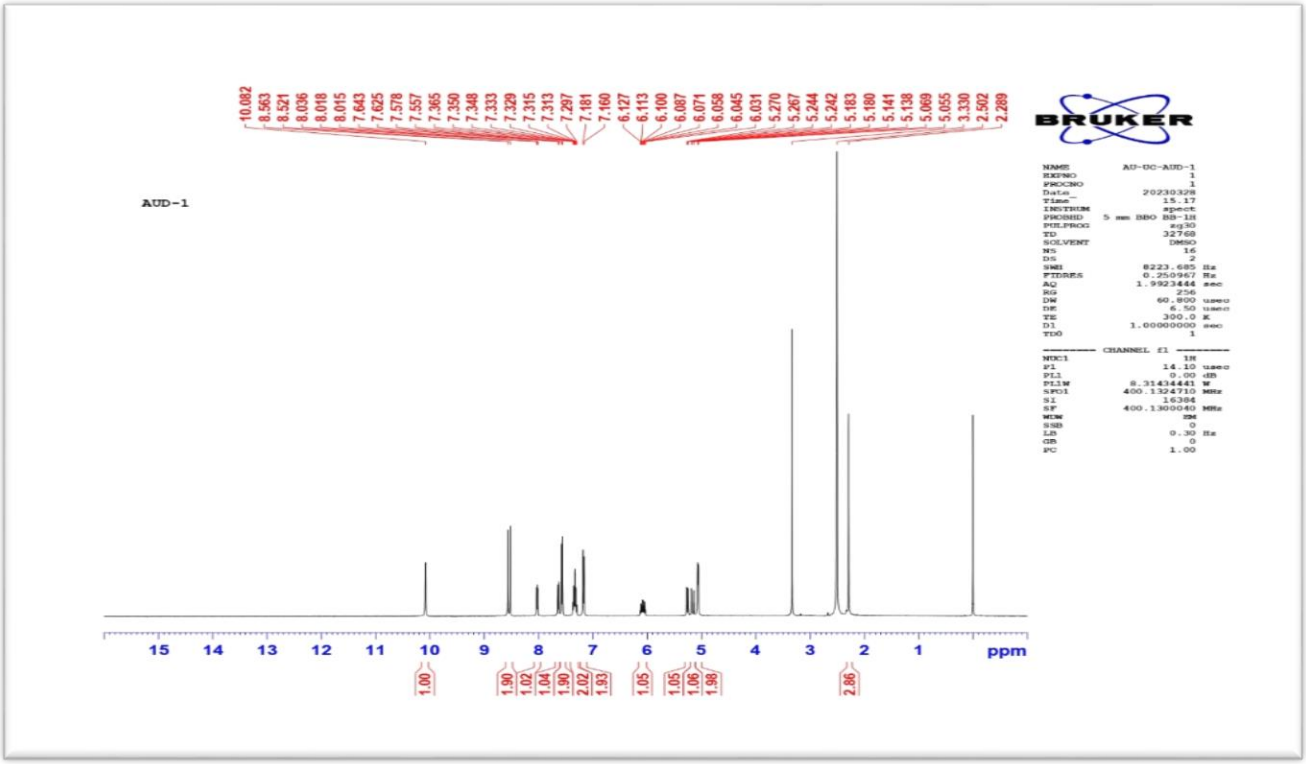
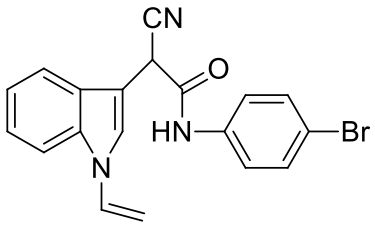


Figure.17 1H-NMR of synthesized Compound

The 1H-NMR is a promising spectroscopic approach conventionally employed for structural assurance of organic molecules in synthetic organic chemistry. The structure of this compound was accustomed by an essential peaks at 10.08 ppm (NH), 7.31 ppm (CH_3), 7.32-7.34 ppm (allyl chloride) and 2.502 ppm (solvent)

5.1.1 N-(4-bromophenyl)-2-cyano-2-(1-vinyl-1H-indole-3-yl)acetamide



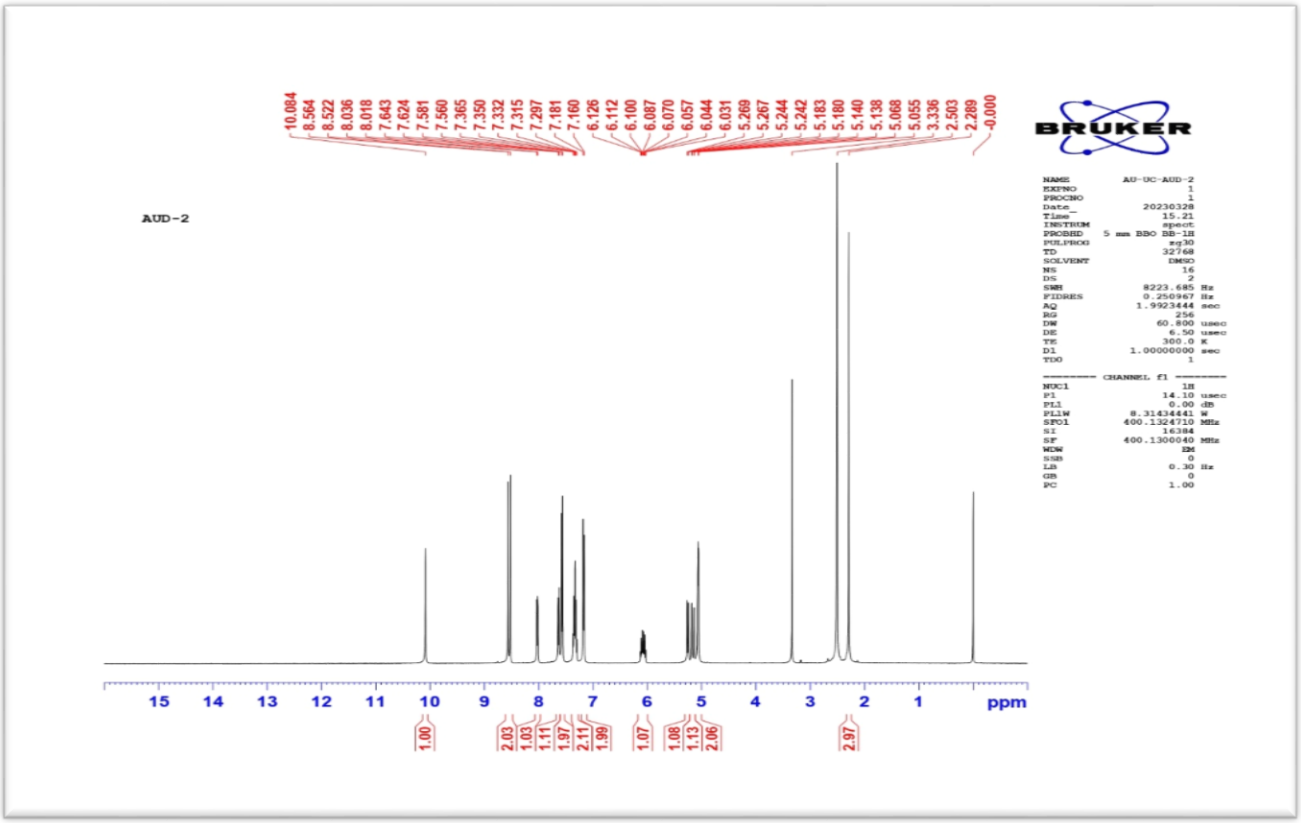
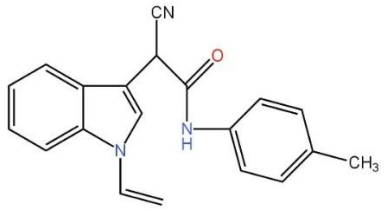


Figure.18 1H-NMR of synthesized Compound

The 1H-NMR is a promising spectroscopic approach conventionally employed for structural assurance of organic molecules in synthetic organic chemistry. The structure of this compound was accustomed by an essential peaks at 7.581 ppm (NH), 5.140-5.180 ppm (CH_3), 6.044-6.057 ppm (allyl chloride) and 2.289 ppm (solvent).

5.1.2 FT-IR analysis of N-(4-bromophenyl)-2-cyano-2-(1-vinyl-1H-indole-3-yl)acetamide



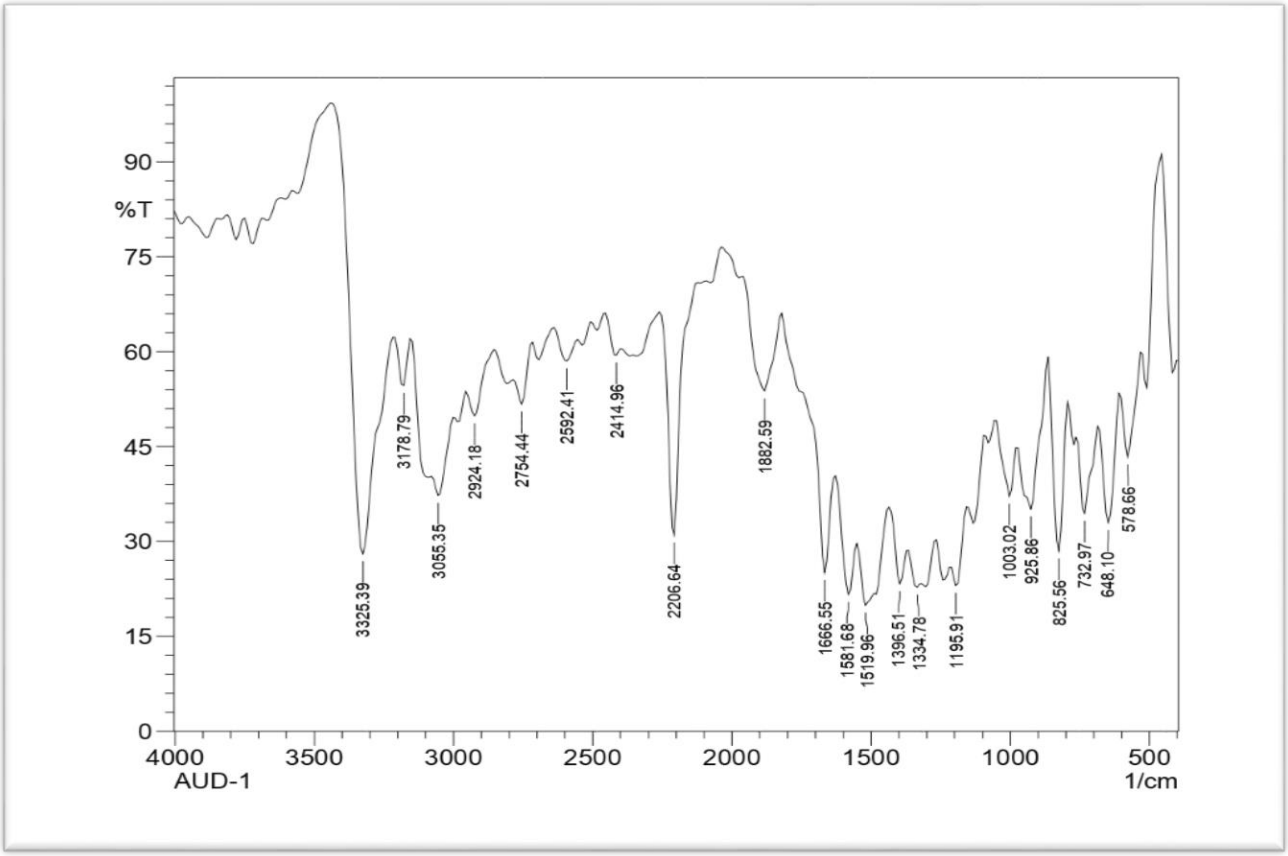
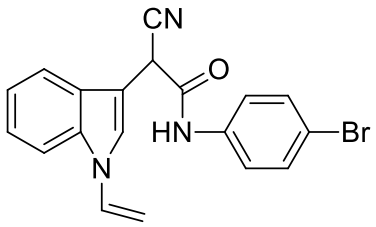


Figure.18 FT-IR of synthesized Compound

FT-IR spectrum of this compound confirm with the presence of 3325.39 cm^{-1} (NH stretching), 2924.18 cm^{-1} (C-H stretching), 2206.64 cm-1 (C=C stretching), 1882.59 cm-1 (indicates the presence of molecule of water), 732.97 cm-1 (disubstitute group), respectively. FTIR spectrum of this compound is given figure.18.

5.1.3 FT-IR analysis of N-(4-bromophenyl)-2-cyano-2-(1-vinyl-1H-indole-3-yl)acetamide



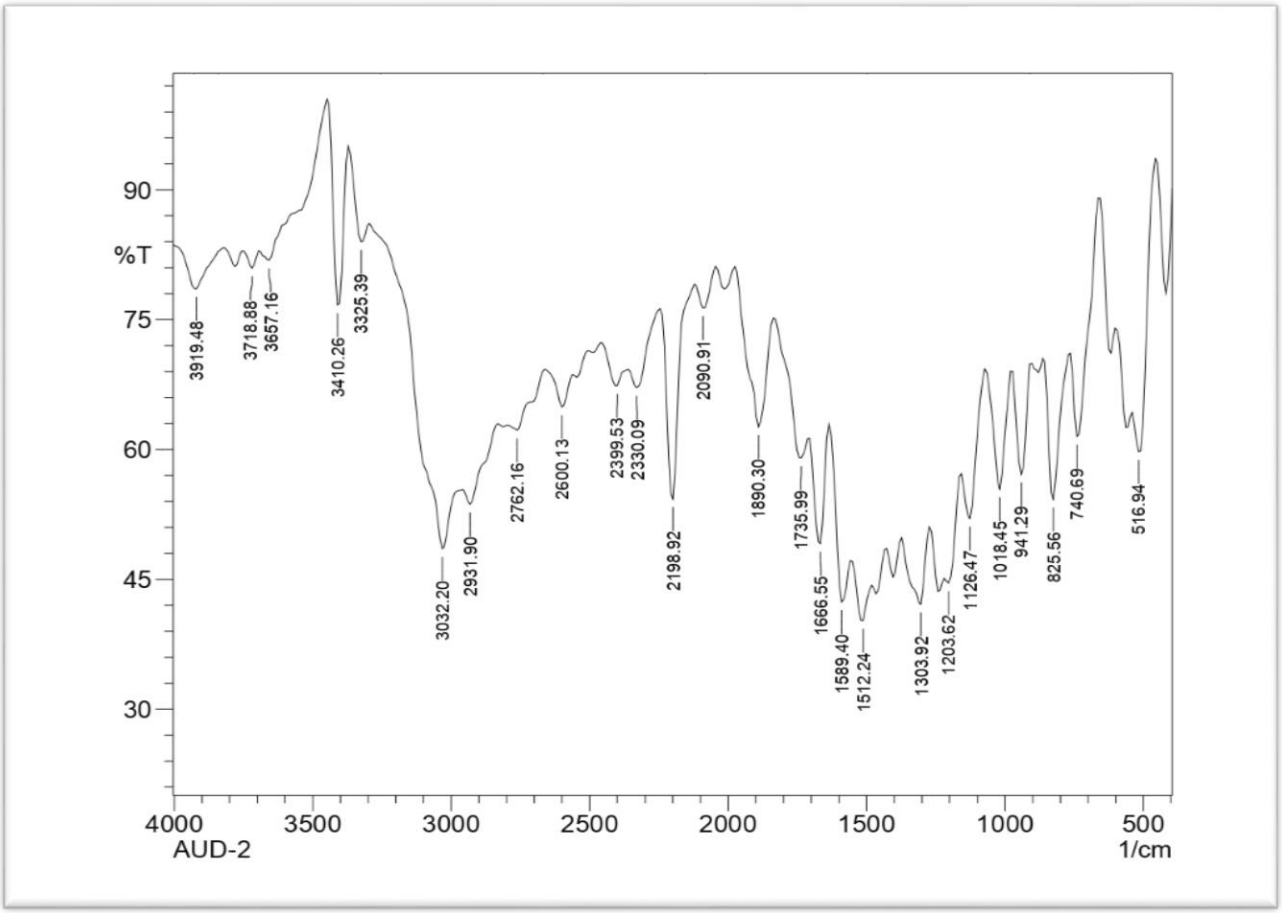


Figure.19 FT-IR of synthesized Compound

FT-IR spectrum of this compound confirm with the presence of 3410.26 cm^{-1} (NH stretching), 3032.20 cm^{-1} (C-H stretching), 1890.30 cm-1 (indicates the presence of molecule of water), 516.94 cm-1 (C-Br stretching), 740.69 cm-1 (disubstitute group) respectively. FTIR spectrum of this compound is given figure.19.

6.0 CONCLUSION

In conclusion, the synthesis of derivatives of indole has been a topic of great interest in the field of organic chemistry. Various synthetic strategies have been developed to access different indole derivatives with diverse chemical and biological properties. From this work, we can conclude that the development of efficient and sustainable synthetic methodologies for the preparation of indole derivatives has opened up a new avenue for the discovery of novel drugs and materials. Overall, the synthesis of indole derivatives has proven to be an exciting and rapidly evolving area of research with promising future prospects.

7.0 REFERENCE

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