SAR Journal of Medical Biochemistry

Abbreviated Key Title: *SAR J Med Biochem*
Home page: https://sarpublication.com/journal/sarjmb/home
DOI: https://doi.org/10.36346/sarjmb.2025.v06i06.001

ISSN 2707-7721 (P)
ISSN 2709-6882 (O)

Original Research Article

Design & Development of Benzimidazole Derivatives for Anti-Inflammatory Activity

Rutuja S. Pawar[1]*, Vilas J. Pise[1], Dr. V.Y. Lokhande[1]
[1]Department of Pharmaceutical Chemistry, Arvind Gavali College of Pharmacy, Jaitapur, Satara 415001, Maharashtra, India

***Corresponding Author:** Rutuja S. Pawar
Department of Pharmaceutical Chemistry, Arvind Gavali College of Pharmacy, Jaitapur, Satara 415001, Maharashtra, India

Article History: | Received: 18.10.2025 | Accepted: 10.12.2025 | Published: 12.12.2025 |

Abstract: The research work presented in my thesis consists of Design & Development of Benzimidazole Derivatives for Anti-Inflammatory Activity**.** Benzimidazole derivatives have been shown to have wide variety of pharmacological activities like Anti helminthic, Anti-inflammatory Diabetes, Cancer. The effective synthesis and detailed characterization of series of thiazolidinedione derivatives using infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry (HRMS). The synthesis was carried out by a multi-step synthetic process that began with starting material Benzimidazole. A combination of spectroscopic methods was used to characterize the synthesized Benzimidazole derivatives. IR spectroscopy gave crucial information on the functional groups present in the compounds, allowing the Benzimidazole-one core structure to be confirmed as well as the presence of additional substituents. The structural connectivity and stereochemistry of derivatives were determined using NMR spectroscopy (1H). Molecular docking studies were carried out to assess the binding affinities of the synthesized Benzimidazole derivatives to inflammation such as Cyclin dependent kinase 1/2/4/6, PPAR- Y and 15-PGDH, tubulin. When compared to the co-crystallized ligand, the selected four compounds showed highest binding energy. This indicates that they have the potential to be effective inhibitors of these biomarkers. For the Biological activity (anti-inflammatory) performed at results showed that Compound 2- (6-bromo-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid showed better anti-inflammatory activity (protein denaturation method). Compound A (6-chloro-1-ethyl- 1H-benzo[d]imidazol-2-yl) acetic acid, B 2-(6-bromo-1H-benzo[d]imidazol-2-yl) acetic acid & C (6-chloro-1-methyl-1H-benzo[d]imidazol-2-yl) acetic acid showed lowest anti- inflammatory activity when compared to positive control.

Keywords: Benzimidazole, Anti-inflammatory Activity, Molecular Docking.

1. INTRODUCTION

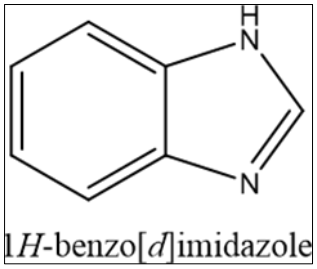
Benzimidazole is an important heterocyclic aromatic organic compound having important pharmacophore and a privileged structure in medicinal chemistry. It is a Bicyclic in nature which consists of an imidazole ring containing two nitrogen atoms at adjacent position fused to benzene ring. Nitrogen atom and the position of N is in 1st and 3rd position of the molecule. Being a major constituent of various natural products, including purine, histamine, histidine and nucleic acid, benzimidazole derivatives have occupied a unique place in the field of medicinal chemistry, thus incorporation of the benzimidazole nucleus to prepare or synthesis novel benzimidazole derivatives has always carried the attention of many medicinal chemist and hence proved to be vital synthetic strategy in drug discovery. Numerous derivatives of benzimidazole have been created and produced. Benzimidazole derivatives fall into several types, such as those that are anti-inflammatory, anti-hypertensive, antimicrobial, antioxidant, anticoagulant, anthelmintic, anti-diabetic, opioid and analgesic, antispasmodic, antidepressant, etc. A flexible heterocyclic compound with a wide range of pharmacological effects is benzimidazole. Research on the synthesis of various benzimidazole derivatives and an assessment of their biological activity is therefore necessary. It is recognized that benzimidazole's are among the best types of heterocyclic organic compounds. They can accommodate an imidazole ring combined with a phenyl ring. A useful formative monogram for the

improvement of biotic or medicinal worry bits is the cyclic benzimidazole framework. Benzimidazoles are widely used as nano sensors in nanomaterials. Because of their distinct anatomy, medications that comprehend benzimidazoles combine to form a variety of therapeutic agents [1, 2].

Heterocyclic analogues are the prime and most distinct group of organic compounds where carbon atoms of the fragment have been substituted by an atom other than carbon. Characteristic hetero atoms comprise of Sulphur, nitrogen and oxygen. On the basis of electronic array, classification of heterocyclic derivatives can be done into two types. Aliphatic heterocyclic compounds and aromatic compounds. Among them, aromatic heterocyclic compounds represent structure motifs like benzimidazole, thiadiazol, pyrrole, pyridine initiate in an immense number of therapeutically active compounds, agrochemicals and medicines [1].

Benzimidazole

Benzimidazoles have been acknowledged to be prime grade of heterocyclic organic, compounds. They accommodate a phenyl ring amalgamated to an imidazole ring. The cyclic benzimidazole framework is applicable formative monogram for the advancement of fragments of medicinal or biotic concern Benzimidazoles possess diverse utilization in nano materials as nano sensors. Owing to their unique anatomic factors benzimidazole comprehending drugs unite to array of remedial agents, thus revealed a comprehensive sphere of bioassays. Benzimidazole substituted at 2ndposition has imperative molecular feature in drug discovery with the bulk of powerful disease, hence this scaffold is renowned as a vibrant molecule in the pharmacodynamic skill along with several channels seemed to plan its derivatives for upcoming search [3].



➢ **CHEMISTRY**

It is a white solid that appears in the form of tabular crystals with chemical formula C7H6N2. The melting point of benzimidazole ranges from 170-172°C. They are freely soluble in water and sparingly soluble in ether, insoluble in benzene and petroleum ether. This molecule also follows 1,3-tautomeric equilibrium depicted in Figure1 [2].

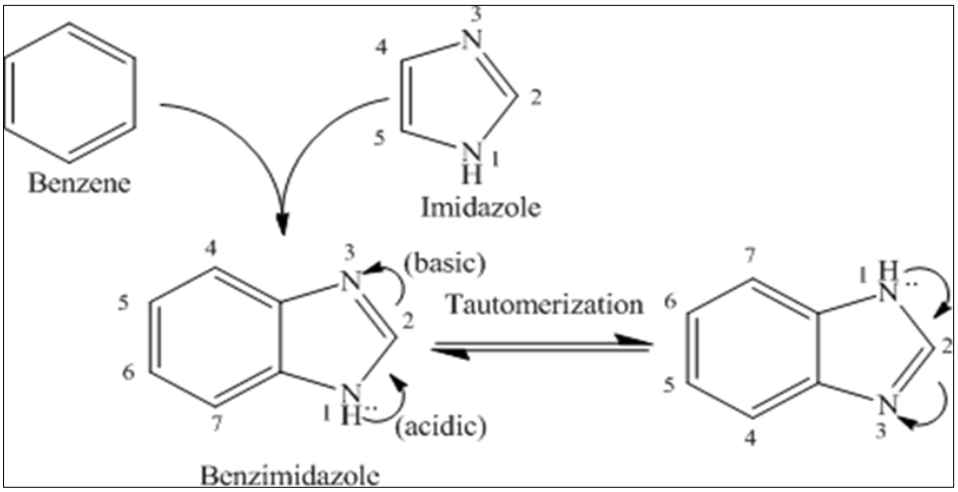


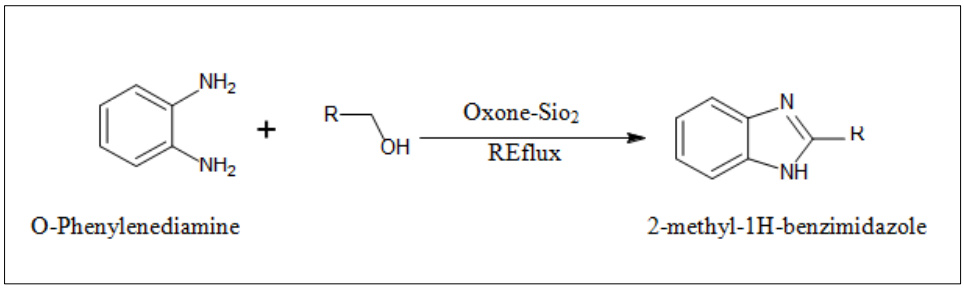
Figure 1: Tautomerism of benzimidazole

Table 1: Physical Properties [1]

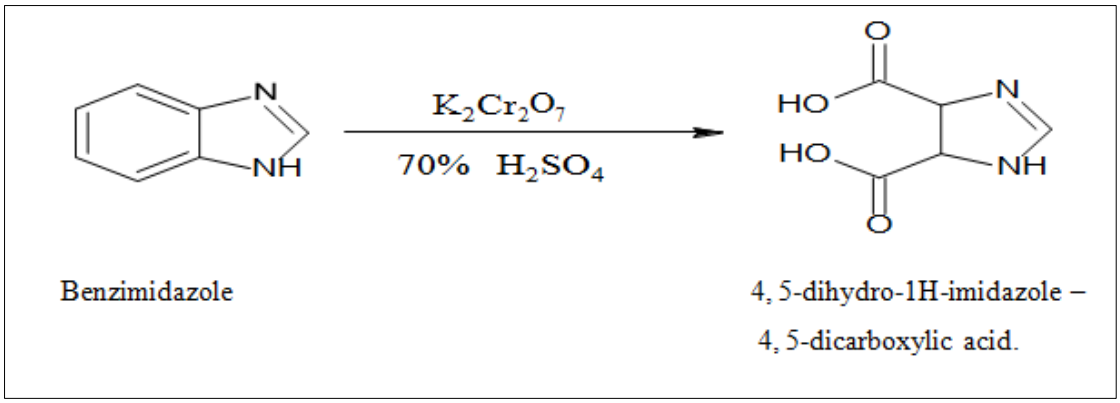
Sr. No	Content	Properties
1.	Amphoteric	Benzimidazole is amphoteric in nature
2.	Molecular formula	C7H6N2
3.	Molecular weight	118.14 g/mol
4.	Melting point	170-172^0 C
5.	Activity (PKa)	12.8(for benzimidazole) & 5.6 (for the conjugate acid)

❖ **CHEMICAL PROPERTIES:** [3, 4]

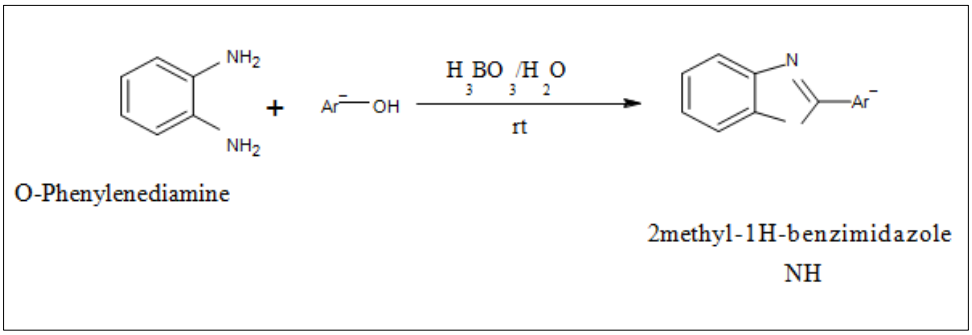
i). Addition Reaction: O-Phenylenediamine addition in the presence of ethanol and silicon oxidation to form a 2-methyl-1H-benzimidazole.



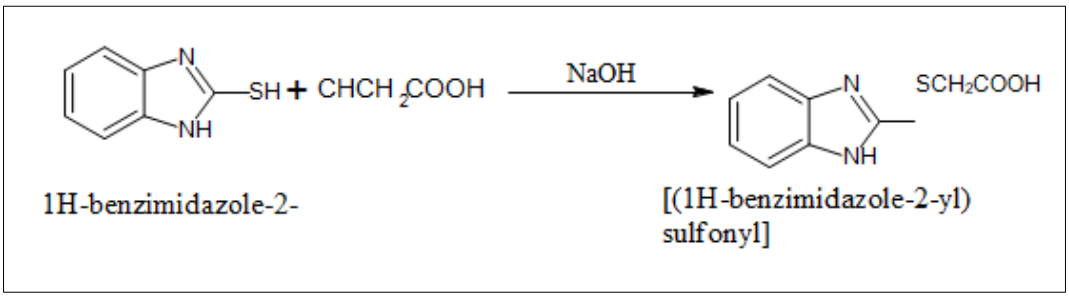
ii). Oxidation Reaction: Benzimidazole oxidation in the presence of Potassium dichromate and 70% H2SO4 to form a 4, 5-dihydro-1H-imidazole -4, 5-dicarboxylic acid.



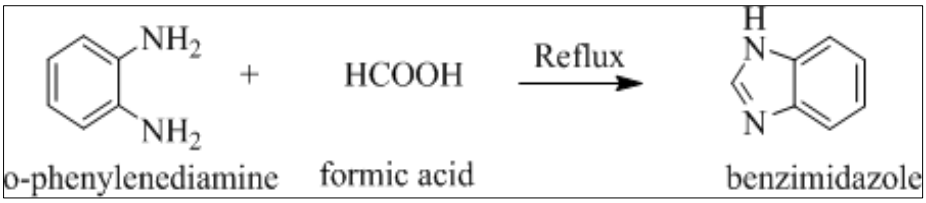
iii). Reduction Reaction: O-Phenylenediamine reduction in the presence of methanol and boric acid to form a 2-methyl- 1H-benzimidazole.



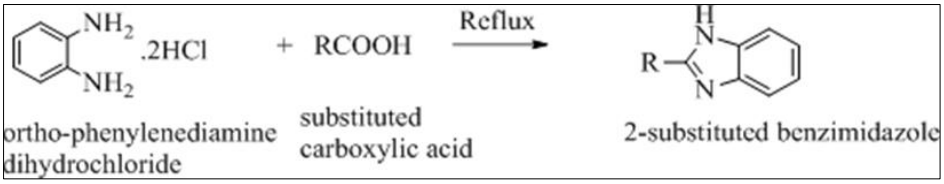
iv). Substitution Reaction: 1H-benzimidazole-2-thiol substitution in the presence of carboxylic acid and NaOH to form a [(1H-benzimidazole-2-yl) sulfonyl] acetic acid.



v). Reaction with Carboxylic Acids: Expected preparation of benzimidazole analogues involves condensation of Ortho-phenylene diamine with formic acid under suitable heating conditions (100°C) endow 2-substituted benzimidazole derivatives in appropriate yield.

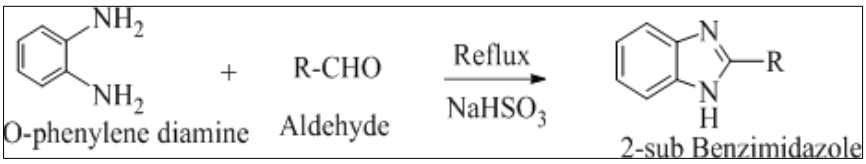


Reaction of O-phenylenediamines in the midst of affixed carboxylic acids (other than formic acid) only by adding hydrochloric or phosphoric acid yield 2-substituted benzimidazole.



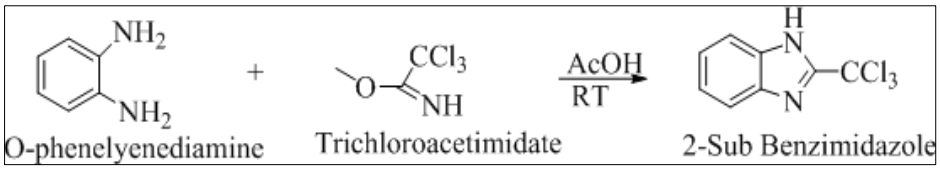
i). Reaction with Sodium Bisulphite Adduct:

Aldehydes on condensation reaction with O-phenylene diamine yield varietyof 2-substituted benzimidazoles. The configuration of benzimidazole was addressed by reacting aryl aldehydes with solution of sodium bisulphite. Precipitate ofbisulphtie was collected and filtered. These precipitates were added to O-phenylene and refluxed in THF. The residue was cooled and filtered.



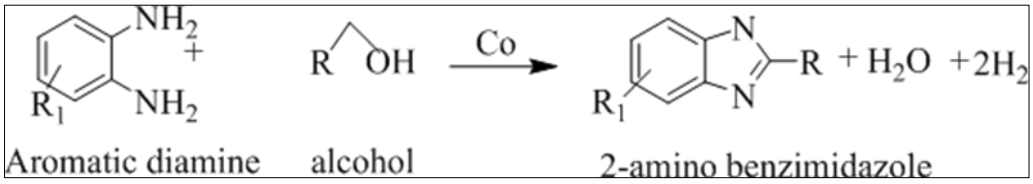
ii). Reaction with Imide Ester:

The condensation reaction of o-phenylene diamine with dichloroacetamide yields 2-trichloromethyl benzimidazole. the reaction mechanism only takes place at room temperature.



iii). Dehydrogenative Coupling:

The coupling reaction involves the reaction between primary alcohols and aromatic diamines in the presence of metal base to liberate 2-amino benzimidazole derivatives with liberation of water and hydrogen gas.



⇒ **Biological Profile of Benzimidazole Analogues: [5]**

Numerous derivatives of benzimidazole demonstrate ample assortment of pharmacological behaviour like anti-HIV anti-hypertensive, anti-convulsant, anti-proliferative, antiviral anti- microbial anti-parasitic. Chemistry and evaluation of an assortment analogues of substituted benzimidazole announce the design of antacid drugs like pantoprazole, lansoprazole, omeprazole and rabeprazole. Thiabendazole, mebendazole, albendazole are broadly anthelmintic drugs that are at present reachable in souk containing benzimidazole in their arrangement. Some of the FDA approved drugs are shown in the **Figure 2.**

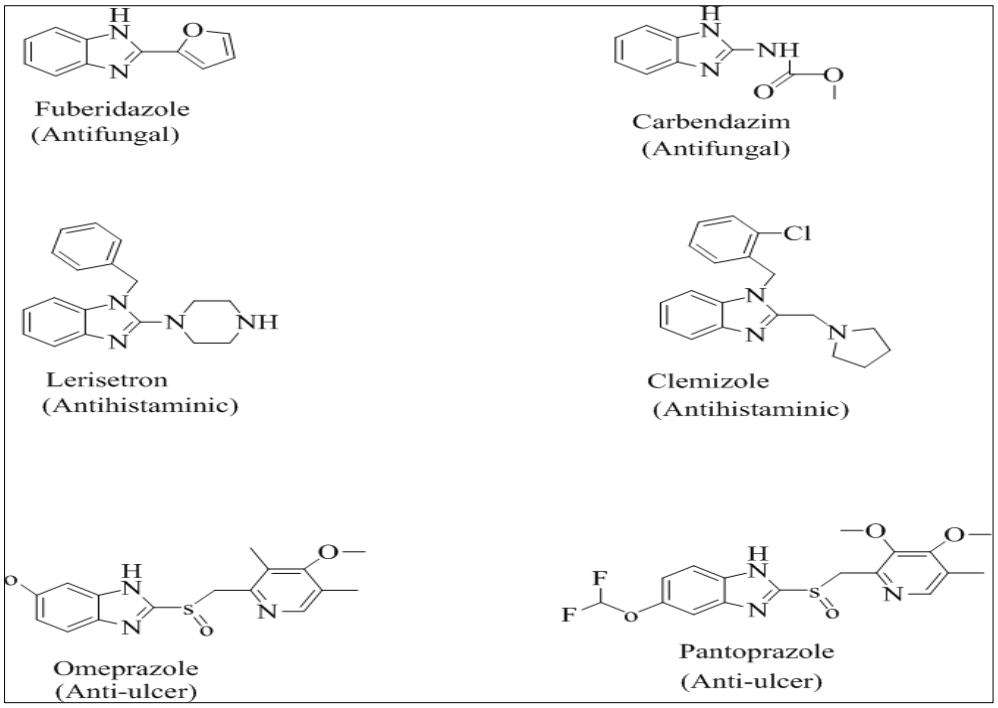
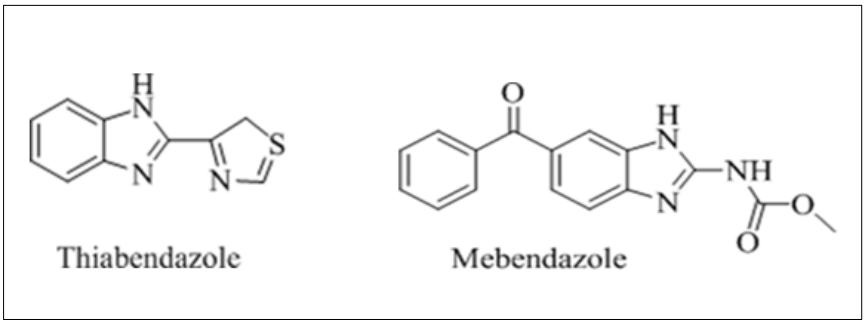
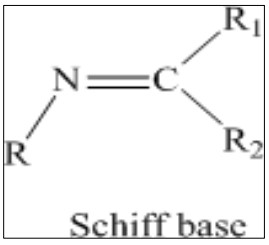


Figure 2: Some of the FDA approved drugs containing benzimidazole scaffold



⇒ **Schiff Bases:** [4]



These substrates are prime class of organic molecules which was announced by Hugo Schiff. These nitrogen analogues are synthesized by replacing the carbonyl derivative with Azomethine nucleus. Condensation reaction occurs between amino group and aldehydes or ketones. Nucleophilic addition reaction between an amine and carbonyl group stalked by designing of an imine i.e., Schiff base. They are generally regarded as sub class of imines where countless solvents are involved in condensation reaction.

⇒ ***Chemistry:***

The common organic solvents used in synthesis of Schiff bases are tetrahydrofuran, methanol and 1,2-dichloro ethane. Protonation of the nitrogen of the C=N yields protonated amine. Protonated imines present in adduct act as electron accepting agents which can easily pull the electrons away from the bonds attached to the analogue. Mechanism of Schiff base is illustrated in **Figure 3.**

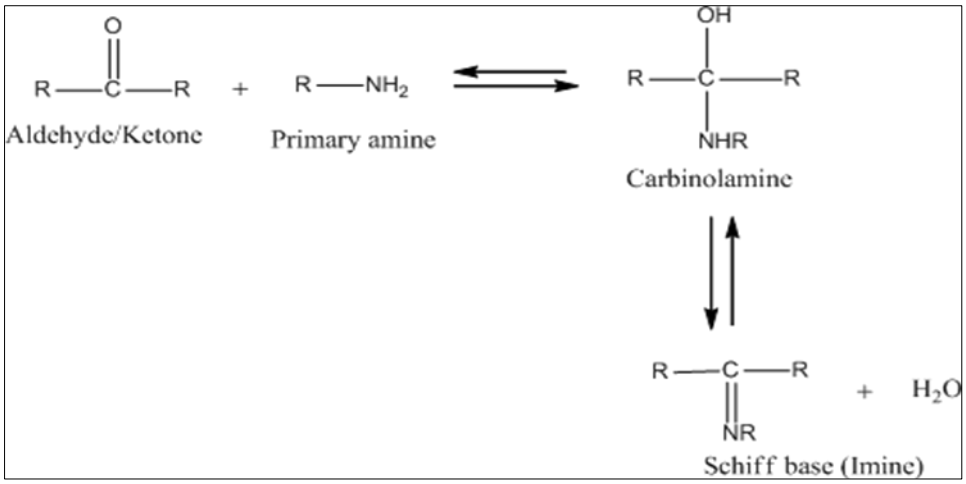
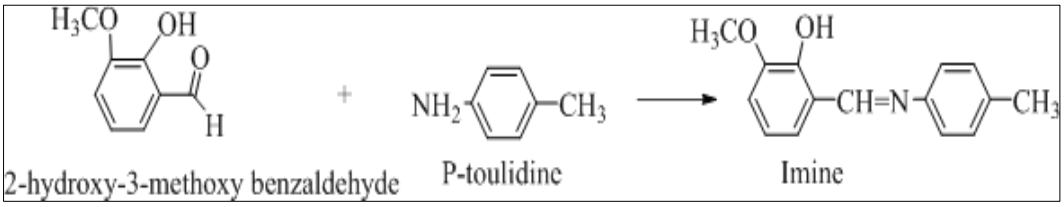


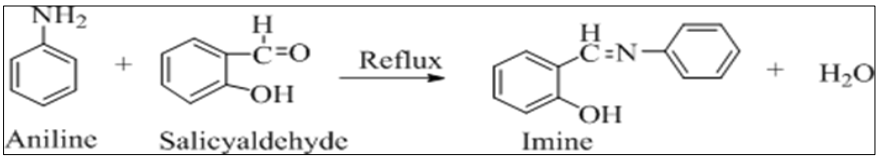
Figure: 3 Reaction mechanism of Schiff base formation

⇒ ***Methods for Synthesis of Schiff base*:** [5]

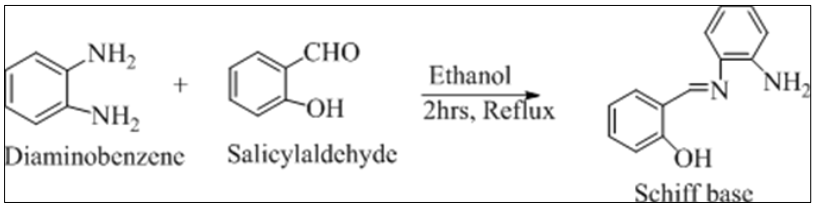
i). Reaction with Toluidine: On refluxing the substituted aldehydes and toluidine in methanol, theformation of imine takes place in high yield.



ii). Reaction with Aniline: Condensation reaction occurs between salicylaldehyde (aldehyde) and anilineto form Schiff base with the liberation of water.



iii). Reaction in Aqueous Medium: To the solution of diaminobenzene added with salicylaldehyde in the presence of ethanol and refluxing for 2-3hrs.The formation of yellow needles of imine were found in high yield.



❖ **Biological Profile of Schiff Base Derivatives:** [6]

They have wide profile of biological actions like antifungal antioxidant anti- inflammatory antibacterial etc. These hetero molecules are abundantly used in the formation of dyes, pigments and also as catalyst and complexing agents. Furthermore, t hese are a dominant division of analogues that construct various industries with countless biological and therapeutic relevance.

Explore for novel aromatic Schiff base analogues have discovered more therapeutic importance due to electron delocalization in the cyclic configuration. Schiff base bearing benzimidazole moiety were found to possess excellent anti-microbial, anticancer age, anti-inflammatory, antioxidant activity. There are numerous medicines in the market having Schiff base linkage and several are in the pipeline for various biological actions e.g.: Thioacetazone and Dantrolene. The biological profile of Schiff bases is depicted in **Figure 4 and 5.**

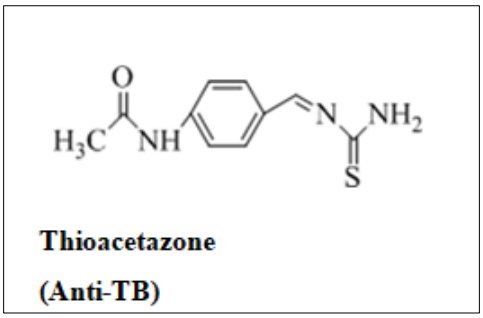


Fig. 4:

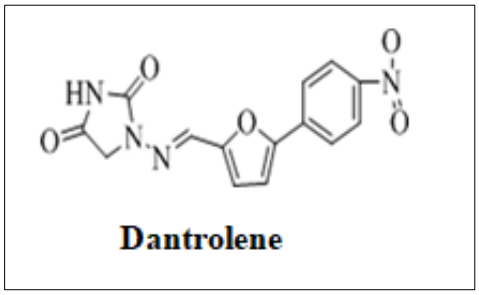


Fig. 5:

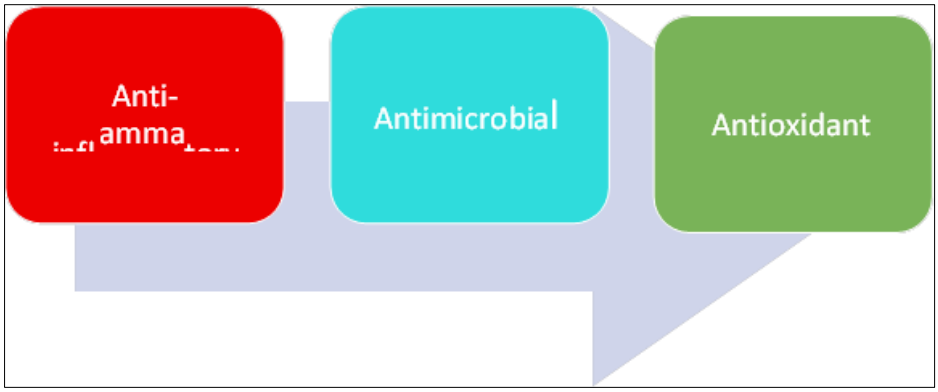
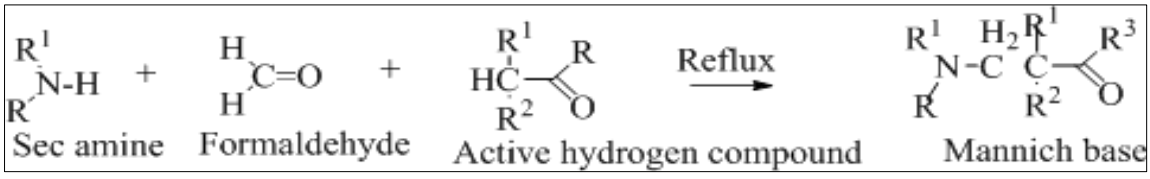


Figure 6: Biological profile of Schiff base

❖ **Manniche Bases:** [3, 4]

These are beta-amino ketones also called as final products of mannich reaction produced by nucleophilic addition reaction between tri molecules i.e. active hydrogen molecule, an aldehyde and an amine group (Primary or secondary).



❖ **Chemistry:**

Formaldehyde and amine react with carbonyl compound to form iminium ion which also follows tautomerism to confer its enol form. This enol form now attacks the iminium ion. Presence of amino alkyl side chain in mannich bases act as a vital bioactive molecule that arrange drugs with high therapeutic value. The reaction mechanism is shown in the **Figure 7** Mechanism of Mannich base [3].

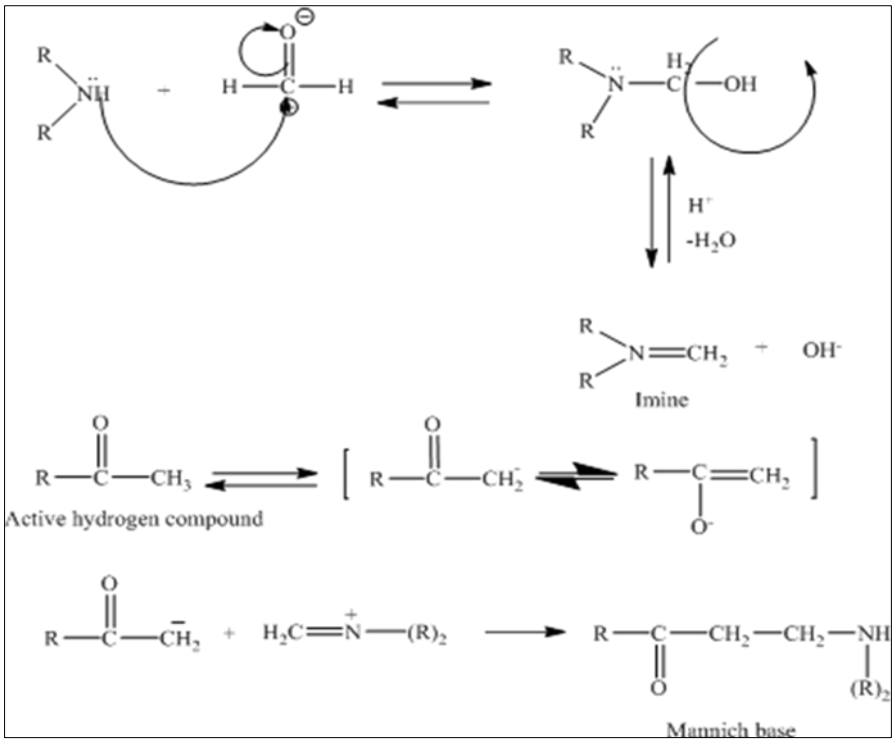


Figure: 7 Reaction mechanism of mannich base

❖ ***Methods for Synthesis of Mannich base:*** [6,7]

i). Reaction with Morpholine:

The mixture of morpholine with furan carbaldehyde and N-phenyl acetamide was refluxed for 4-5hrs in ethanol and this reaction mixture was poured on crushed ice, which further separated crystals of mannich base **Figure 8.**

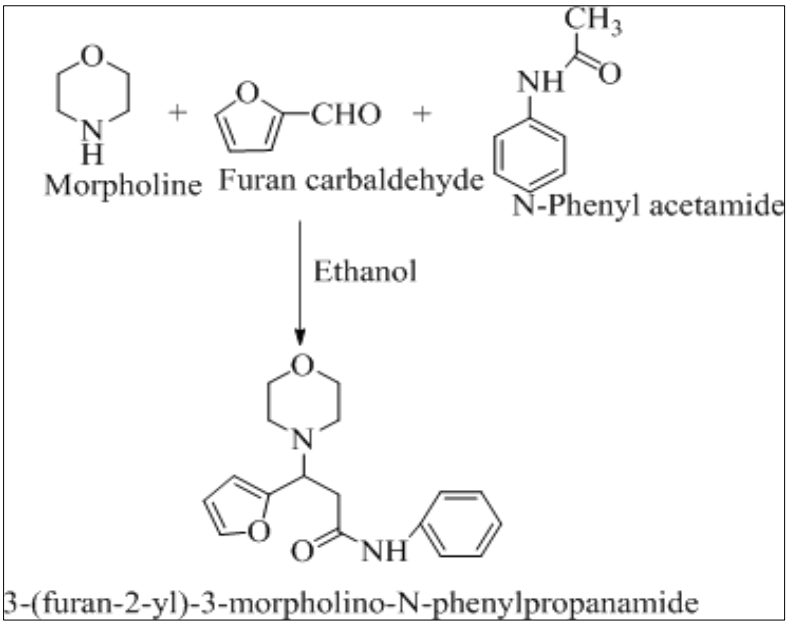


Figure 8:

ii). Reaction with 2-Phenyl Benzimidazole

N-Mannich bases can be primed by the synthetic reaction of 2-phenyl benzimidazole with secondary amine and formaldehyde. Reflux assembly was set up and reaction continued for 7- 8hrs in the existence of ethanol. Needle shaped crystals clarified the formation of N-Mannich base **Figure 9.**

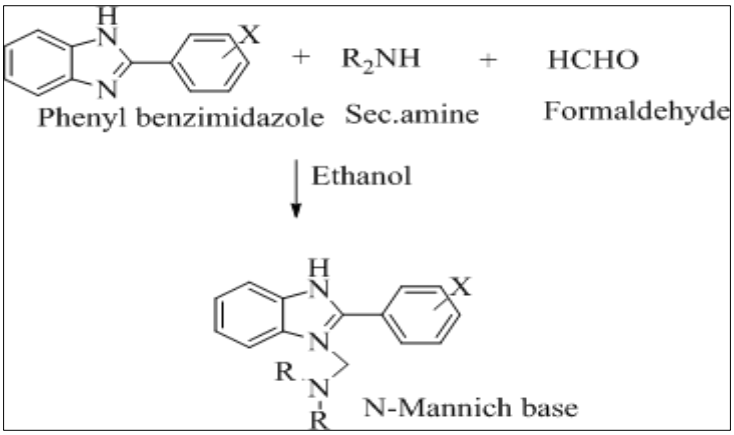


Figure 9:

❖ **Biological Properties of Mannich Base:**

The reactive character of mannich base announces them as promising anticancer, antimicrobial, anti-inflammatory, antiviral agents, antioxidant agents. Besides, developed mannich bases from 2-amino benzimidazole would probably upshot in derivatives possessing extraordinary biotic actions about several diseases. Cocaine, ranitidine, atropine drugs mark them as good mannich bases with amino alkyl side chain that make them reactive in nature. Mannich bases are accredited to participate in an energetic task in the succession of synthetic pharmaceutical chemistry drugs formed via mannich reaction are shown in **Figure 10** [7].

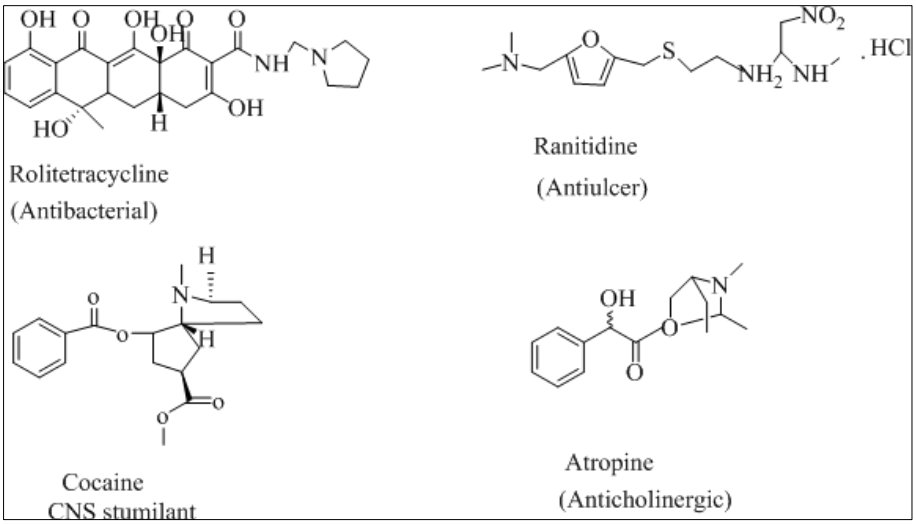


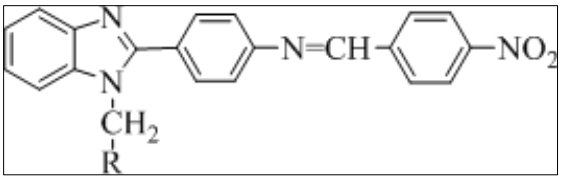
Figure 10: FDA approved drugs containing mannich base scaffold

❖ **Schiff Mannich Bases:** [8]

Mannich reaction is a blueprint of condensation reaction of carbonyl group and an amine later on with loss of water to outline Schiff base. Electrophilic addition reaction occurs in step 2 with derivatives accommodating a proton which is acidic in character. Outgrowth of Mannich bases as of two-substituted benzimidazoles can actually figure up in analogues with elevated therapeutic actions. The Schiff bases were put all the way through mannich reactions with diverse secondary amines and formaldehyde to produce Mannich Schiff base derivatives. Mannich base are of remedial effect from past frequentyears and also practiced the analyst for intend of neoteric heterocyclic hybrids for progression of innovative biodegradable expertise.

⇒ **GENERAL STRUCTURE**:

R = Dimethylamine, diethyl amine



⇒ **INFLAMATION:** [8, 9]

Inflammation is a normal essential response to any noxious stimulus thus threatens the host and may vary from a localized response to a generalized response. It is a body's defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissues. Inflammation involves two basic processes with some overlapping i.e. early inflammatory response and later followed by healing. Though both these processes generally have protective role against injurious agents, inflammation and healing may cause considerable harm to the body.

➢ **Inflammatory Factors:**

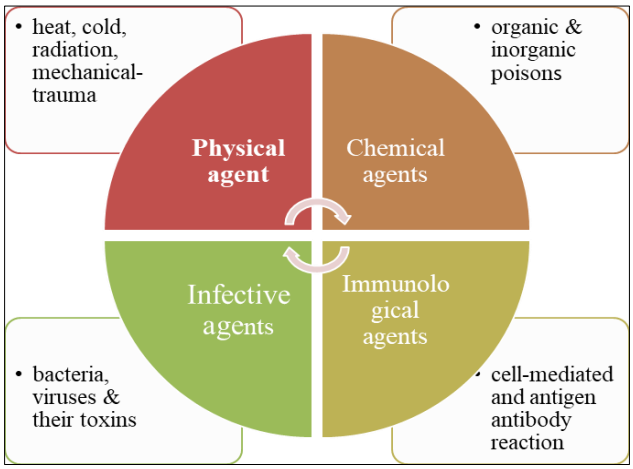


Figure 11: The triggers for the inflammation

➢ **Inflammation is recognized by following symptoms: Figure 12** [10]

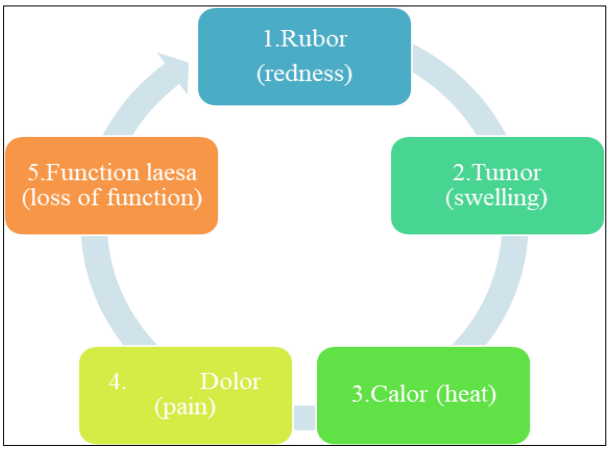


Figure 12:

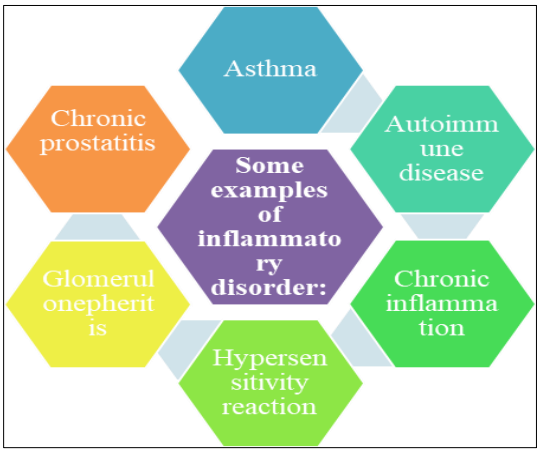


Figure: 13 Example of Inflammatory Disorder [10]

Table 2: Comparison of acute and chronic inflammation: [10]

Parameters	Acute inflammation	Chronic inflammation
Causative agent	Pathogens, injured tissue	Chronic inflammation following acute inflammation, recurrent attacks of acute inflammation, also infection with organism of low pathogenicity is chronic from the beginning.
Major cells involved	Neutrophils	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells)
Primary mediators	Vasoactive amines, eicosanoids	Cytokines, reactive oxygen species
Onset	Immediate	Delayed
Duration	Few days	Up to many months, or years
Outcomes	Healing, abscess formation, chronic information	Tissue destruction, fibrosis

➢ **MECHANISM OF INFLAMMATION: [**12]

Arachidonic acid is does not occur free in cell but is normally esterified in membrane phospholipids. Particularly in the carbon 2 position of phosphatidylcholines and phosphatidyl inositol. It is relaxed from phospholipids through the activation of cellular phospholipases by mechanical, chemical and physical stimuli. Arachidonic acid is further metabolized by cyclooxygenase and lipo-oxygenase **Figure 14.**

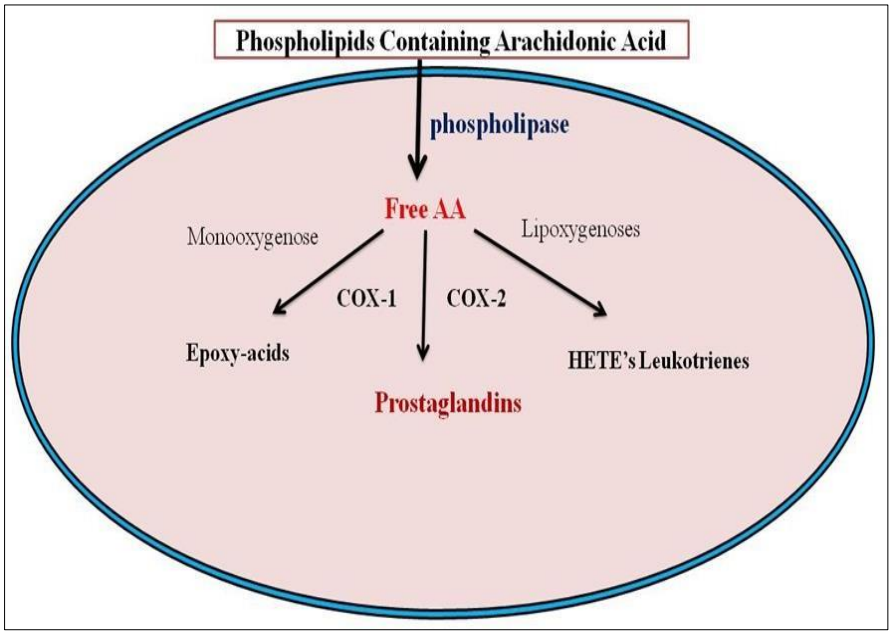


Figure 14: Mechanisms of Inflammation

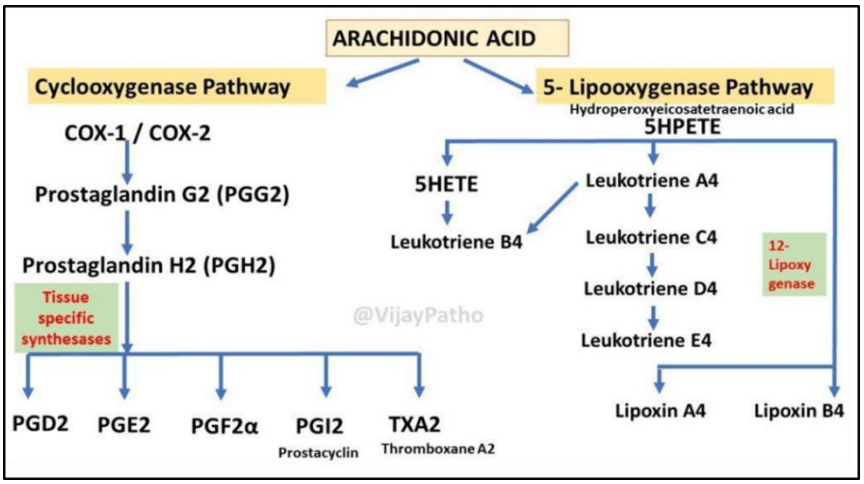


Figure 15:

- **Cyclooxygenase and Lipo-oxygenase Pathway: Figure: 15** [13]
- **Materials and Equipment:**

❖ ***Material:***

1. Drugs, Chemicals and Solvents
2. Instruments
3. Glassware
4. Docking Software

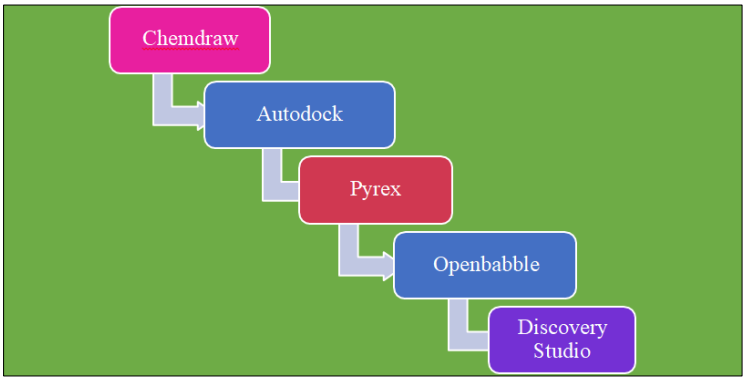


Figure 16:

Docking Software

❖ ***Identification and Characterization of Synthesized Compound:***

The synthesized compounds were scaled for yield and purified by recrystallization with suitable solvent. The purified compound was assigned for further spectral analysis and physical constant determination. Determination of compound as determined by using Thin Layer Chromatography, Nuclear Magnetic Resonance spectroscopy, infrared spectroscopy, Mass spectrophotometry.

1. Melting Point Determination:

The melting point of synthesized compound were determined by using Equiptronics digital melting point/boiling point apparatus EQ730.

2. Thin Layer Chromatography:

Thin Layer Chromatography was done by using precoated silica gel plates with suitable solvent system. The Rf value were recorded accordingly.

3. Infrared Spectroscopy:

Infrared Spectra of synthesized compound were recorded by using IR spectrometry. Instrument used: Bruker Alpha-II ATR FT-IR.

4. Nuclear Magnetic Resonance:

1HNMR spectra of synthesized compound were taken using internal standard Tetra Methyl Silane. 1HNMR spectra were recorded by using DMSO d6 as a solvent and the chemical shift data were recorded in the term of δ value related to TMS.

Instrument Used: NEO 500MHz FT-NMR spectrometer.

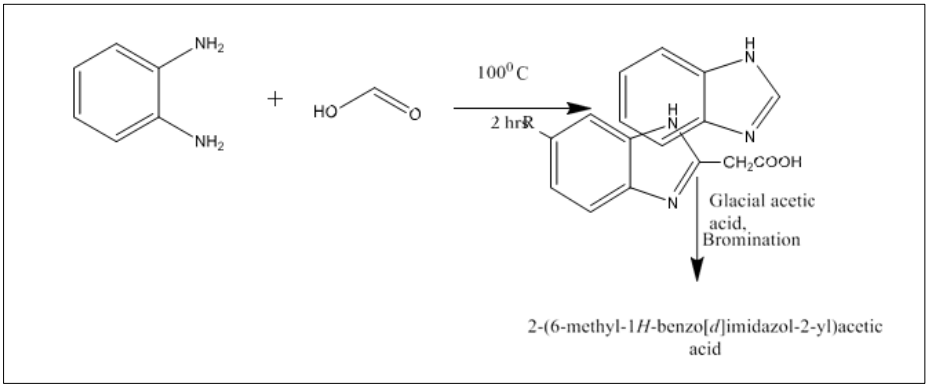
5. Mass Spectrophotometer:

Mass spectrophotometry of synthesized compound was recorded by using Gas Chromatography-Mass Spectrometer Common facility Centre (CFC) – Hyderabad. Model: MS-TQ050 HS-20.

6. In Vitro Anti-Inflammatory Study

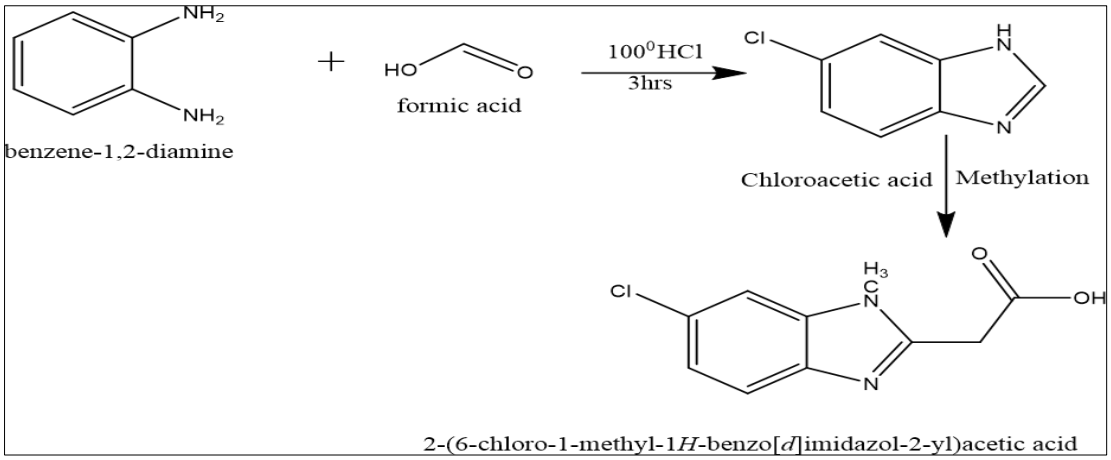
Biocyte institute of research & development, sangali.

7. Scheme:



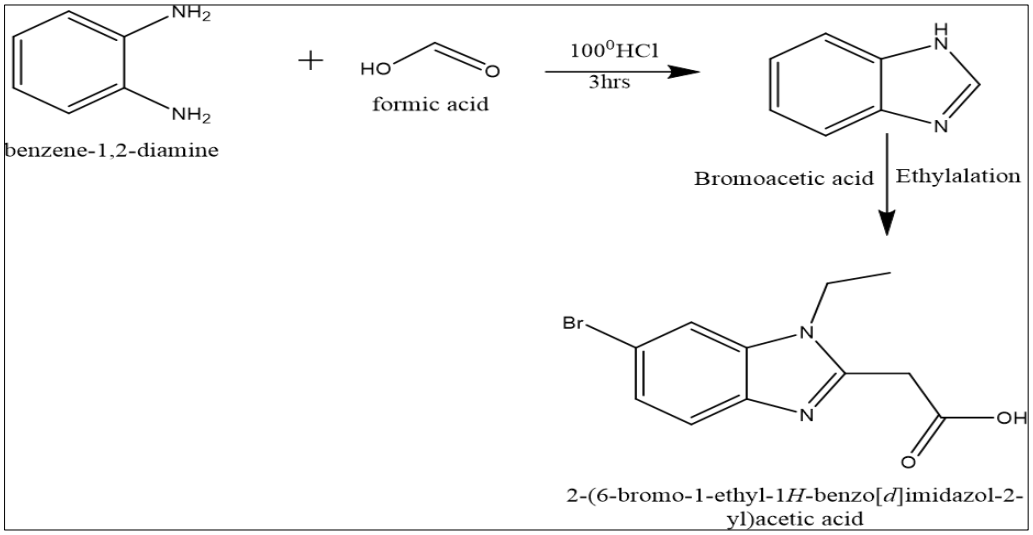
Reaction and Procedure:

- **Compound: 1**



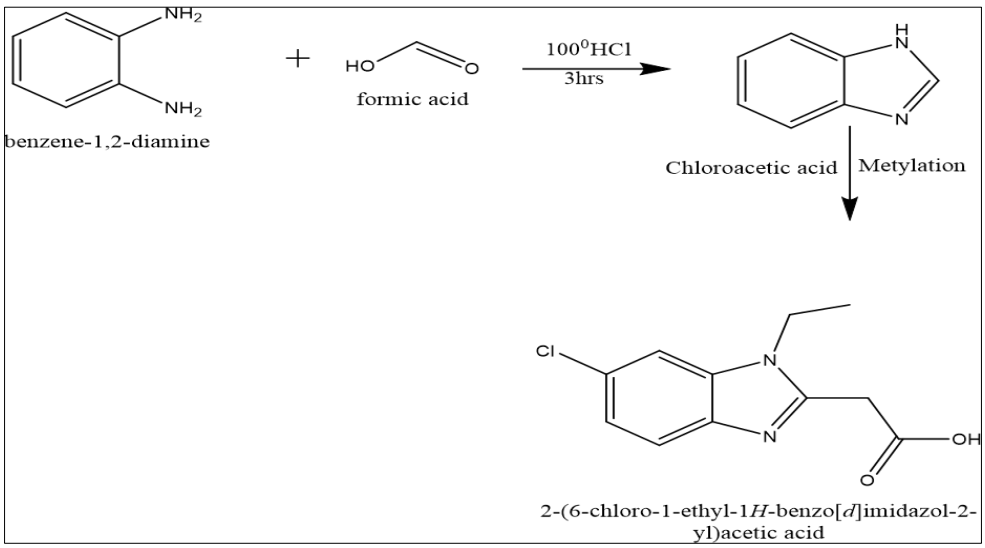
2-(6-chloro-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)acetic acid

- **Compound: 2**



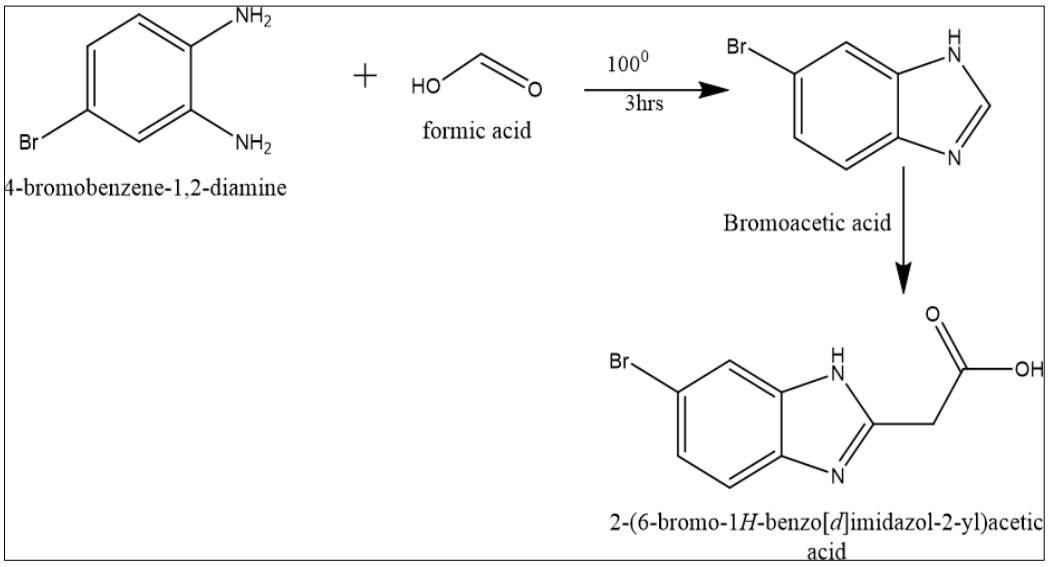
2-(6-bromo-1-ethyl-1*H*-benzo[*d*]imidazol-2-yl)acetic acid

- **Compound: 3**



2-(6-chloro-1-ethyl-1*H*-benzo[*d*]imidazol-2-yl)acetic acid

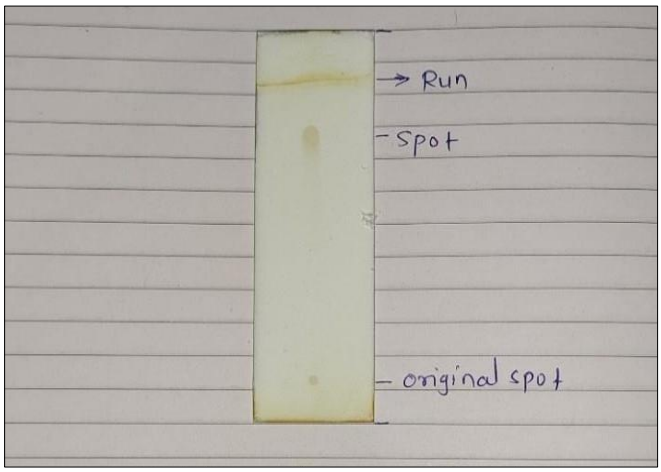
Compound: 4



➢ ***Thin Layer Chromatography:***

Chromatography is a process for breaking down mixtures of substances into their individual components. Silica gel G is an adsorbent used in TLC. A silica gel slurry was produced in distilled water. The slurry was applied to a glass plate measuring 7.5 by 2.5 cm to form a 0.2 mm-thin layer. Before activation at 110 o C for an hour, the Plates were air dried. The sample was applied via an applicator. The slide was kept for the creation of a solvent system. To increase component resolution, a range of solvent solutions were used [7].

Trial and error in the solvent system containing chloroform: methanol (8:2) led to the ultimate solvent system's discovery· [8, 9].



- Absorbent: Silica gel G (activated)
- Thickness: 0.2mm
- Plate size: 7.5 × 2.5 cm
- Activation temperature: 105°C for 1 hr.
- Solvent system: chloroform: methanol (8:2)

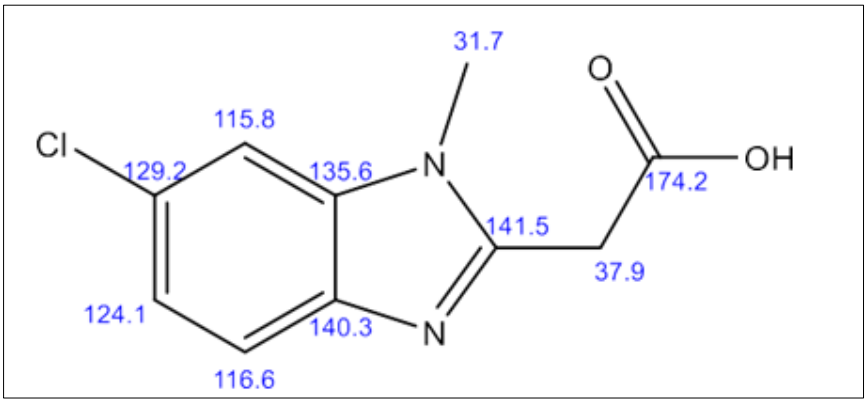
Calculation:

➢ Retention Factor (RF Value)

$$\text{RF Value} = \frac{\text{Distance travelled by solute (cm)}}{\text{Distance travelled by solvent (cm)}}$$

⇒ RESULT & DISSCUTION

➢ ***Compound-1***



- **IR Spectra: 2-(6-chloro-1-methyl-1H-Benzo[d]Imidazol-2-yl) Acetic Acid**

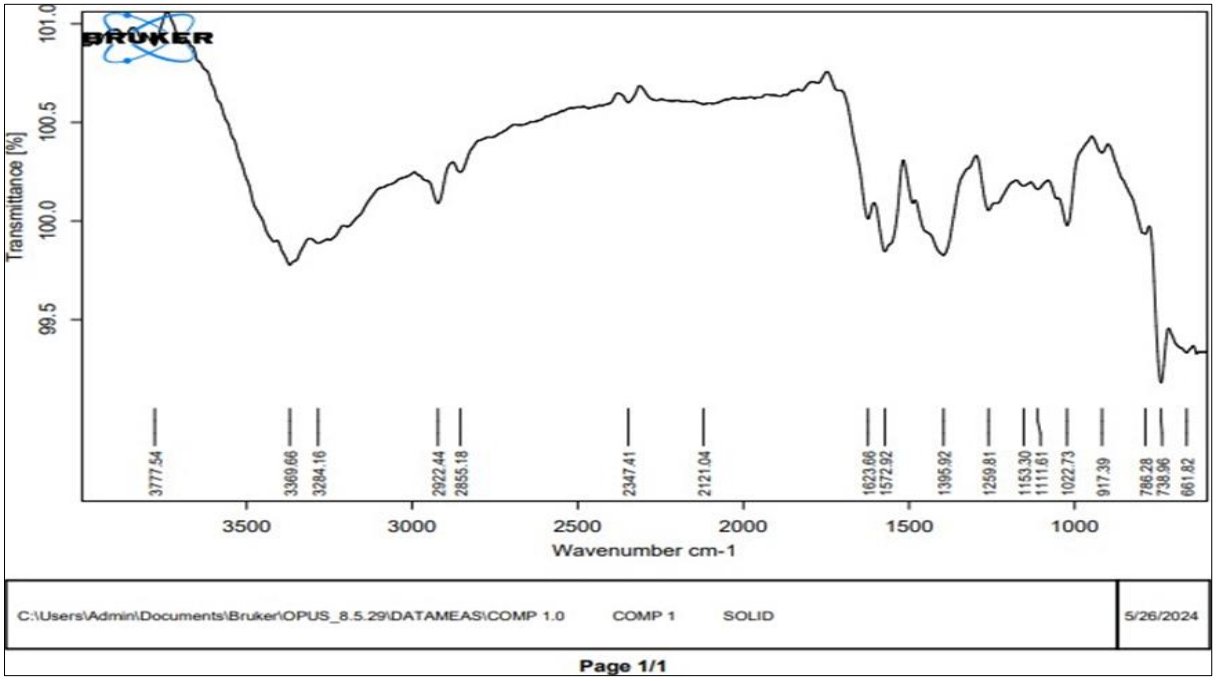


Fig. 16: IR spectra of 2-(6-chloro-1-methyl-1H-benzo[d]imidazol-2-yl) acetic acid

Table 3: IR Interpretation of 2-(6-chloro-1-methyl-1H-benzo[d]imidazol-2-yl) acetic acid

Functional group	Observed value (cm^{-1})	Actual Range(cm^{-1})
O-H (Stretch)	3284	3600-3200
C=O (stretch)	1671	1680-1630
C-O (stretch)	1259	1350-1000
C-H(stretch)	2986	3000-2840
C=C (aromatic)	1572	1600-1475
C-H (Bending)	786	1000-650
C-Cl (stretch)	738	850-550

• NMR –

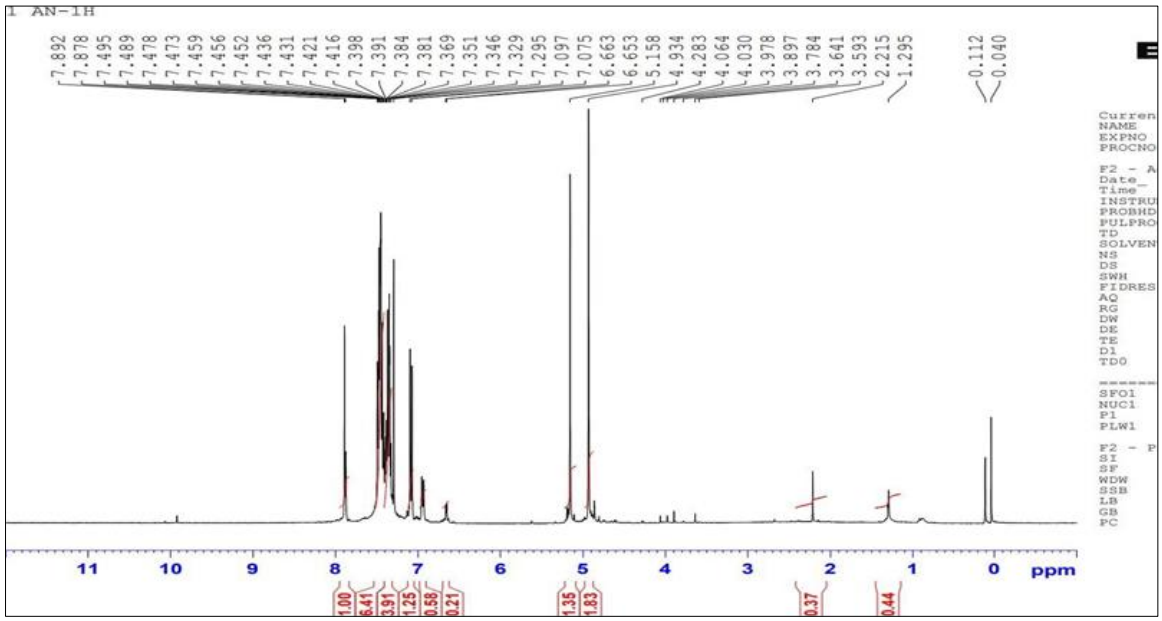


Fig. 17: NMR spectra of 2-(6-chloro-1-methyl-1H-benzo[d]imidazol-2-yl) acetic acid

Table 4: NMR Interpretation 2-(6-chloro-1-methyl-1H-benzo[d]imidazol-2-yl) acetic acid

Protons	Chemical shift Observed (ppm)	Standard value(ppm)	Splitting proton
CH_2	4.534	3.0-5.0	Singlet
Cl	5.158	4.0-7.0	Singlet
CH3	2.531	2.0-4.0	Doublet
COOH	11.087	10-12	Singlet
Ar-H	7.890	7.0-9.0	Multiple

- **MASS:**

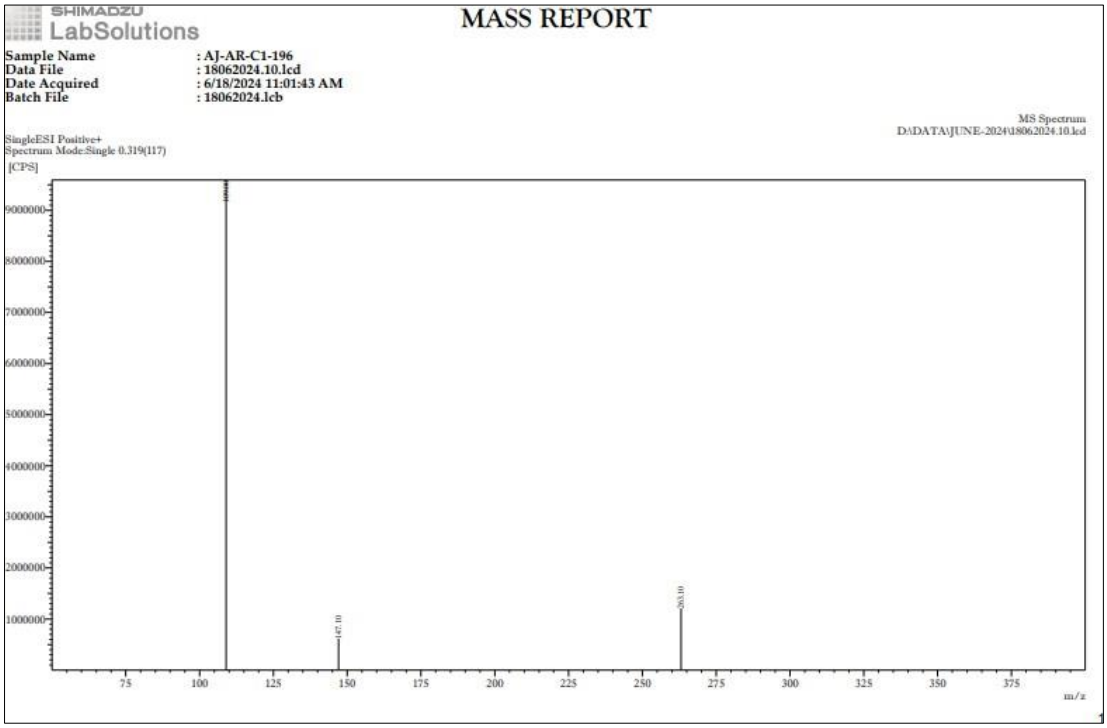
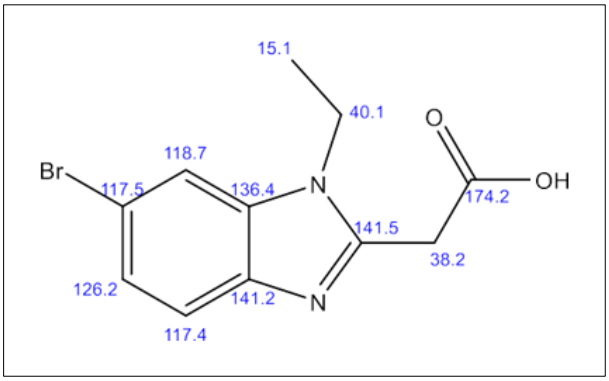


Fig. 18: Mass spectra of 2-(6-chloro-1-methyl-1H-benzo[d]imidazol-2-yl) acetic acid

Table 5: Mass interpretation of 2-(6-chloro-1-methyl-1H-benzo[d]imidazol-2-yl) acetic acid

Compound	Experimental Mass	Molecular formula	Theoretical mass	Major fragments
2-(6-chloro-1-methyl-1H-benzo[d]imidazol-2-yl) acetic acid	236.65 g/mol	C11H9ClN2O2(M+H)	233.65 g/mol	270.11 250.76 228.89

➢ ***Compound-2***



- **IR Spectra: 2-(6-bromo-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid:**

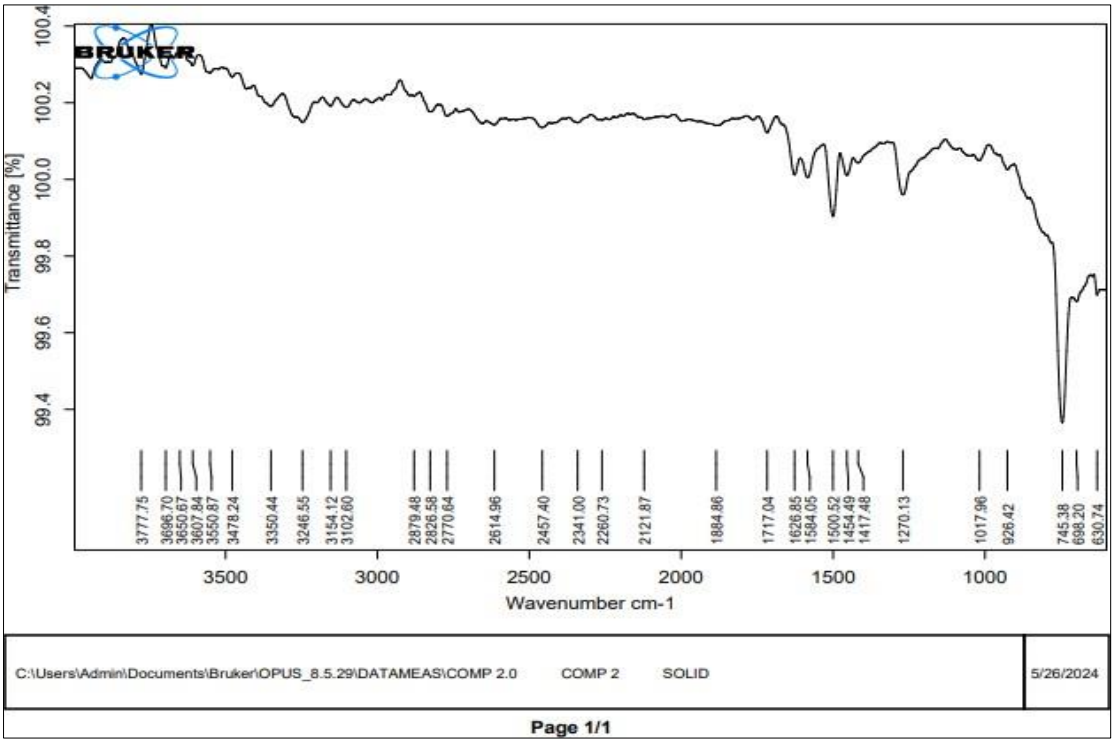


Fig. 19: IR spectra of 2-(6-bromo-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid

Table 6: IR Interpretation 2-(6-bromo-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid

Functional group	Observed value (cm^{-1})	Actual Range(cm^{-1})
OH (stretch)	3323	3600-3200
C=O stretch	1671	1680-1630
C-H (stretch)	2986	3000-2840
C=C (aromatic)	1590	1600-1475
C-Br stretch	545	690-515

- **NMR:**

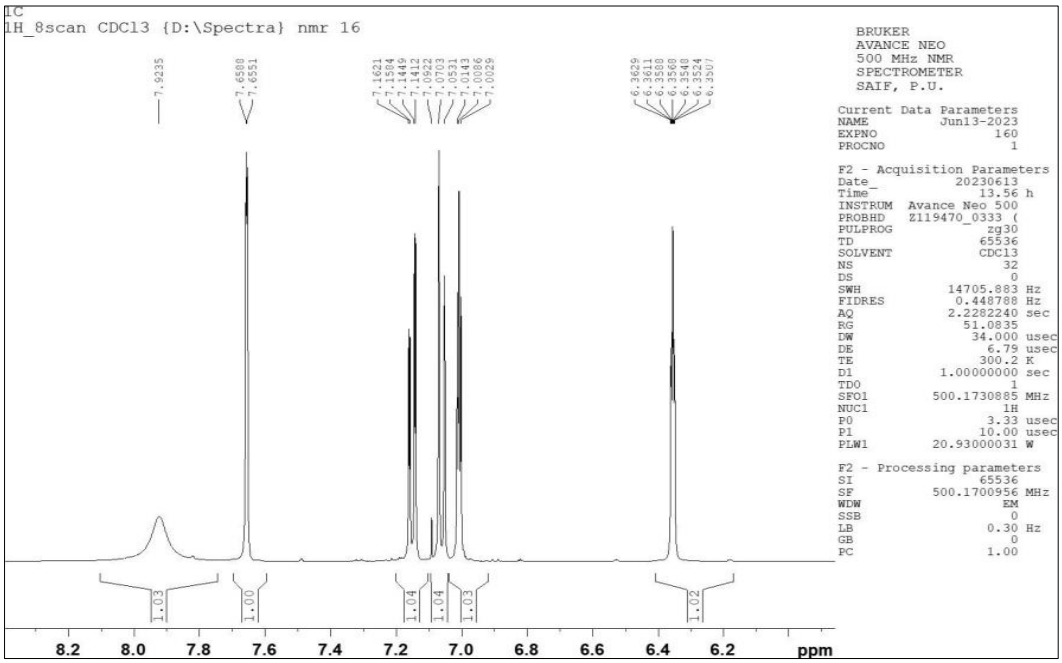


Fig. 20: NMR Spectra 2-(6-bromo-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid

Table 7: NMR Interpretation of 2-(6-bromo-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid

Protons	Chemical shift Observed (ppm)	Standard value(ppm)	Splitting proton
Ar-H	7.14	6.5-8.0	Multiplet
Ar-H	7.0029	6.5-8.0	Doublet
CH3	1.2	1.0-2.0	Triplet
CH2	3.2	2.5-4.0	Quartet
CH2	4.1	3.5-5.0	Singlet
COOH	11.2	10-12	Singlet

- **MASS:**

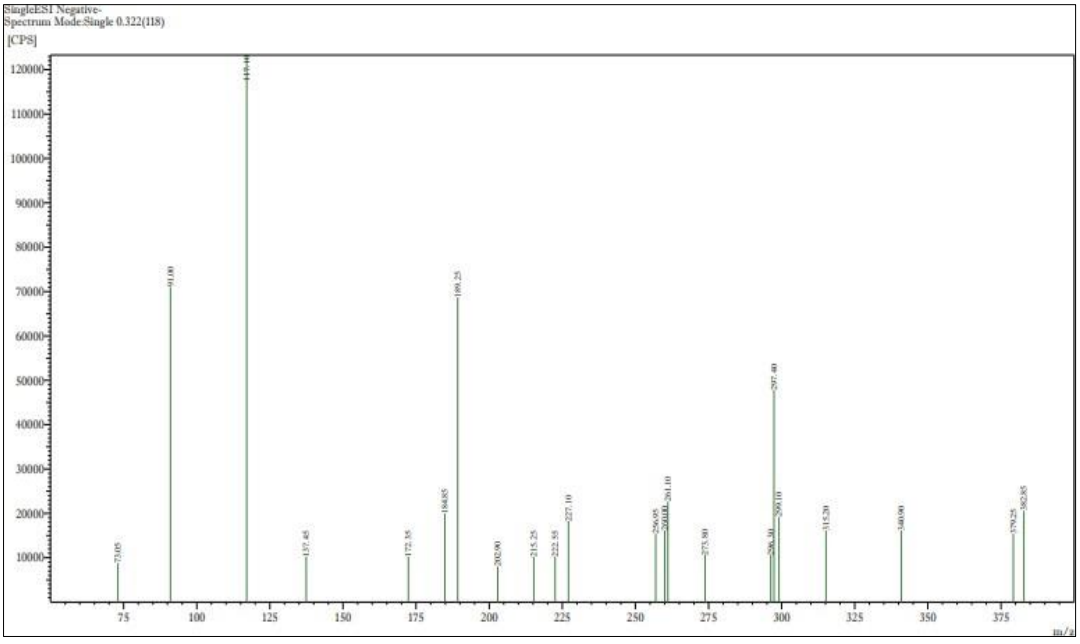
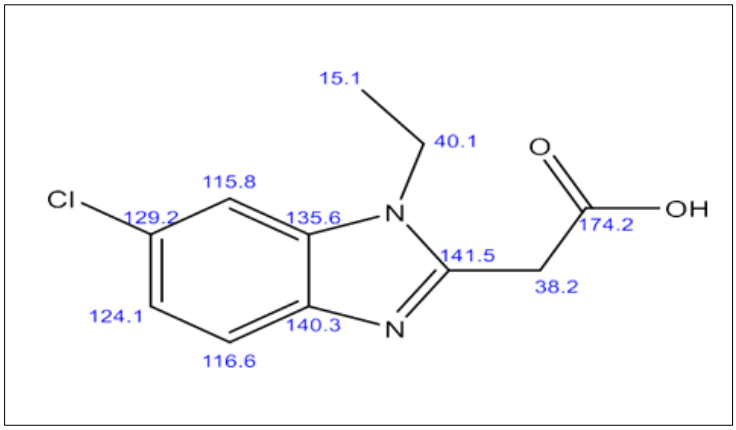


Fig. 21: Mass spectra of 2-(6-bromo-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid

Table 8: Mass interpretation of 2-(6-bromo-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid

Compound	Experimental Mass	Molecular formula	Theoretical mass	Major fragments
2-(6-bromo-1-ethyl- 1H-benzo[d]imidazol-2- yl) acetic acid	264.12 g/mol	$C_{11}H_{11}BrN_2O_2$ (M+2H)	262.12 g/mol	297.93 280.56 241.036

➢ ***Compound-3***



- **IR Spectra: 2-(6-chloro-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid:**

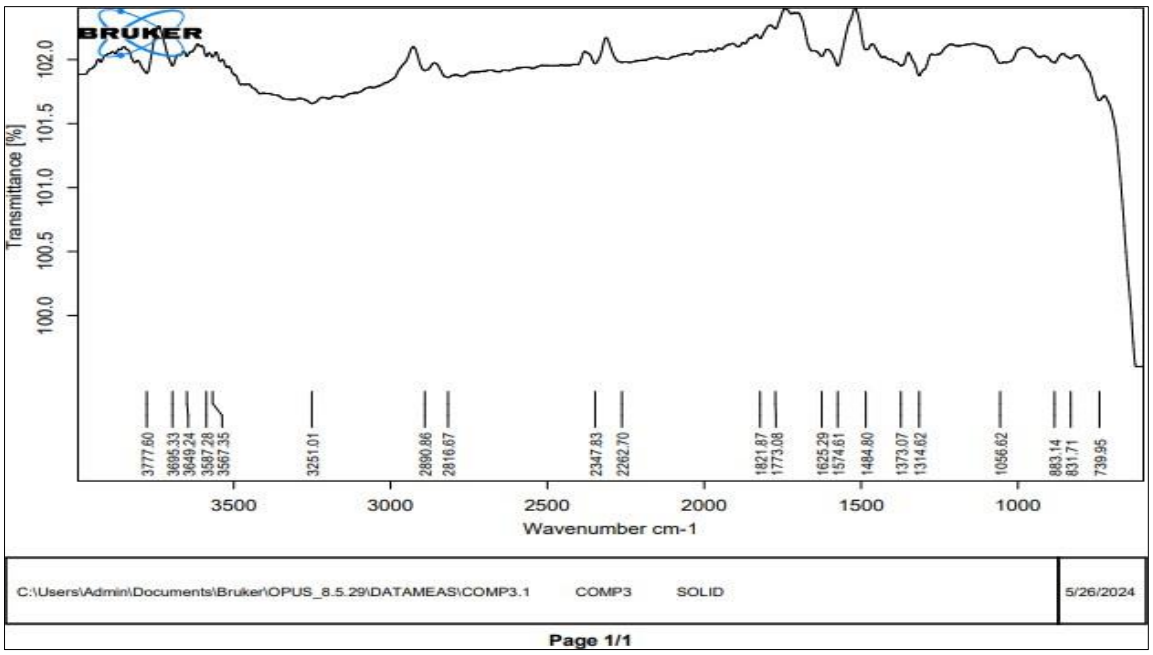


Fig. 22: IR spectra of 2-(6-chloro-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid

Table 9: IR Interpretation of 2-(6-chloro-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid

Functional group	Observed value (cm^{-1})	Actual Range(cm^{-1})
OH	3463	3600-3100
C-H (aromatic)	2915	3000-2840
CH2 (stretch)	3100	3100-3095
C=O (amide)	1662	1680-1630
C-H	2950	3000-2850
C-N	1308	1350-1000
C-Cl (stretch)	819	850-600
N-H (stretch)	3200	3500-3100

NMR:

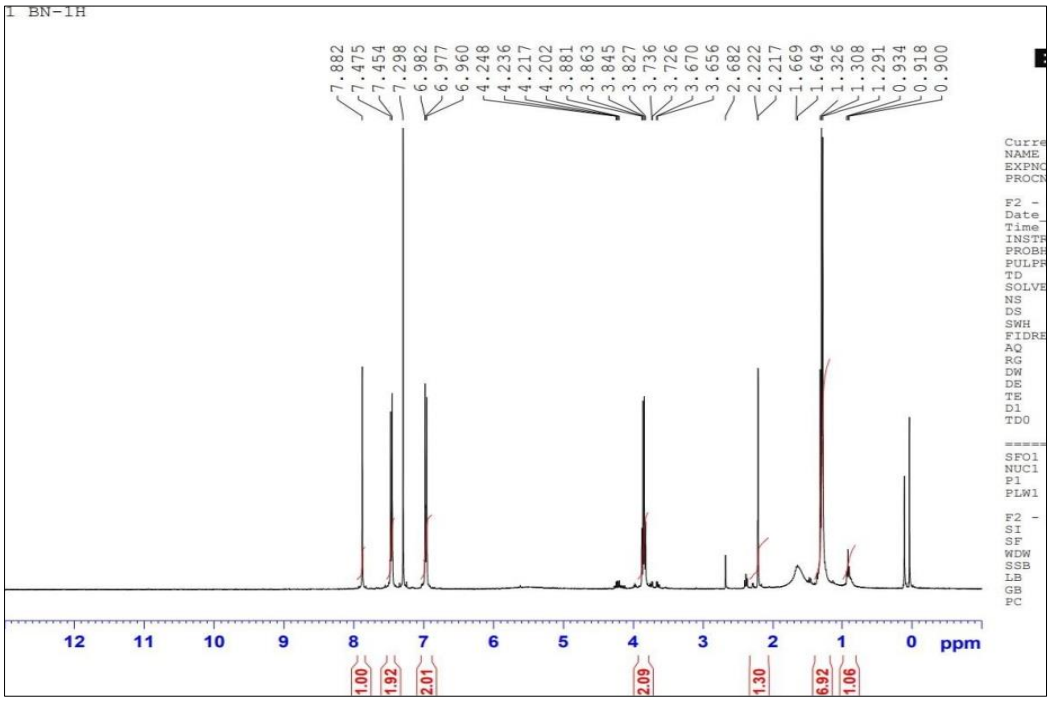


Fig. 23: NMR spectra of 2-(6-chloro-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid

Table 10: NMR interpretation of 2-(6-chloro-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid

Protons	Chemical shift Observed (ppm)	Standard value(ppm)	Splitting protons
R-CH_3	0.918	0.7-1.3	Triplet
R-CH_2	1.308	1.2-1.4	Quartet
CH2	3.5	3.0-5.0	Singlet
Ar-H	6.977	6.5-8.0	Doublet
Ar-H	7.454	6.5-8.0	Doublet
COOH	10.5	10-12	Singlet

Mass:

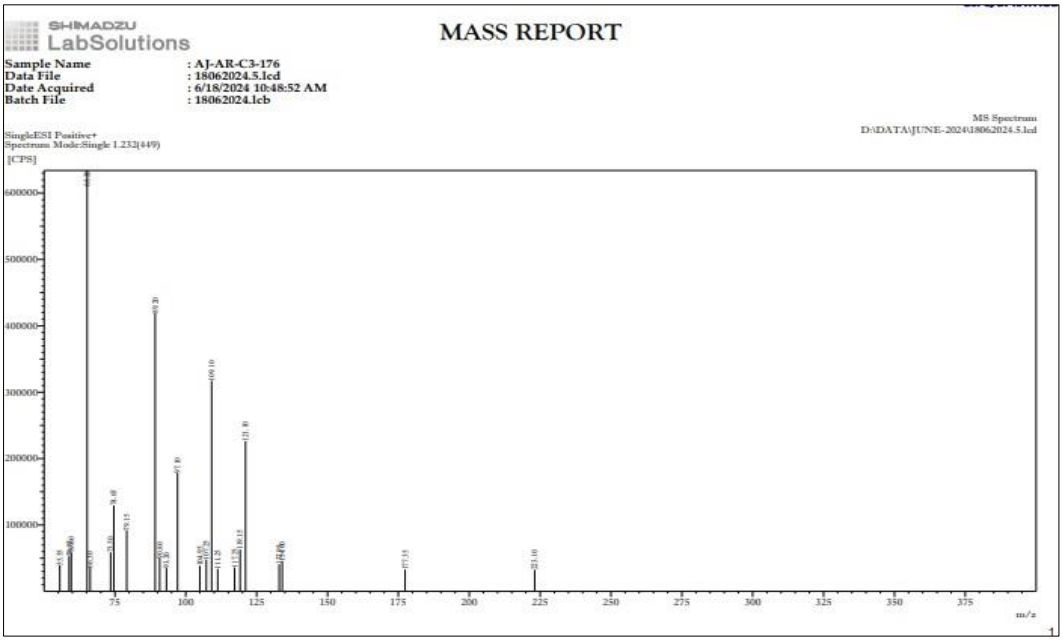
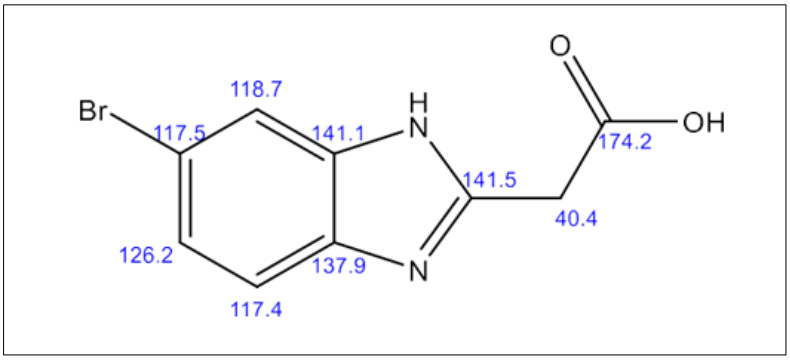


Fig. 24: Mass spectra of 2-(6-chloro-1-ethyl-1H-benzo[d]imidazol-2-yl) acetic acid

Table 11: Mass interpretation of 2-(6-chloro-1-ethyl-benzo[d]imidazol-2-yl) acetic acid

Compound	Experimental Mass	Molecular formula	Theoretical mass	Major fragments
2-(6-chloro-1-ethyl- 1H-benzo[d]imidazol-2- yl) acetic acid	252.05 g/mol	C12H12ClN2O2 (M+H)	249.84 g/mol	245.07 254.11 259.25

Compound-4:



- **IR Spectra: of 2-(6-bromo-1H-benzo[d]imidazol-2-yl) acetic acid**

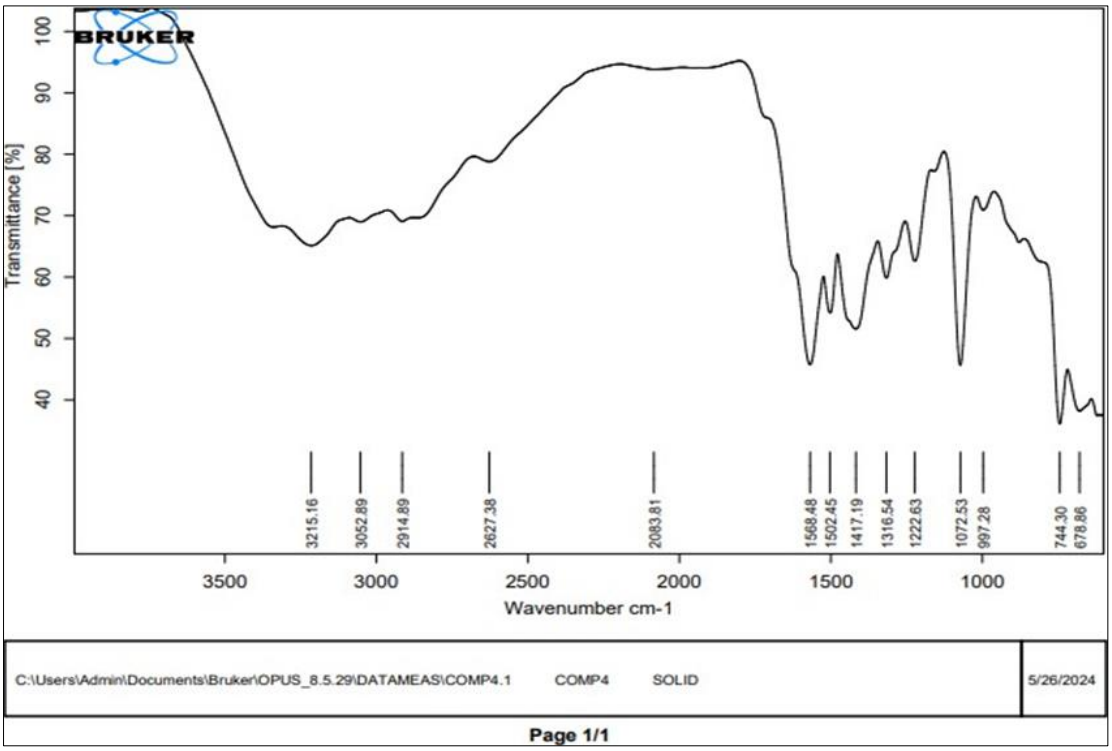


Fig. 25: IR spectra of 2-(6-bromo-1H-benzo[d]imidazol-2-yl) acetic acid

Table 12: IR Interpretation of 2-(6-bromo-1H-benzo[d]imidazole-yl) acetic acid

Functional group	Observed value (cm^{-1})	Actual Range(cm^{-1})
OH	3635	3600-3200
C=O (Stretch)	1675	1750-1650
C-H (Stretch) Aromatic	3105	3100-3095
N-H (Stretch)	3209	3500-3100
C=C (aromatic)	1442	1600-1475
C-Br	551	600-500

- **NMR-**

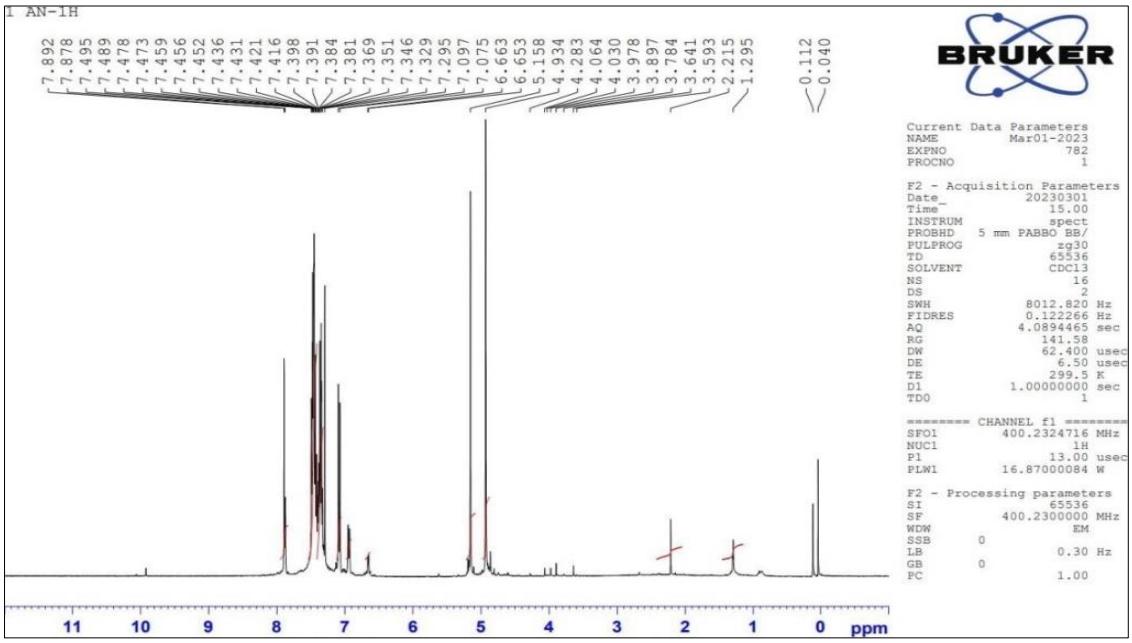


Fig. 26: NMR spectra of 2-(6-bromo-1H-benzo[d]imidazol-2-yl) acetic acid

Table 13: NMR Interpretation 2-(6-bromo-1H-benzo[d]imidazol-2-yl) acetic acid

Protons	Chemical shift Observed (ppm)	Standard value(ppm)	Splitting proton
Ar-H	7.097	6.5-8.0	Singlet
N-H	11.29	10-13	Singlet
Ar-CH	5.097	4.0-6.0	Doublet
COOH	11.07	11-12	Singlet
CH2	4.5	3.5-5.5	Singlet

- **MASS:**

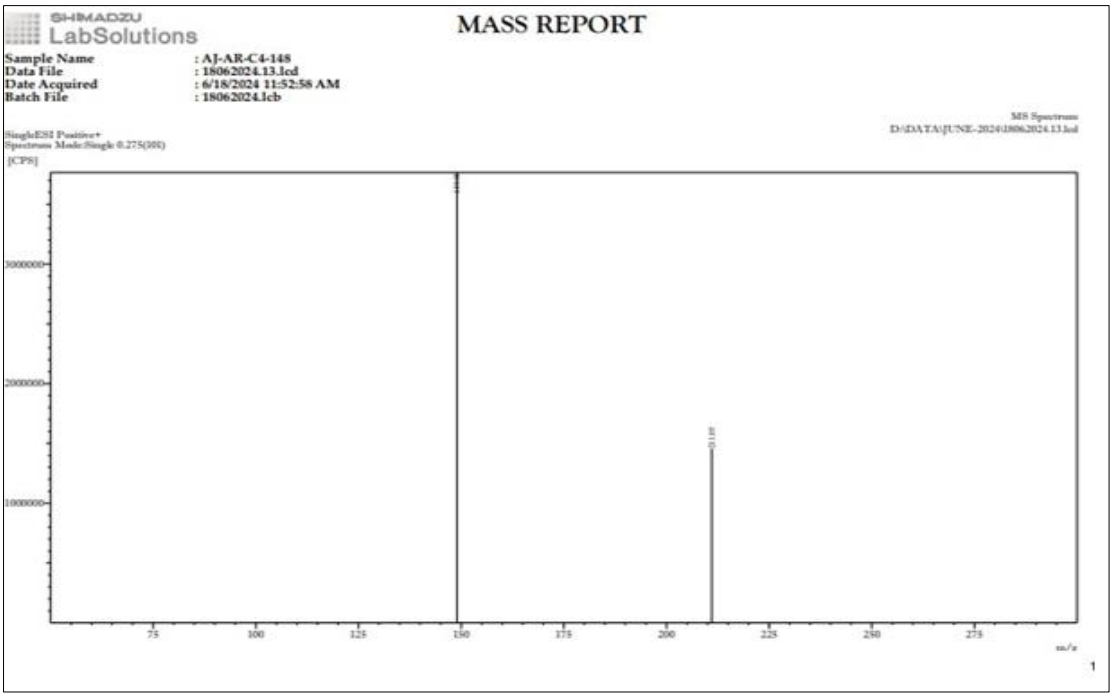


Fig. 27: Mass spectra of 2-(6-bromo-1H-benzo[d]imidazol-2-yl) acetic acid

Table 14: Mass interpretation of 2-(6-bromo-1H-benzo[d]imidazol-2-yl) acetic acid

Compound	Experimental Mass	Molecular formula	Theoretical mass	Major fragments
2-(6-bromo-1H- benzo[d]imidazol-2- yl) acetic acid	255.08 g/mol	C9H7BrN2O2(M+H)	254 g/mol	270.50 250.23 248.19

Thin layer chromatography: [Chloroform: Methanol 8:2]

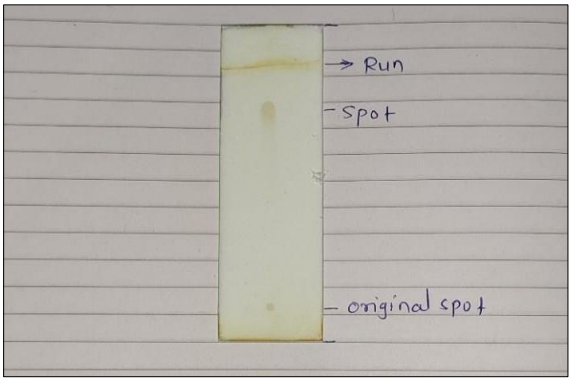


Fig. 7.13 Thin Layer Chromatography

- **Observation:**
  1. Distance travelled by solvent = 6.6cm
  2. Distance travelled by solute = 5.5 cm

- **Calculation:**

$$\text{Rf Value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

- **Result:** Rf Value of sample was found to be **0.82.**

- **ANTI-INFLAMMATORY ACTIVITY:**

A protective host response is inflammation. This is an effective self-defence strategy which is effective with foreign and transmissible diseases in order to treat the development of additional illnesses. Unrestrained inflammatory reaction, however, causes more harm than good. Numerous non-communicative diseases, notably neurodegeneration, cancer, diabetes, immunological diseases, heart issues, renal and liver problems, are associated with persistent inflammation. Infectious diseases become more severe by severe inflammation. It is essential to continuously look for novel, diversified combinations with anti-inflammatory characteristics in order to encourage derivatives with anti-inflammatory effects as potentially bioactive compounds. Derivatives with anti-inflammatory actions may have a variety of pharmacological profiles [2].

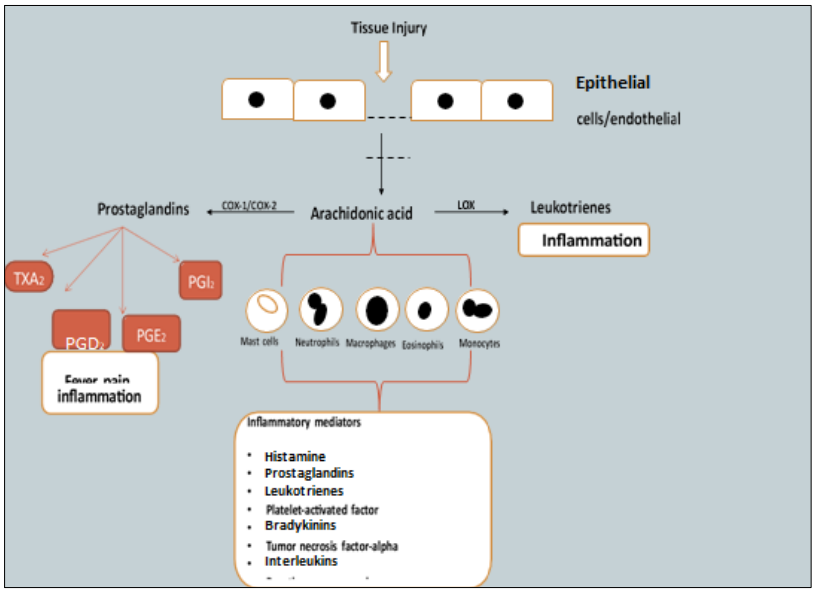


Figure 28: Depiction of mechanism of inflammation: PGI2: Prostacyclin, PGD2: Prostaglandin D, TXA2-Thromboxane A2, PGE2: ProstaglandinE2[8]

- ***In Vitro* Anti-Inflammatory Activity by Protein Denaturation Method:**

The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 4 mL of Synthetic compound (1000µg/ml). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (370c ±2) in an incubator for 15 min and then heated at 70oc for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at concentration 1000 µg/ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula, **%** inhibition = absorbance of control - absorbance of test / absorbance of control x 100.

Sr. no.	Sample (1000µg/ml)	Conc.	O. D.	Mean	Percent inhibition
1	Control	-	0.42	0.43	
			0.43		
			0.46		
2	Standard Diclofenac sodium	250µg/ml	0.12	0.12	73.09
			0.11		
			0.15		
		500µg/ml	0.10	0.09	79.06
			0.09		
			0.08		
		1000µg/ml	0.07	0.06	86.04
			0.06		
			0.05		
3	Compound- A F12	250µg/ml	0.25	0.25	44.86
			0.28		
			0.24		
		500µg/ml	0.20	0.18	59.13
			0.18		
			0.17		
		1000µg/ml	0.15	0.13	70.95
			0.14		
			0.12		
	Compound- B F13	250µg/ml	0.29	0.31	29.90
			0.30		
			0.35		
		500µg/ml	0.30	0.29	34.58
			0.31		
			0.28		
		1000µg/ml	0.25	0.22	48.83
			0.22		
			0.21		

Fig. 29: Protein Denaturation Reading

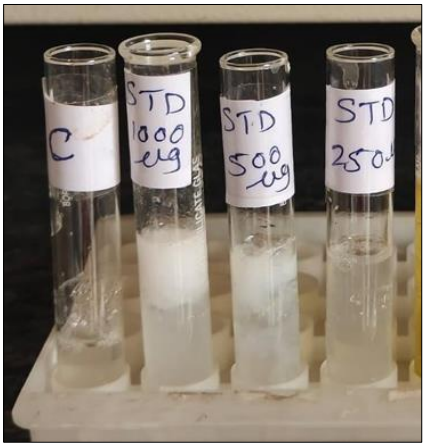


Fig. 30: Standard Compound

Fig. 31: Test Compound

➢ ***Result:***

Denaturation of tissue protein is one of the well documented causes of inflammatory and arthritic diseases. Production of auto antigen in certain arthritic diseases may be due to denaturation of protein in vivo. Agents that can prevent protein denaturation therefore could be worthwhile for anti-arthritic and anti-inflammatory drug development Compound-A F12, Compound-B F13 showed good activity as compared with standard drug.

REFERENCES

1. Walia R, Hasibullah MD, Naaz SF *et al.*, Benzimidazole derivatives–an overview. Int. J. Res.Pharm. Chem. 2011; 1(3):565-74.
2. Gajanan, Gavande, *et al.*, "A Review on Benzimidazole and it's Biological Activities" Journal of Pharmaceutical Chemistry and Drug Formulation 3.1 (2021).
3. Juan Valdez *et al.,* Synthesis and Antiparasitic Activity of 1H-Benzimidazole Derivatives Bio organic & amp; Medicinal Chemistry Letters 12 (2002) 2221–2224.
4. Afaf H. et.al. Synthesis and Antimicrobial Activity of Some New Benzimidazole Derivatives Molecules 2000, 5, 1429–1438.
5. HS. Lamba *et al.*, Benzimidazole derivatives: Spectrum of pharmacological activity and toxicological properties. Pharmaceutical Chemistry Journey.
6. Achar KC HR *et al.,* In-vivo analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives. European journal ofmedicinal chemistry. 2010 May1; 45(5):2048- 54.
7. Hanan M. Refaat *et al.,* Synthesis and anticancer activity of some novel 2 -substituted benzimidazolederivatives European Journal of Medicinal Chemistry 45 (2010) 2949e2956.
8. O¨ zden O¨ zel Gu¨ven, a, *et al.,* Synthesis and antimicrobial activity of some novel phenyl & benzimidazole substituted benzyl ethers Bioorganic & Medicinal Chemistry Letters 17(2007)2233–2236.
9. Asma Eswayah, *et al.*, Synthesis and Analgesic Activity Evaluation of Some New Benzimidazole Derivatives American Journal of Chemistry and Application 2017; 4(5): 30-35.
10. Muhammad Tahar, *et al.*, Synthesis, α-glycosidase inhibitory potential and molecular docking study of benzimidazole derivatives.
11. Azize Gizem Ulua *et al.*, Benzimidazole derivatives as potent and isoform selective tumor- associated carbonic anhydrase IX/XII inhibitors Bioorganic Chemistry 95 (2020) 103544.
12. Thakur Desai, Prasad A., Sudhir G. Wadodkar, and Chandrabhan T. Chopade. "Synthesis and anti-inflammatory activity of some benzimidazole-2-carboxylic acids." Pharmacologyonline 1 (2007): 314-329.
13. Tianrong Zhang, *et al.*, Design, synthesis and biological activity evaluation of novel methyl substituted benzimidazole derivatives T. Zhang *et al.*, / Tetrahedron 76 (2020) 131027.