SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL SCREENING OF SUBSTITUTED BENZIMIDAZOLE

A Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI-600 032

In partial fulfilment of the requirements for the award of the degree of

MASTER OF PHARMACY

in

PHARMACEUTICAL CHEMISTRY

Submitted by

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Under the Guidance of

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Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)
Institutional Animal Ethics Committee (IAEC)
Reg No: 688 /PO/Re/S/02/CPCSEA

CERTIFICATE

This is to certify that the project **Proposal No: NCP/IAEC/2021-22/04** entitled **"Synthesis, characterisation and Pharmacological (Analgesic activity) screening of 1,2 substituted benzimidazoles"** submitted by Dr./Mr./Ms. **P. Haritha** has been approved/recommended by the IAEC of Nandha College of Pharmacy in its meeting held on 12/08/2021 and **Swiss Albino Mice: 30** (Number and Species of animals) have been sanctioned under this.

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Chairperson	Dr. T. Sivakumar		12/8/21
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CERTIFICATE

This is to certify that the Dissertation entitled **"SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL SCREENING OF SUBSTITUTED BENZIMIDAZOLE"** submitted to The Tamilnadu Dr. M.G.R Medical University, Chennai, was carried out by P.HARITHA (**261915402)** in the Department of Pharmaceutical Chemistry, Nandha College of Pharmacy Erode-52 in the partial fulfilment of the degree of Master of Pharmacy in Pharmaceutical Chemistry under my direct supervision and guidance.

This work is original and has not been submitted in part or full for any degree or diploma of this or any other university.

Place: Erode **Dr. K. SRINIVASAN, M.Pharm., Ph.D.,**

Date:

,

EVALUATION CERTIFICATE

The work presented in this thesis entitled "**SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL SCREENING OF SUBSTITUTED BENZIMIDAZOLE**" was carried out by me in the Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode-52 under the supervision and guidance of **Dr. K. SRINIVASAN, M.Pharm., Ph.D.,** Professor, Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode-52.

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Internal Examiner **External Examiner**

DECLARATION

The work presented in this thesis entitled "**SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL SCREENING OF SUBSTITUTED BENZIMIDAZOLE"** was carried out by me in the Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode-52 under the supervision and guidance of **Dr. K. SRINIVASAN, M.Pharm., Ph.D.,** Professor, Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode-52.

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Place: Erode . **P.HARITHA**

Date: **(261915402)**

ACKNOWLEDGEMENT

"Success is the progressive realisation of worthy goal many able people failed to achieve anything worldwide because they have not been properly guided. Success of any

project depends solely on support, guidance and encouragement received from the guide and well-wishers".

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Place: Erode **P.HARITHA**

Date: **(261915402)**

CONTENTS

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

INTRODUCTION

Heterocyclic Chemistry is an integral part of organic chemistry. A large number of heterocyclic compounds both synthetic and natural, are pharmacologically active and are in clinical use. Several heterocyclic compounds have applications in agriculture as insecticides, fungicides, herbicides, pesticides etc. They also find applications as sensitizers, developers, antioxidants, co polymers etc. They are used as vehicles in the synthesis of other organic compounds. Chlorophyll-photosynthesizing and haemoglobin-oxygen transporting pigments are also heterocyclic compounds. [1]

New advances in synthetic methodologies that allow rapid access to a wide variety of functionalized heterocyclic compounds are of critical importance to the medicinal chemist as it provides the ability to expand the available drug-like chemical space and drive more efficient delivery of drug discovery programs.

The development of robust synthetic routes that can readily generate bulk quantities of a desired compound help to accelerate the drug development process. While establishing synthetic methodologies are commonly utilized during the course of a drug discovery program, the development of innovative heterocyclic syntheses that allow for different bond forming strategies are having a significant impact in the pharmaceutical industry.

The synthesis and functionalization of heterocycles, there remains a great need for further advances in this area. In the early drug discovery phase, medicinal chemistry design hypotheses continually require speedy access to new chemical space – novel heterocycles or substitution patterns that, for example, satisfy strictly physicochemical requirements, provide new vectors in structure-based drug design and can afford access to novel intellectual properties.[2]

The process of drug discovery begins with the identification of new, previously undiscovered, biologically active compounds, often called “hits,” which are typically found by screening many compounds for the desired biologic properties. We will next explore the various approaches used to identify “hits” and to convert these “hits” into “lead” compounds and subsequently, into drug candidates suitable for clinical trials.

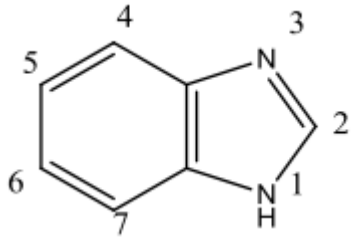
Chemical or functional group modifications of the “hits” are performed in order to improve the pharmacologic, toxicological, physiochemical, and pharmacokinetic properties of

a “hit” compound into a “lead” compound. The lead compound to be optimized should be of a known chemical structure and possess a known mechanism of action, including knowledge of its functional groups (pharmacophoric groups) that are recognized by the receptor/active site and are responsible for that molecule’s affinity at the targeted receptor site.

“Lead optimization” is the process whereby modifications of the functional groups of the lead compound are carried out in order to improve its recognition, affinity, and binding geometries of the pharmacophoric groups for the targeted site (a receptor or enzyme); its pharmacokinetics; or its reactivity and stability toward metabolic degradation.[3]

BENZIMIDAZOLE:

Benzimidazole is a heterocyclic aromatic organic compound. It is an important pharmacophore and privileged structure in medicinal chemistry. It plays a very important role with plenty of therapeutic activities such as: antiulcers, antihypertensives, analgesic, anti-inflammatory, anti-viral, antifungals, anticancers, and antihistaminics.



This compound is bicyclic in nature which consists of the fusion of benzene and imidazole. [4]

Benzimidazole nucleus is a constituent of many bioactive heterocyclic compounds that are of wide interest because of their diverse biological and clinical applications. Moreover, benzimidazole derivatives are structural isosters of naturally occurring nucleotides, which allows them to interact easily with the biopolymers of the living System. [5]

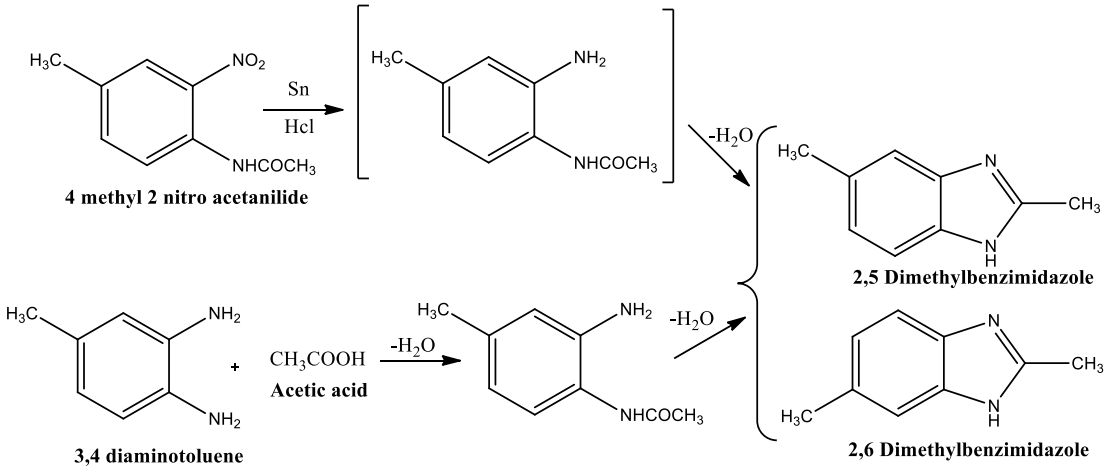
The benzimidazole ring system as a nucleus from which to develop potential chemotherapeutic agents was established in the 1950s when it was found that

5, 6-dimethyl-l-(a-D-ribofuranosyl) benzimidazole is an integral part of the structure of the vitamin B_{12}.

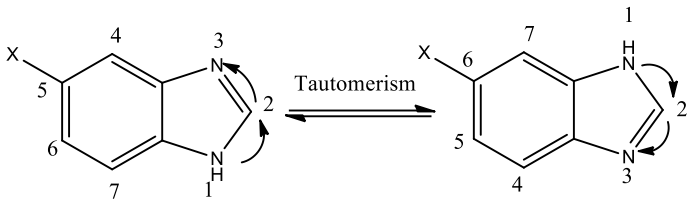
Modifications in the position 2 and 5 of the molecule provide a number of active drugs. The synthetic pathway to the various benzimidazole usually proceeds through

two steps; first, the construction of desired benzene ring containing 1–2 diamino groups followed by the ring closer of the compound (*O*-phenylenediamine) to construct the imidazole ring.[6]

The very first benzimidazole (2, 5 or 2, 6-dimethylbenzimidazole) was prepared in 1872 by Hoebrecker through reduction of 2-nitro-4-methylacetanilide. Several years later, Ladenburg obtained the same compound by refluxing 3, 4-diamino toluene with acetic acid.



Benzimidazoles were also known as benziminazoles and benzoglyoxalines. These were also named as derivatives of *o*-phenylenediamine, for example, benzimidazole was called methenyl-*o*-phenylenediamine and 2- methyl benzimidazole was called as ethenyl-*o*-phenylenediamine and so on. They were also named as derivatives of groups composing imidazole portion of ring, for example, benzimidazole has also been called as *o*-phenylene formamidine. 2(3H)-benzimidazolone and 2(3H)-benzimidazolethione has been known as *O*-phenylurea and *O*-phenylenethiourea, respectively. Hydrogen atom attached to N-1 of the nucleus readily tautomerises.[7]

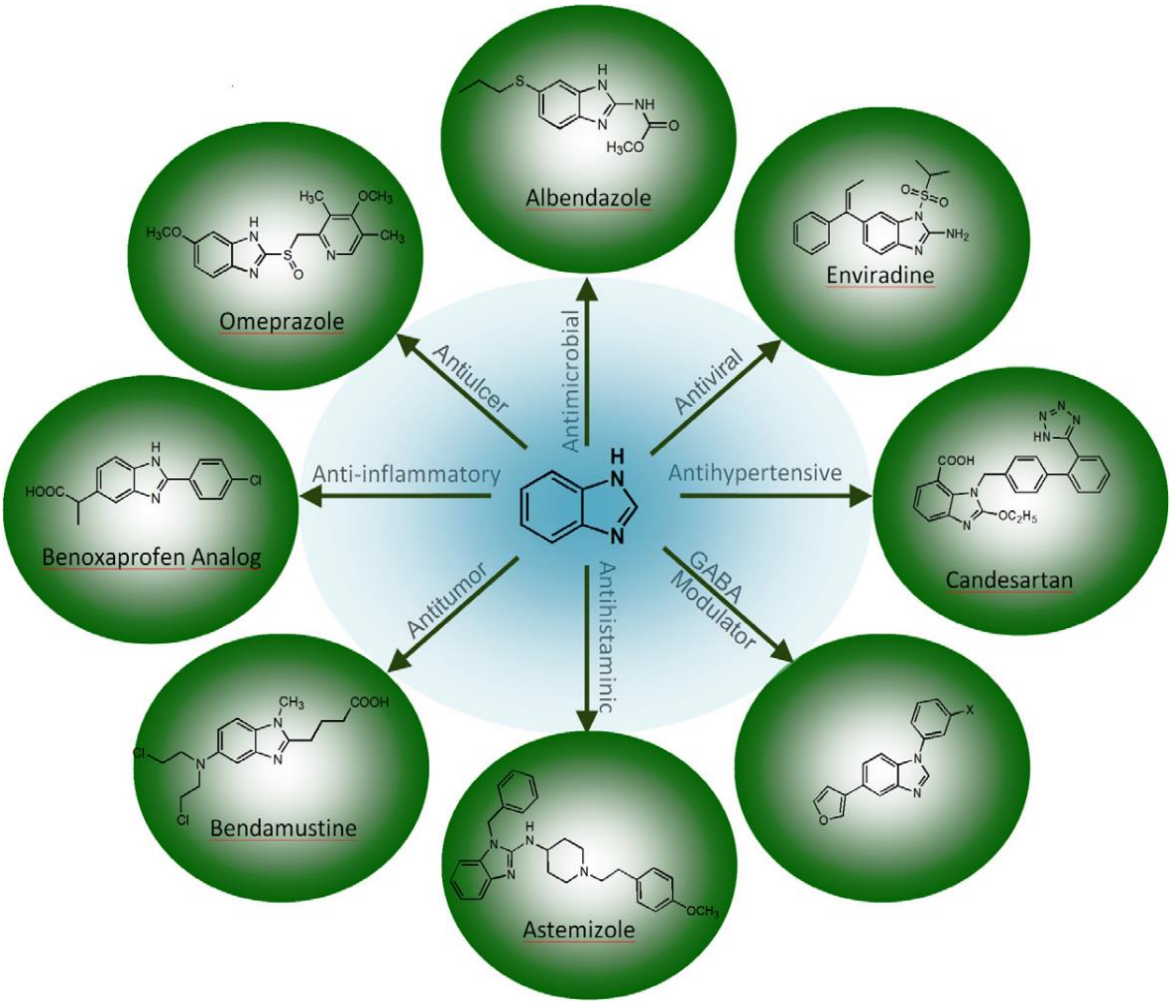


Though all seven positions in the benzimidazole nucleus can be substituted with a variety of chemical entities, but most of the biologically active benzimidazole based compounds bear functional groups at 1, 2 and/or 5(or 6) positions. Accordingly, the compounds may be mono-, di- or tri-substituted derivatives of the nucleus.

History of benzimidazole: [8]

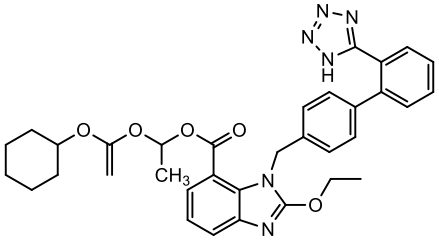
Year	Biological activity reported
1943	Goodman and Nancy Hart published the first paper on antibacterial properties of benzimidazole.
1944	Woolley published their work on benzimidazoles He also reported the antibacterial activity of synthesized benzimidazoles against *E. coli* and *Streptococcus lactic.*
1950	CIBA pharmaceutical (now Novartis) were discovered benzimidazole derivative opioid agonist etonitazene
1960	Fort *et al*., reported the discovery of benzimidazole derivatives as proton pump inhibitors
1965	Burton *et al*., Reported 2-trifluoro benzimidazoles are potent decouplers of oxidative phosphorylation in mitochondria. They are also inhibitors of photosynthesis, and some exhibit appreciable herbicidal activity.
1971	Mebendazole was discovered by Janssen pharmaceutical in Belgium
1975	Albendazole was invented by Robert J. Gyurik and Vassilios J. Theodorides and assigned to SmithKline Corporation
1977	Astemizole was discovered by Janssen pharmaceutical
1989	Lackner *et al*., reported the anti-inflammatory activity of benzimidazole. Omeprazole was developed by Astra AB (now AstraZeneca)
1991	Telmisartan was discovered and developed by Boehringer ingelheim *et al*.,
1992	Candesartan is a benzimidazole which was developed at Takeda pharmaceutical.
1994	Devivar *et al*., reported that 6- dichlorobenzimidazole-1-βD-ribofuranoside (DRB) and its 2-substituted derivatives Show activity against human cytomegalovirus.
2001	Most recently, the antiprotozoal activity of substituted 2-trifluoro benzimidazoles has been reported by Navarette-Vazquez *et a*l.,

Biological activities of benzimidazole[7]

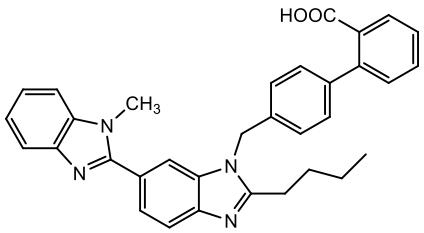


Antihypertensive action:

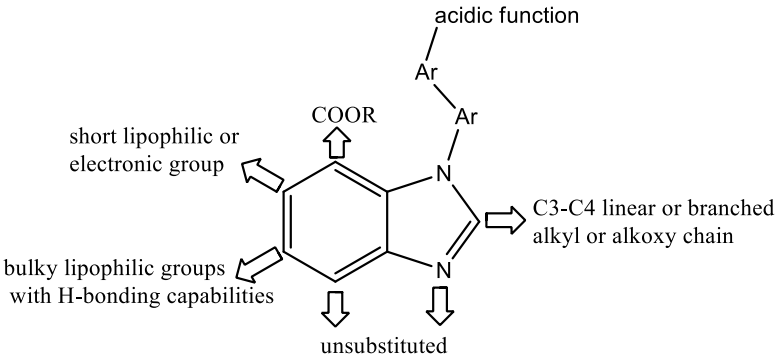
Benzimidazole based compounds act as antihypertensives by intercepting with Renin–Angiotensin System (RAS). Angiotensin II (Ang II) is an octapeptide which is active pressor produced by RAS cascade. Angiotensinogen, a polypeptide, is cleaved by rennin to produce a decapeptide, Ang I, which is further acted upon by Angiotensin converting enzyme (ACE) to generate Ang II. The latter acts on angiotensin receptor 1 (AT1) resulting in vasoconstriction, Na^+ retention and aldosterone release to cause hypertensive action.



Candesartan cilexitil



Telmisartan



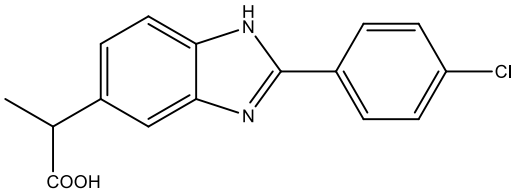
SAR OF BENZIMIDAZOLE MOIETY AS AT_1 RECEPTOR ANTAGONIST

Anti-inflammatory activity:

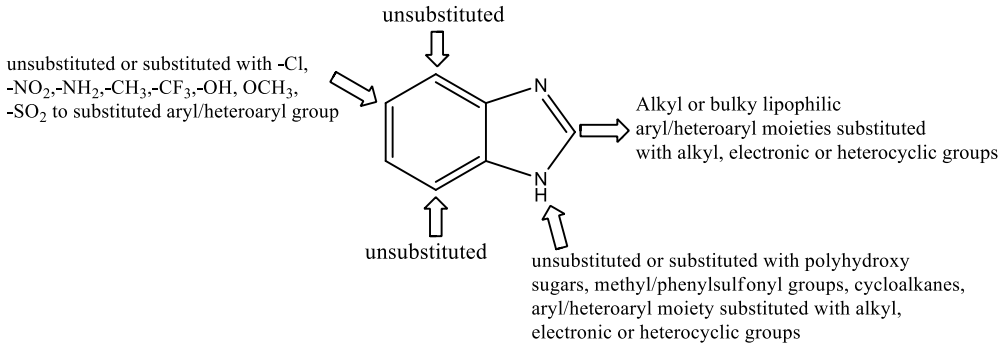
Control of inflammation has become of prime importance due to its association with numerous diseased states like Alzheimer's disease, asthma, atherosclerosis, Crohn's disease, gout, multiple sclerosis, osteoarthritis, psoriasis, rheumatoid arthritis, diabetes mellitus, carcinoma, bacterial or viral infections, etc. which results in chronic inflammation.

The most common and widely explored points for control of inflammation include inflammatory mediators like plasma proteases, prostaglandins, histamine, serotonin, nitric oxide, interleukins 1–16 (IL-1 to IL-16), tumor necrosis factor-a (TNF-a), chemokines (CXC, CC and C subsets) and colony stimulating factors (CSF).These mediators are produced through various processes involving cyclooxygenases, caspases and kinases like cyclin dependent kinases (CDK1 and CDK5), mitogen activated protein kinase 38 (MAP38), c-Jun N-terminal kinase (JNK), serine threonine kinases (IKK1 and IKK2), interleukin receptor associated kinase 4 (IRAK-4), Janus kinases

(JAK1- JAK3and Tyk2), kinase insert domain receptor (KDR), lymphocyte specific kinase (Lck), spleen tyrosin kinase (Syk) and TNF-a kinase (TNFK).



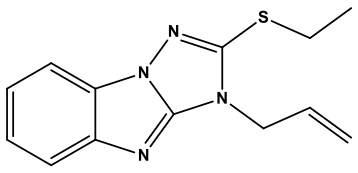
2-(2-(4-chlorophenyl)-1*H*-benzimidazol-5-yl) propanoic acid



SAR OF BENZIMIDAZOLE MOIETY AS ANTI-INFLAMMATORY ACTIVITY

Antimicrobial agents:

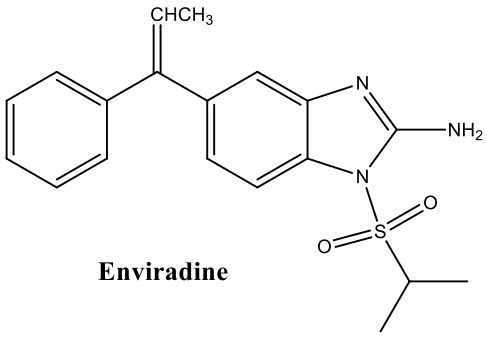
Antimicrobial agents constitute a diverse group of chemical entities acting against varied kinds of microbes including bacteria, protozoa, helminths (worms), fungi and viruses. Various research groups have evaluated antibacterial, antiprotozoal, anthelmintic and/or anti-fungal activities concomitantly while evaluation of antiviral compounds remains solitary. Antimicrobials from benzimidazole nucleus have been taken up after the year 2000.



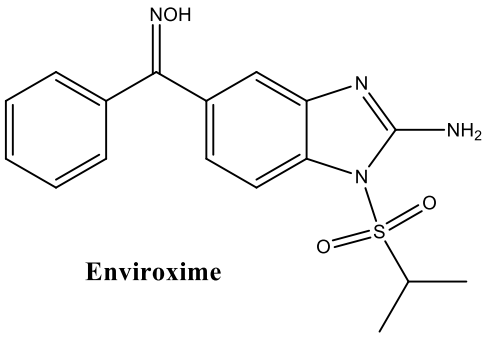
3-allyl-2-(ethylthio)-3*H*-benzo[4,5]imidazo[1,2-*b*] [1,2,4]triazole

Antiviral activity:

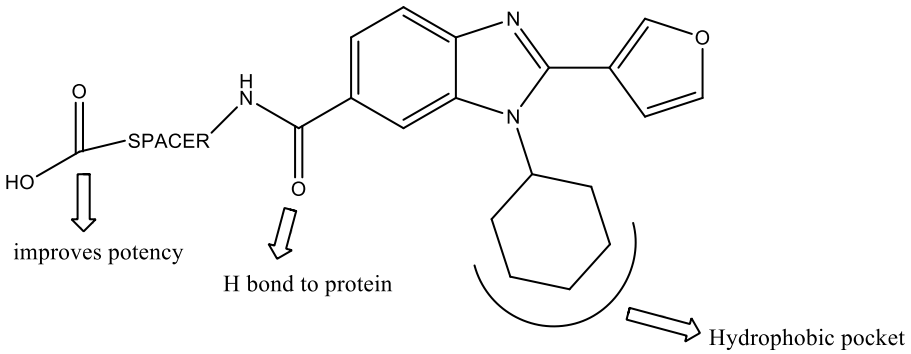
Antiviral properties of various benzimidazole derivatives have been evaluated using different virus strains, such as human cytomegalovirus (HCMV), human herpes simplex virus (HSV-1), human immunodeficiency virus (HIV), and hepatitis B and C virus (HBV and HCV).



Enviradine



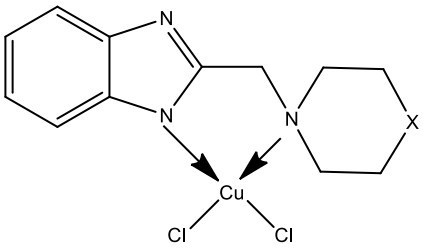
Enviroxime

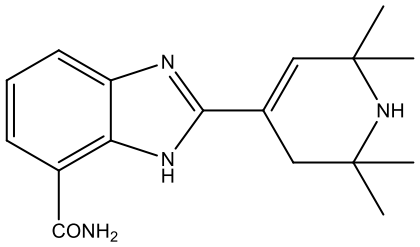


SAR OF BENZIMIDAZOLE FOR HEPATITIS C VIRUS

Antioxidant activity:

The drugs possessing antioxidant and free radical scavenging activity have been implicated in treatment of various diseases like cancer which are directly related to lack of antioxidant capacity of organism.

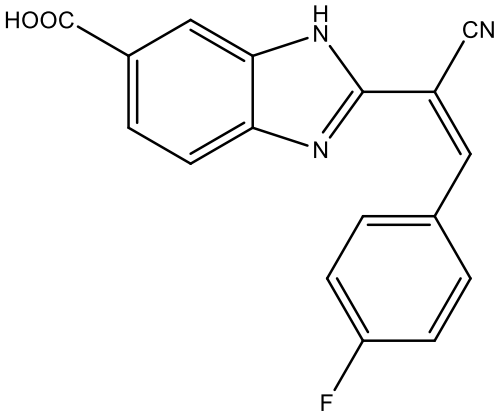




2-(2,2,6,6-tetramethyl-1,2,3,6-tetrahydropyridin-4-yl)-1*H*-benzo[*d*]imidazole-7-carboxamide

Anticancer activity:

Cancer is one of the leading health hazards which are affecting a wide majority of world population. Benzimidazole being an isostere of purine based nucleic acid and an important scaffold in various biologically active molecules is widely explored for development of anticancer agents.

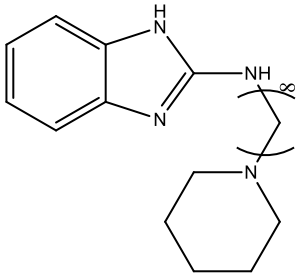


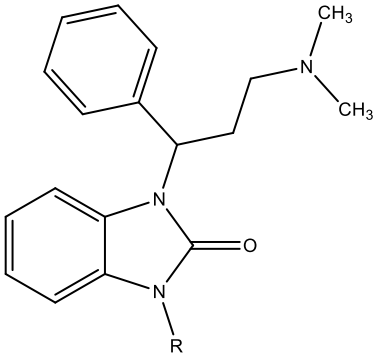
(*Z*)-2-(1-cyano-2-(4-fluorophenyl)vinyl)-1*H*-benzo[*d*]imidazole-6-carboxylic acid

Psychoactive agents:

The H_3 receptors in CNS are associated with central disorders such as impaired cognitive functions. A series of H_3-antagonists composed of an imidazole ring

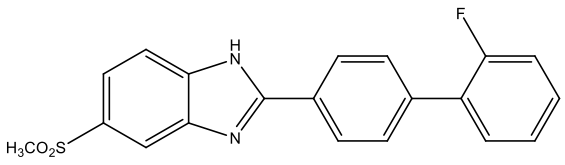
connected through an alkyl spacer to a 2-aminobenzimidazole moiety was designed and synthesized. Its QSAR and quantitative structure property relationship (QSPR) analysis suggested a three carbon atoms chain length optimum for the antagonistic activity.



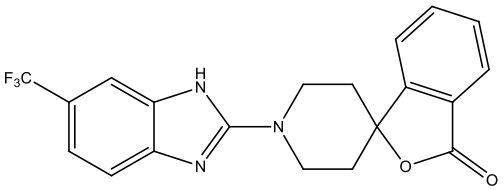


Lipid modulating activity:

Lipids play an important role in pathophysiology of many metabolic diseases like diabetes, dyslipidemia, CVS related disorders, cancer, etc., the various targets that modulate lipid levels, HMG-CoA reductase is the most widely explored target and many drugs inhibiting this enzyme are clinically available. In addition to this, many new targets are identifies which are being exploited for development of novel drugs for modulation of lipid levels.



2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-5-(methylsulfonyl)-1*H*-benzo[*d*]imidazole

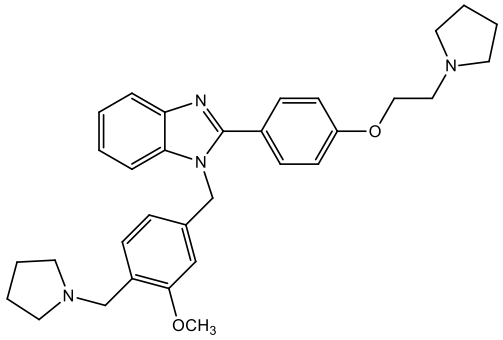


1'-(6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)-3*H*-spiro[isobenzofuran-1,4'-piperidin]-3-one

Anticoagulants:

Thrombin causes proteolytic cleavage of fibrinogen, induces platelet activation and triggers a wide range of effects secondary to thrombosis, for example, vascular smooth muscle cell and fibroblast proliferation, monocyte chemotaxis, and neutrophil

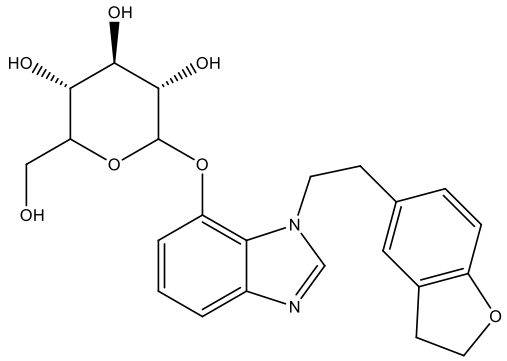
adhesion. Inhibition of thrombin is an important mechanism for inhibition of coagulation. Benzimidazole nucleus act as an appropriate template to place the varied substitutents required for interaction with thrombin.



1-(3-methoxy-4-(pyrrolidin-1-ylmethyl)benzyl)-2-(4-(2-(pyrrolidin-1-yl) ethoxy)phenyl)-1*H*-benzo[*d*]imidazole

Antidiabetic agents:

Diabetic mellitus is a metabolic disorder that is characterized by insulin resistance and relative insulin deficiency. Non-insulin dependent diabetes mellitus (NIDDM) is the most prevalent type. The primary goal of treatment of NIDDM is controlling the levels of blood glucose. The sodium-glucose co transporters (SGLTs) in the proximal tubules are responsible for glucose reabsorption in the intestine (SLGT1) and kidney (SLGT2) and hence, provide a novel target for treatment of NIDDM through inhibition of renal glucose reabsorption[7].



(3*S*,4*R*,5*R*)-2-((1-(2-(2,3-dihydrobenzofuran-5-yl)ethyl)-1*H*-benzo[*d*]imidazol-7-yl)oxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol

SYNTHESIS OF BENZIMIDAZOLE: [8]

Synthesis of Benzimidazole can be possible from various starting material viz.

- *O*-phenylene diamine
- *O*-nitroarylamines and *O*-dinitroarenes
- *O*-substituted-N-benzylidene aniline
- Amidine
- Using green chemistry
- Miscellaneous

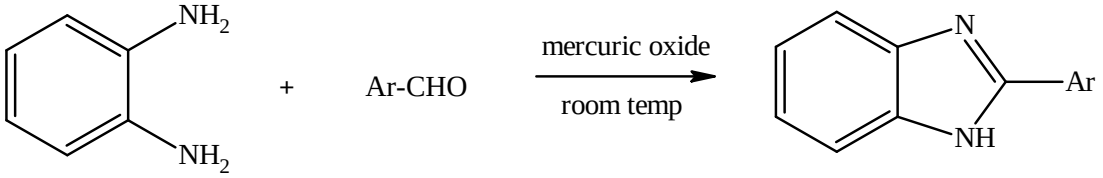
From *O*-phenylene diamine:

Benzimidazole can be synthesized by the reaction of *O*-phenylene diamine with

- Carboxylic acids and its derivatives
- Various substitute aldehydes
- Ketones
- Urea
- Aminoethers
- Lactones

Reaction with substituted aldehydes:

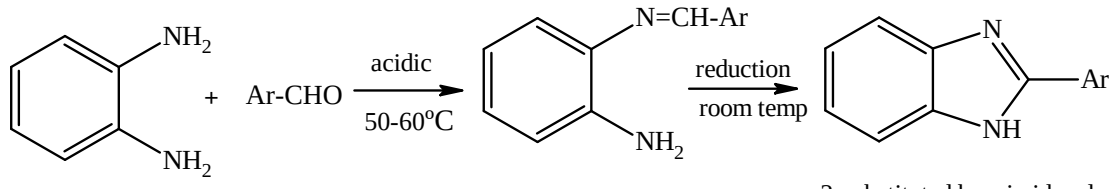
The reaction between *O*-phenylenediamine and aryl aldehydes in the presence of the oxidising agents like- cupric acetate, mercuric oxide, chlorine, lead tetra acetate, manganese dioxide, Nickel peroxide at room temperature. This synthetic method is ecofriendly and gives good yield of about 85%.



O-phenylenediamine

2-substituted benzimidazole

When *O*-phenylenediamine is reacted with aromatic aldehydes in the presence of acidic medium at 50ºC to 65ºC, it yields an intermediate 2- (benzylideneamino) aniline which is converted into 2-substituted benzimidazole by treating with reducing agents which gives 78% yield.

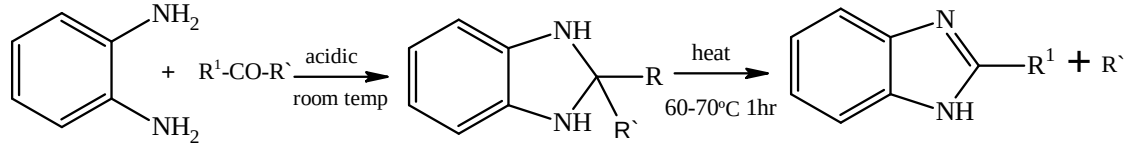


O-phenylenediamine

2-substituted benzimidazole

Reaction with ketones:

O-phenylenediamine reacted with the substituted ketones in the presence of acidic medium at room temperature gives a 2, 2-disubstituted-benzimidazoles, which on heating 60 to 70ºC for 1 h, breaks down into 2-substituted benzimidazole and a hydrocarbon.

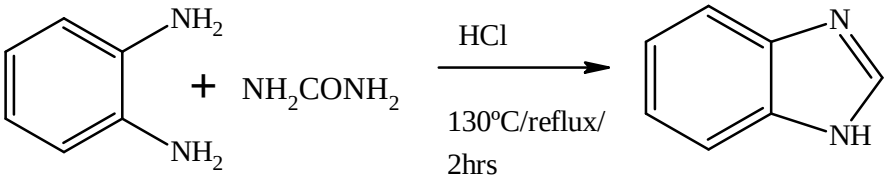


O-phenylenediamine

2-substituted benzimidazole

Reaction with urea:

O-phenylenediamine reacts with urea in the presence of hydrochloric acid at 130ºC for 2 h gives a 78% yield of benzimidazole.

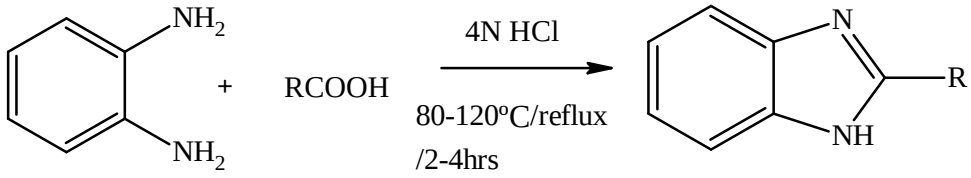


O-phenylenediamine

1H benzimidazole

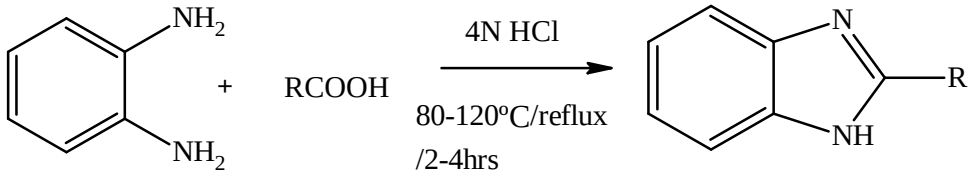
Reaction with various acids and its derivatives:

O-phenylenediamine was refluxed with formic acid under the acidic conditions (4N HCl) at 120ºC for 2 to 4 h to give 75% yield of benzimidazole.



O-phenylenediamine 2 subtituted benzimidazole

O-phenylenediamine was refluxed with aliphatic acid in the presence of 4N-HCl at 80-120ºC temperature for 2 to 4 h. This method yields 80 to 90% of 2-substituted benzimidazole.



O-phenylenediamine 2 subtituted benzimidazole

Till now, benzimidazole nucleus was synthesized from many monobasic acids and some of the dibasic acids like succinic, gutaric, pimelic, adipic, azelaic, maleic, fumaric, 2,5 furan dicarboxylic acid etc., They have not synthesized from phthalic acid.

Here, benzimidazole was synthesized from condensation of *O*-phenylenediamine with phthalic acid [9]

ANALGEIC ACTIVITY: [10, 11]

The term pain, derived from the Latin ***poena*** for punishment, reflects the deleterious effects that can be inflicted upon the body. Pain has been described by the International Association for the Study of Pain as an "***unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage***." The ability to sense noxious stimuli associated with injury or threatened injury is therefore a protective response against harm. Sensory receptors for pain (nociceptors) are found in all tissues of the body. A variety of noxious stimuli (thermal, chemical, mechanical or electrical) cause them to respond leading to pain. The chemical mediators which initiate nociception (e.g. bradykinin, serotonin, acetylcholine, histamine, H+, K+), and sensitise nociceptors (e.g. prostaglandins, leukotrienes, substance P) are numerous.

The nature of pain is highly subjective. Pain has both sensory (somatic) and psychological (affective) components. One person may feel pain in response to noxious stimuli, while another person may disregard the stimuli. The affective (psychological) aspects of pain play a critical role in pain perception.

Pain can be described as either acute or chronic. Acute pain, which does not outlast the initiating painful stimulus, has three generally encountered origins. The most common type of acute pain is of superficial origin from wounds, chemical irritants, and thermal stimuli, such as burns. Acute pain of deep somatic origin usually arises from injection of chemical irritants or from ischemia, such as with myocardial infarction. Acute pain of visceral origin is most often associated with inflammation. Chronic pain, by contrast, outlasts the initiating stimulus, which in many cases is of unknown origin. Chronic pain is often associated with diseases such as cancer and arthritis.

The transmission of pain impulses occurs via series of afferent neurons:

First order neurons

Second order neurons

Third order neurons

First-order neurons are of two types*:*

Aδ – rapidly conducting (12–30 m/s), small diameter myelinated fibres. These respond to pinprick and sudden onset of heat and are responsible for rapid pain sensation and reflex withdrawal.

C fibres – slow (0.5–2 m/s), non-myelinated fibres. These respond to heat, mechanical and chemical stimuli and are responsible for slow pain sensation and immobilisation of the affected area.

Second-order neurons:

Branches from both Aδ and C fibres synapse with cells in the dorsal horns of the spinal cord. A network of cells in this area, which includes the substantia gelatinosa, integrates these inputs to form second-order neurons. Second-order neurons travel to the thalamus in the ascending spinothalamic pathways on the contralateral side. Transmission of pain impulses may be inhibited by interneuron's within the substantia gelatinosa as well as from higher centres. This is known as the 'gate theory' of pain.

***Third-order neurones*:**

Conveys pain impulses to the somatosensory cortex.

The mechanism of action of traditional NSAIDs involves blockade of the production of prostaglandins by inhibition of the enzyme cyclooxygenase (COX) at the site of injury in the periphery, thus decreasing the formation of mediators of pain in the peripheral nervous system.

Pain is not simply a harmless and inevitable consequence of surgery and trauma but can result in a number of adverse consequences. Inadequate administration of relief to a patient in distress should therefore be avoided. Fears that the use of opioids to treat severe pain will result in masking of a diagnosis, or in drug addiction, are outdated and incorrect.

ANTI-INFLAMMATORY ACTIVITY: [12]

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent, followed by removal of the necrosed cells and tissues.

Inflammation begins when a stimulus, such as infection, physical stress, or chemical stress, produces cellular damage. This damage initiates the activation of transcription factors that control the expression of many inflammatory mediators. *Among the more important inflammatory mediators are the eicosanoids, biological oxidants, cytokines, adhesion factors, and digestive enzymes* (proteases, hyaluronidase, collagenase, and elastase).

The agents causing inflammation may be as under:

Infective agents like bacteria, viruses and their toxins, fungi, parasites.

Immunological agents like cell-mediated and antigen-antibody reactions.

Physical agents like heat, cold, radiation, mechanical trauma.

Chemical agents like organic and inorganic poisons.

Inert materials such as foreign bodies.

Signs of inflammation:

The Roman writer Celsus in 1st century A.D. named the famous 4 *cardinal signs of inflammation* as:

Rumor (redness)

Tumor (swelling)

Color (heat) and

Dolor (pain)

To these, fifth sign *functio laesa* (loss of function) was later added by Virchow. The word inflammation means burning. This nomenclature had its origin in old times but now we know that burning is only one of the signs of inflammation.

Types of inflammation:

Depending upon the defense capacity of the host and duration of response, inflammation can be classified as acute and chronic.

A. ***Acute inflammation*** is of short duration (lasting less than 2 weeks) and represents the early body reaction, resolves quickly and is usually followed by healing.

The main features of acute inflammation are:

Accumulation of fluid and plasma at the affected site;

Intravascular activation of platelets; and

Polymorpho nuclear neutrophils as inflammatory cells.

Sometimes, the acute inflammatory response may be quite severe and is termed as *fulminant acute inflammation.*

B. ***Chronic inflammation*** is of longer duration and occurs either after the causative agent of acute inflammation persists for a long time, or the stimulus is such that it induces chronic inflammation from the beginning. A variant, *chronic active inflammation* is the type of chronic inflammation in which during the course of disease there are acute exacerbations of activity.

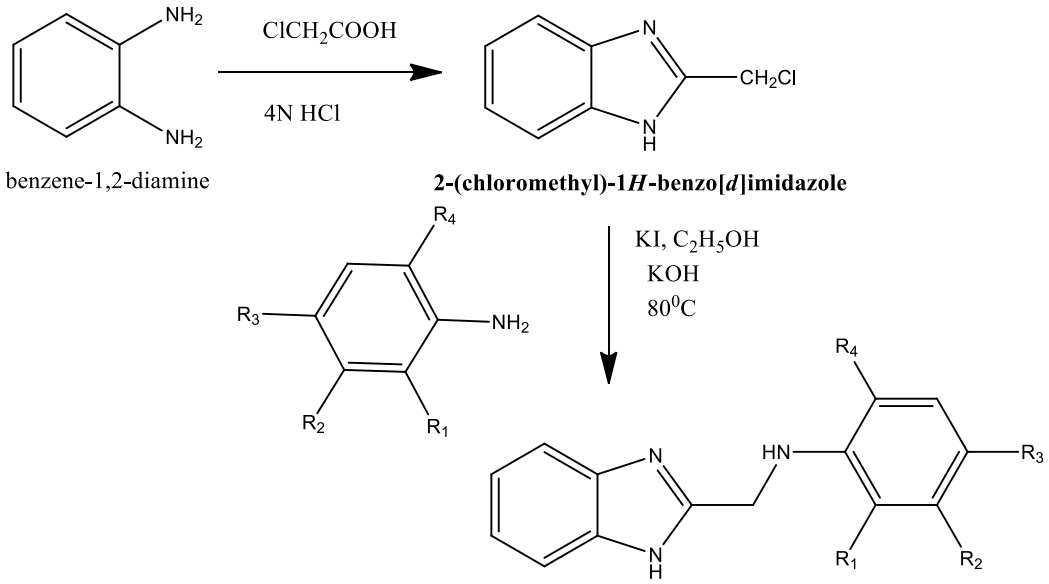
The characteristic feature of chronic inflammation is presence of chronic inflammatory cells such as lymphocytes, plasma cells and macrophages, granulation tissue formation, and in specific situations as granulomatous inflammation.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Srivastava *et al.,* studied the analgesic screening of benzimidazole nucleus having a derivatives of 2-phenylhydrazinomethyl and 2-(2-hydroxyphenyl) groups. In these series of derivatives suggested that 2-phenylhydrazinomethyl benzimidazole has a biologically active pharmacophore.[13]

Mariappan *et al.*, synthesized a 2-substituted benzimidazole using *O*-phenylenediamine and chloro acetic acid formed a derivatives of 2 chloromethyl benzimidazole with substituted primary aromatic amines.[14]

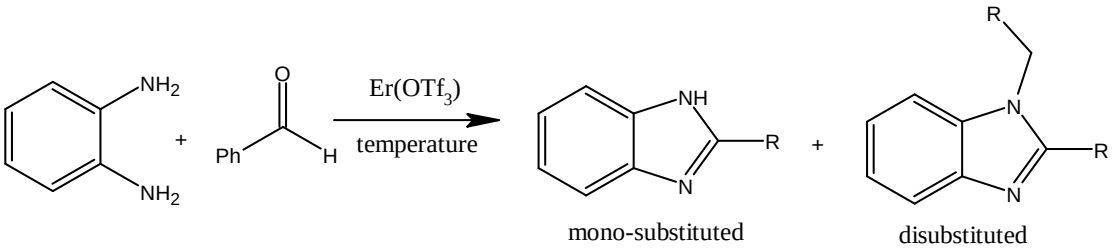


Ueda *et al.,* developed a convenient method for the synthesis of certain poly (benzimidazoles) of high molecular weights. These polymers were prepared readily by direct polycondensation of activated dicarboxylic acids with 3, 3'-diaminobenzidine tetrahydrochloride using phosphorus pentoxide/methanesulfonic acid (PPMA) as condensing agent and solvent.[15]

Nurul H. Ansari, Bjorn C.G. Soderberg Synthesized a N-alkoxy-2H-benzimidazoles by the Treatment of 2-nitro-N-(2-methyl-1-propen-1-yl)benzenamines with potassiumtert-butoxide in tert-butanol followed by the addition of n electrophile.[16]

Herrera Cano *et al.,* Synthesized a benzimidazole derivatives from *O*-phenylenediamine using different aldehydes reported. Double-condensation products were

selectively obtained when $Er(OTf)_3$ was used as the catalyst in the presence of electron-rich aldehydes. The formation of mono-condensation products was the preferred path in absence of this catalyst.[17]



Parmender Singh *et al.,* synthesized a two series of novel benzimidazole derivatives. The first one comprise of 2-methyl, the second one comprise of 2-phenyl substitution on benzimidazole moiety.[18]

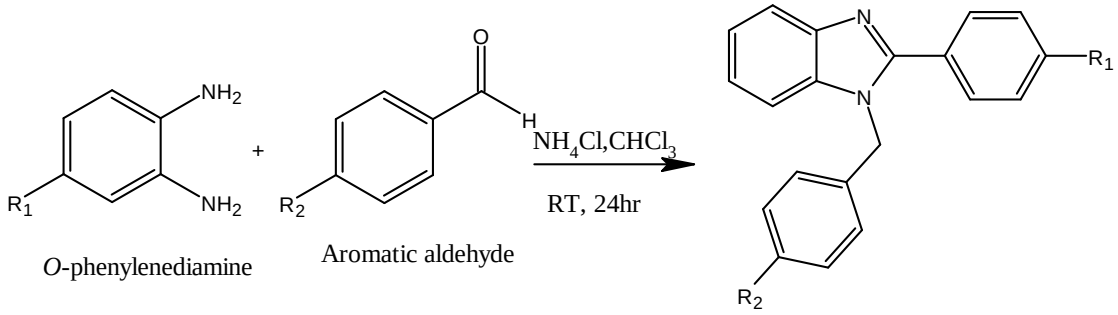
Ajani *et al.*, synthesized 2-substituted and 1, 2-disubstituted benzimidazole derivatives to investigate their antibacterial diversity for possible future drug design. The structure based design of precursors 2-(1H-benzimidazol-2-yl)aniline, 2-(3,5-dinitro phenyl)-1H benzimidazole and 2-benzyl-1H-benzimidazole were achieved by the condensation reaction of *o*-phenylenediamine with anthranilic acid, 3,5-dinitrophenylbenzoic acid, and phenylacetic acid, respectively.[19]

Hirashima *et al.,* discovered a new series of benzimidazole derivatives bearing a diarylmethyl group as inhibitors of hepatitis C virus NS5B RNA-dependent RNA polymerase (HCV NS5B RdRp). They extended the structure-activity relationship (SAR) study to analogues bearing a substituted biphenyl group and succeeded in a significant advancement of activity.[20]

M. Shaharyar *et al.,* used *O*-phenylenediamine and phenoxyacetic acid as a starting material through series of steps 2-[2-(phenoxymethyl)-1H-benzimidazol-1-yl]acetohydrazide 5 was Obtained. Various derivatives of 2-[2-(phenoxymethyl)-1H-benzimidazol-1-yl]-N -[(Z)-phenylmethylidene]acetohydrazide and some compounds containing oxadiazole bearing benzimidazole were synthesized by using various aromatic aldehyde, cyanogens bromide and carbon disulfide/potassium hydroxide.[21]

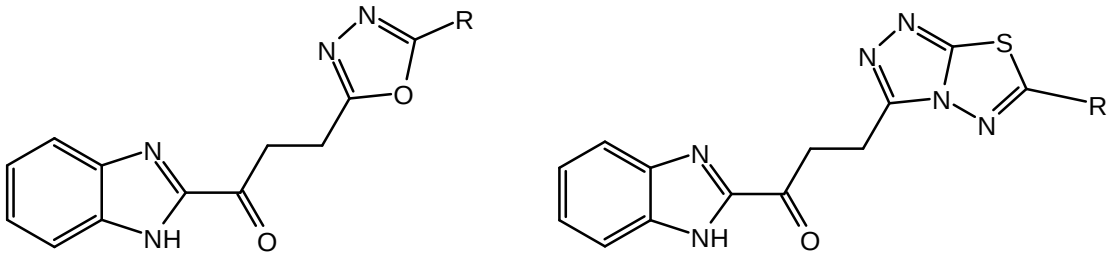
Y. M. Shaker *et al.*, Synthesized a novel series of 5-nitro-1H-benzimidazole derivatives substituted at position 1 by heterocyclic rings and Cytotoxicity and antiviral activity of the new compounds were tested.[22]

Saha *et al.*, Synthesized disubstituted benzimidazoles through condensation of *O*-phenylenediamine compounds with aromatic aldehydes in the presence of ammonium salt as a catalyst.[23]



O-phenylenediamine Aromatic aldehyde Disubstituted benzimidazole

A. Husain *et al.*, Synthesized a Two new series of benzimidazole bearing oxadiazole[1-(1H-benzo[d]imidazol-2-yl)-3-(5-substituted- 1,3,4-oxadiazol-2-yl)propan-1-ones (4a–l)] and triazolo-thiadiazoles[1-(1H-benzo[d]imidazol-2-yl)-3- (6-(substituted)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)propan-1-one] from 4-(1H-benzo[d]imidazol-2-yl)-4-oxobutanehydrazide (3) with an aim to produce promising anticancer agents.[24]

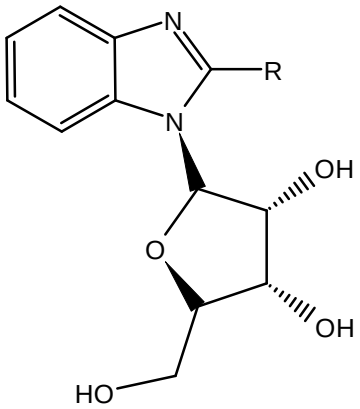


Salahuddin *et al.*, used o-phenylenediamine and naphtene-1-acetic acid/2-naphthoxyacetic acid as a starting material through a series of steps and 2-(naphthalen-1-ylmethyl/Naphthalen-2-yloxymethyl)-1H-benzimidazol-1-yl]acetohydrazide were obtained.In the first series 1,3,4-oxadiazole derivatives have been synthesized from Schiff base of the corresponding hydrazide i.e. 2-[2-(naphthalen-1-ylmethyl)-1H-benzimidazol-1-yl]acetohydrazide by using Chloramin-T. In the second series 1, 3,4-oxadiazole has been

synthesized from 2-{2-[(naphthalen-2-yloxy)- methyl]-1Hbenzimidazol-1-yl}acetohydrazide by using phosphorous oxychloride and aromatic acid. [25]

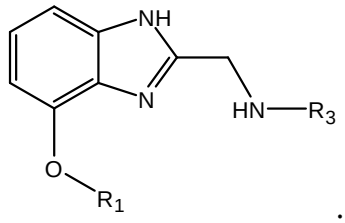
Z. Wu *et al.,* were designed, synthesized and pharmacologically evaluated a series of new 6-substituted aminocarbonyl benzimidazole derivatives with 1, 4-disubsituted or 1, 5-disubsituted indole moiety and benzoic acid moiety.[26]

V.S. Shinde *et al.,* synthesized an efficient route for the benzimidazole nucleosides 1–8 from readily available D-glucose via 3, 5- dihydroxy-1, 2-O-isopropylidene-α-D-ribofuranose and 3-azido-3-deoxy-1, 2-O-isopropylidene-α-D-xylofuranose intermediates has been adopted. Ribofuranosyl nucleosides 1–4 with different benzimidazole bases, and 3′deoxy-3′-azido-ribofuranosyl nucleosides 5–8, as another series, were obtained. All these newly synthesized analogs were evaluated for anticancer activity using MDA-MB-231 cell line. [27]



Manforte *et al.,* were synthesized and evaluated a series of novel N1-aryl-2-arylthioacetamido-benzimidazoles as inhibitors of human immunodeficiency virus type-1 (HIV-1).[28]

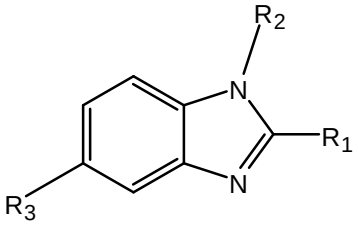
P.R. Boggu *et al.,* were synthesized a series of novel hydroxyethylaminomethyl benzimidazole analogs and evaluated for their IL-5 inhibitory activity using pro-B Y16 cell line.[29]



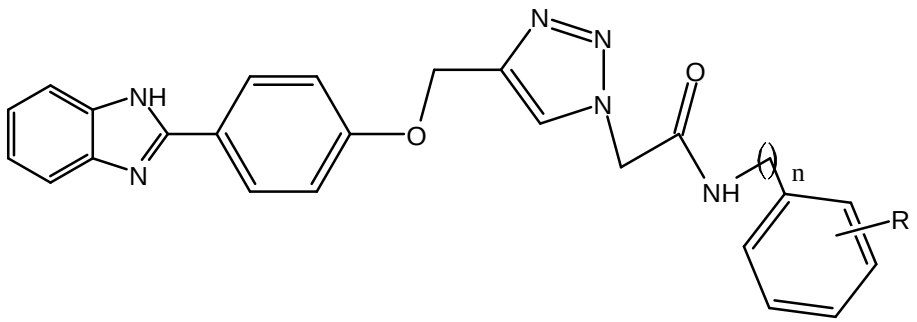
Vashist *et al.,* Developed a series of benzimidazole derivatives using 2 amino benzimidazole with substituted aromatic aldehydes and its chemical scaffolds were authenticated by NMR, IR, elemental analyses and physicochemical properties. The synthesized compounds were screened for their antimicrobial and antiproliferative activities.[30]

Q. Zhou, P. Yang et al., synthesized A new Cu(II) complex of $CuL(ClO_4)_2$ characterized by elemental analyses, UV–Vis, FT-IR, cyclic voltammogram and X-ray single crystal diffraction. [31]

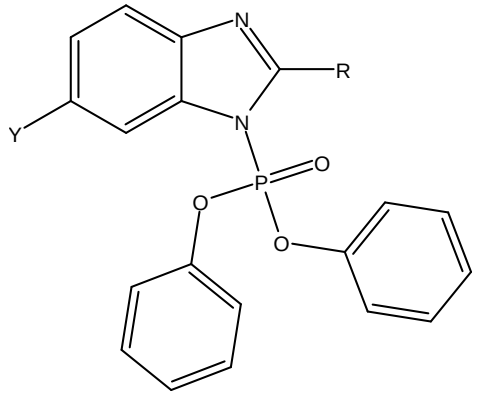
Thakurdesai *et al.,* synthesized some benzimidazole-2-carboxylic acid derivatives they were tested for acute anti-inflammatory activity against carrageenan induced rat paw edema model. The test compounds were found to be safe upto 2000 mg/kg, p.o. doses and exhibited good anti-inflammatory activity at 100 mg/kg p.o. and higher doses. Their activity largely depends on substituents at position 5 and chain length at position 2 of benzimidazole moiety. With 1-benzyl substitution, activity was found to increase.[32]



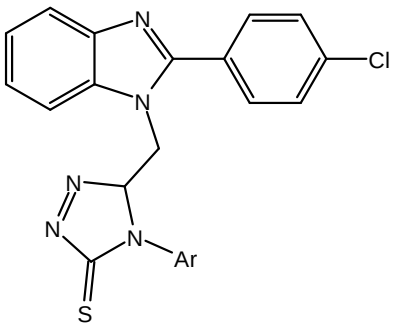
Asemanipoor N *et al.,* synthesized a series of benzimidazole-1, 2, 3-triazole hybrids as new α-glucosidase inhibitors, In vitro α-glucosidase inhibition activity results indicated that all the synthesized compounds exhibited more inhibitory activity in comparison to standard drug acarbose. The docking study was performed in order to evaluation of interaction modes of the synthesized compounds in the active site of α-glucosidase and to explain structure-activity relationships of the most potent compounds and their corresponding analogs. [33]



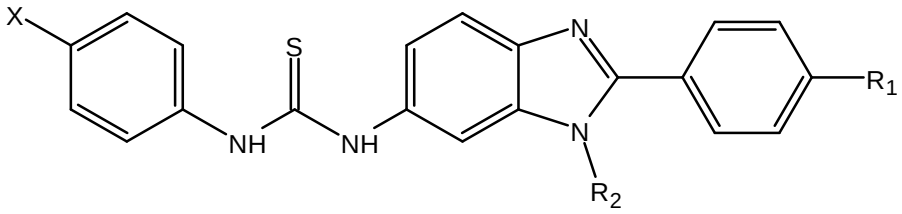
Yılmaz U *et al.,* synthesized a Novel heterocyclic phosphoramidates by a nucleophilic substitution reaction of 2-substituted benzimidazoles and diphenyl chlorophosphate ($ClPO_3Ph_2$) in the presence of potassium hydroxide (KOH) under an argon atmosphere. [34]



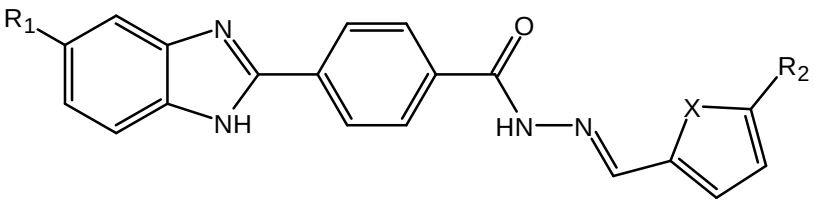
Ayhan-kilcigil G et al synthesized Some novel benzimidazole derivatives carrying thiosemicarbazide and triazole moieties at the N1 position were synthesized and their in vitro effects on rat liver microsomal NADPH-dependent lipid peroxidation (LP) levels determined by measuring the formation of 2-thiobarbituric acid reactive substance. The free radical scavenging properties of the compounds were also examined in vitro by determining the capacity to scavenge superoxide anion formation and the interaction with the stable free radical 2, 2-diphenyl-1 picrylhydrazyl (DPPH). [35]



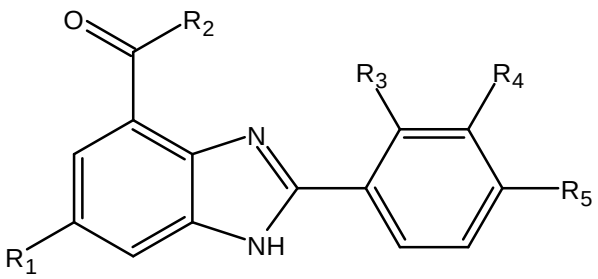
G. Ayhan kilcigil, N. Altanlar *synthesized* Some benzimidazole derivatives and tested there in vitro antifungal activities against *Candida albicans, Candida glabrata* and *Candida krusei*.[36]



Ulviye Acar Çevik *et al.*, synthesized benzimidazole-hydrazone derivatives by aiming at the identification of new chemical entities as potent anticancer agents.[37]



Bukhari, Lauro, Jantan *et al.*, studied the anti-inflammatory activities of a new series of benzimidazole derivatives by investigating their inhibition of secretory phospholipase A_2, lipoxygenase, COXs and lipopolysaccharides-induced secretion of TNF-α and IL-6 in mouse RAW264.7 macrophages.[38]



K.C.S. Achar *et al.,* synthesised a series of 2-methylaminobenzimidazole derivatives by the reaction of 2-(chloromethyl)-1H-benzimidazole derivatives with primary aromatic amines. The newly synthesized compounds were screened for analgesic and anti-inflammatory activities on acetic acid induced writhing in mice and carrageenan induced paw edema in rats. In these Compounds, 2 compound showed a potent analgesic (89% at 100 mg/kg b.w) and anti-inflammatory (100% at 100 mg/kg b.w) activities compared with standard drug Nimesulide (100% at 50 mg/kg b.w) respectively.[39]

AIM AND OBJECTIVE

AIM AND OBJECTIVE

To design a better medicinal agents, the relative contribution that each functional group (i.e., pharmacophore) makes to the overall physicochemical properties of the molecule must be evaluated. Studies of this type involves modification of the molecule in a systematic fashion followed by a determination of how these changes affect biologic activity.

Benzimidazoles are regarded as a promising class of bioactive compounds exhibits a large range of biological activities.

Benzimidazole ring is an important pharmacophore in modern drug discovery. It is extensively used in the clinics for preventing and treating various types of diseases with low toxicity, high bioavailability and good biocompatibility & curative effects. Benzimidazole moiety has been emerged as a pharmacophore of choice for this designing analgesic and anti-inflammatory agents. Pain and inflammation has been recognized as an overwhelming burden to the healthcare system.

Synthesis of benzimidazole has received an increasing attention in the development of new drug molecules. It paved the way for the development of novelty in the synthesis of benzimidazole derivatives having an analgesic and anti-inflammatory activity. Here, new method was developed for the synthesis of benzimidazole moieties which are expected to give the potent analgesic and anti-inflammatory action.

MATERIALS AND METHODS

MATERIALS AND METHODS

MATERIALS

Chemicals used:

- *O*-Phenylenediamine
- Phthalic acid
- Hydrazine hydrate
- Phenylhydrazine
- 2, 4 dinitro phenylhydrazine
- Dimethylamine
- Diethylamine
- 4N HCl
- Methanol
- Ethanol
- Acetone
- Benzene
- Ethylacetate
- Hexane
- Chloroform
- Water
- DMSO.

METHODS

For synthesis, characterization and pharmacological screening:

SYNTHESIS

Step 1: 2-substituted benzimidazole (compound-1) from the Condensation of *O*-phenylenediamine and Phthalic acid.

Step 2: Substitution of hydrazine containing compounds (hydrazine hydrate, phenyl hhydrazine, 2,4 dinitro phenylhydrazine) at 2nd position of 2-substituted benzimidazole from step 1.

Step 3: Substitution of secondary amines (dimethylamine & diethylamine) at 1st position of synthesized benzimidazole derivatives from step 2 through mannich reaction.

CHARACTERIZATION

Solubility

Thin Layer Chromatography

IR Spectroscopy

NMR Spectroscopy

Mass Spectroscopy

PHARMACOLOGICAL SCREENING

Analgesic activity:

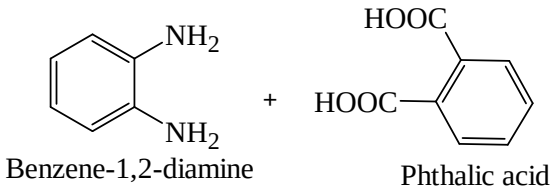
- Tail immersion method (*centrally acting*)
- Acetic acid induced writhing method (*peripherally acting*)

Anti-inflammatory activity:

- Carrageenan induced paw oedema method (*acute inflammation*)

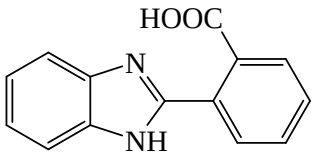
SCHEME

SCHEME



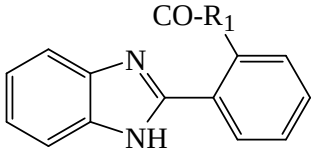
Benzene-1,2-diamine + Phthalic acid

STEP-1 4N HCl, $-2H_2O$



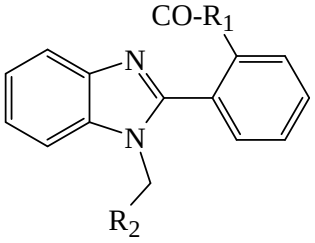
(Compound-1)

STEP-2 R_1 + CH_3OH, $-CH_3OH$



(Compound-1a,1b,1c)

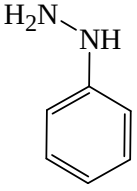
STEP-3 R_2 + HCHO + C_2H_5OH



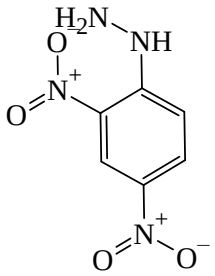
(Compound-$1a_1$-$1a_2$,$1b_1$-$1b_2$,$1c_1$-$1c_2$)

R_1=

NH_2-$NH_2.H_2O$
Hydrazine hydrate
(Compound-1a)

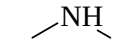


Phenyl hydrazine
(Compound-1b)

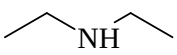


2,4-dinitro phenyl hydrazine
(Compound-1c)

R_2=

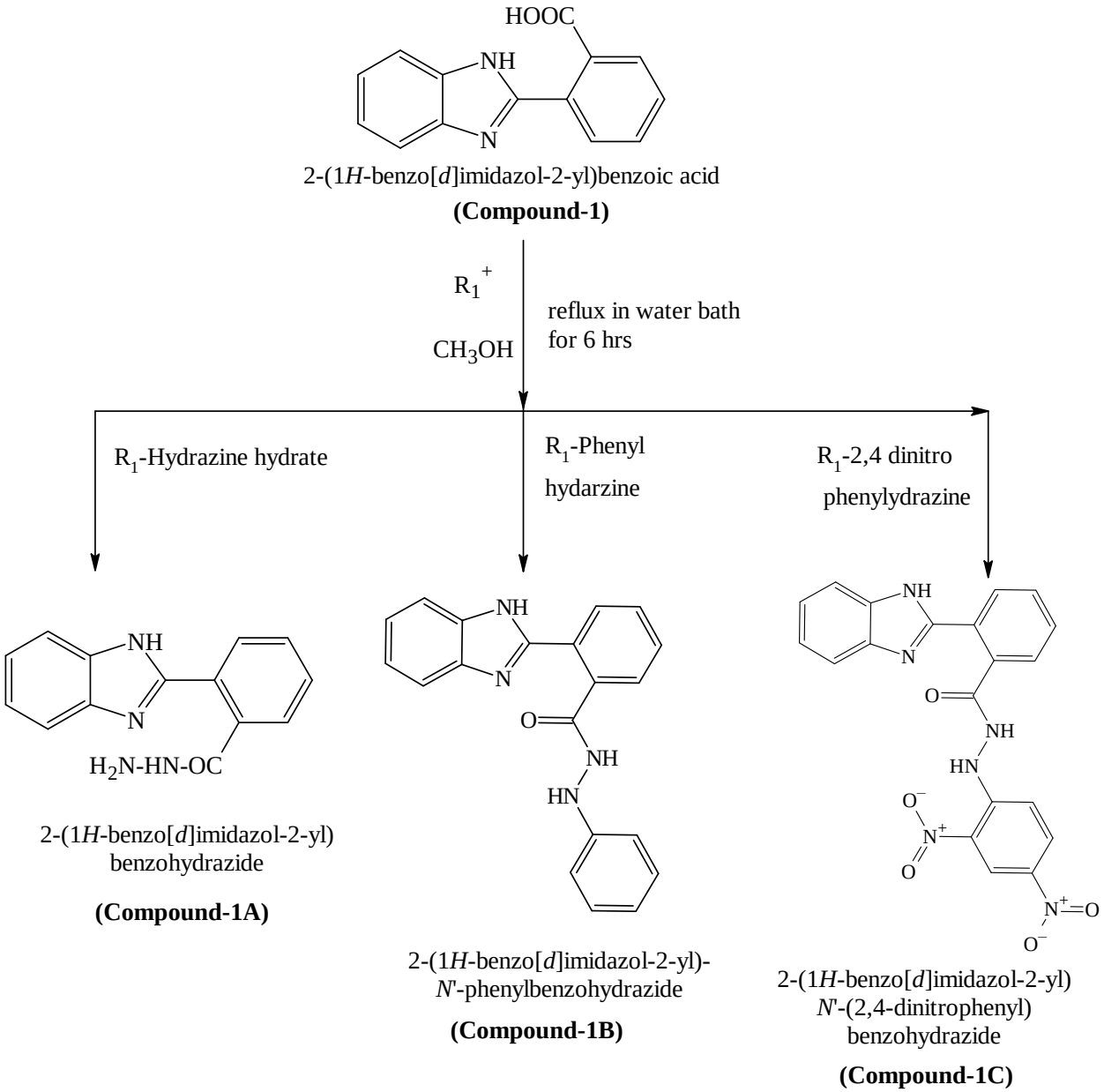


Dimethyl amine
(Compound-$1a_1$,$1b_1$,$1c_1$)

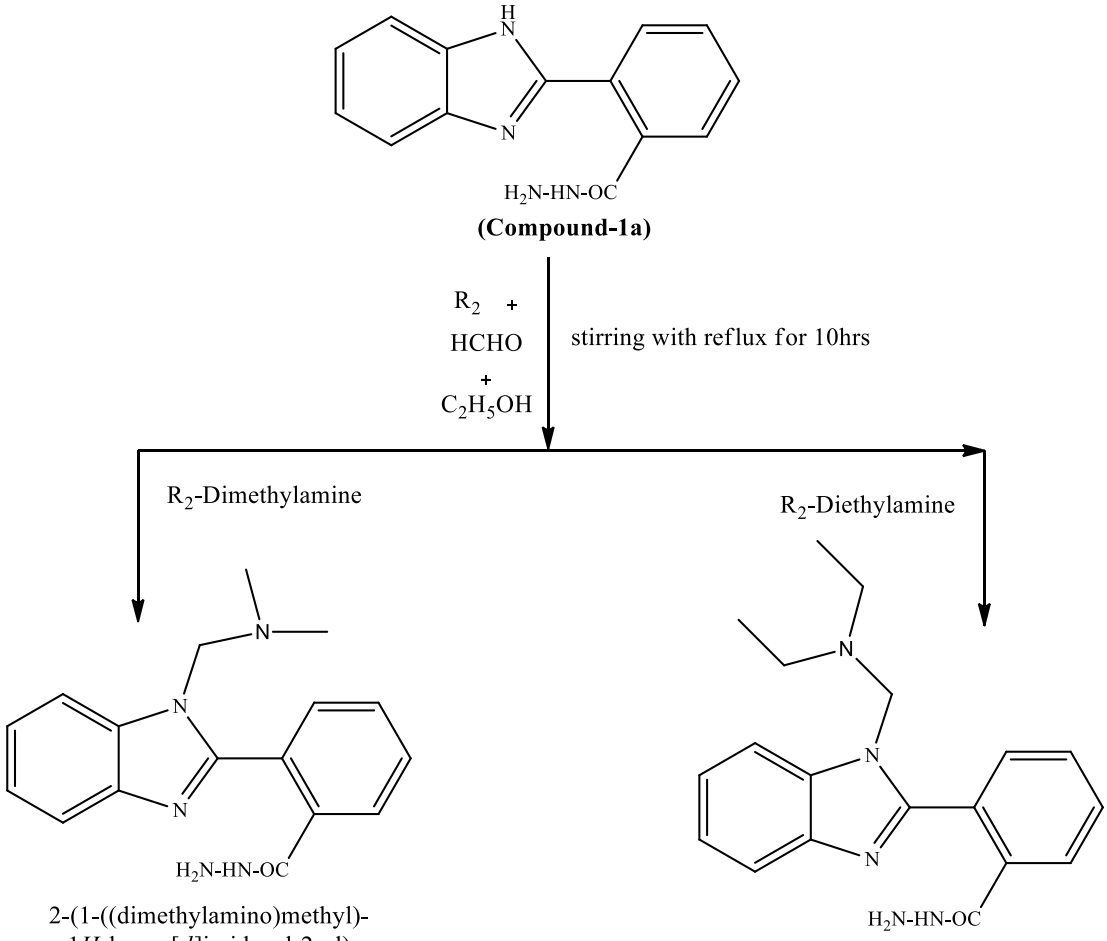


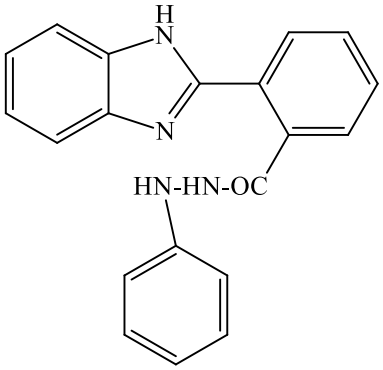
Diethyl amine
(Compound-$1a_2$,$1b_2$,$1c_2$)

STEP 2:


HOOC
NH
N
2-(1*H*-benzo[*d*]imidazol-2-yl)benzoic acid
(Compound-1)
R_1^+
CH_3OH
reflux in water bath
for 6 hrs
R_1-Hydrazine hydrate
R_1-Phenyl
hydarzine
R_1-2,4 dinitro
phenylydrazine
H_2N-HN-OC
2-(1*H*-benzo[*d*]imidazol-2-yl)
benzohydrazide
(Compound-1A)
O
NH
HN
2-(1*H*-benzo[*d*]imidazol-2-yl)-
N'-phenylbenzohydrazide
(Compound-1B)
O^-
N^+
$N^+=O$
2-(1*H*-benzo[*d*]imidazol-2-yl)
N'-(2,4-dinitrophenyl)
benzohydrazide
(Compound-1C)

STEP 3:


H
N
N
H2N-HN-OC
(Compound-1a)
R2 +
HCHO
+
C2H5OH
stirring with reflux for 10hrs
R2-Dimethylamine
R2-Diethylamine
N
N
N
H2N-HN-OC
2-(1-((dimethylamino)methyl)-1H-benzo[d]imidazol-2-yl) benzohydrazide
(Compound-1a1)
N
N
N
H2N-HN-OC
2-(1-((diethylamino)methyl)-1H-benzo[d]imidazol-2-yl) benzohydrazide
(Compound-1a2)



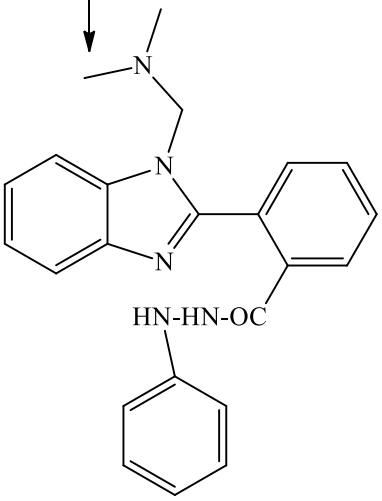
(Compound-1b)

R_2 +
HCHO
+
C_2H_5OH

stirring with reflux for 10hrs

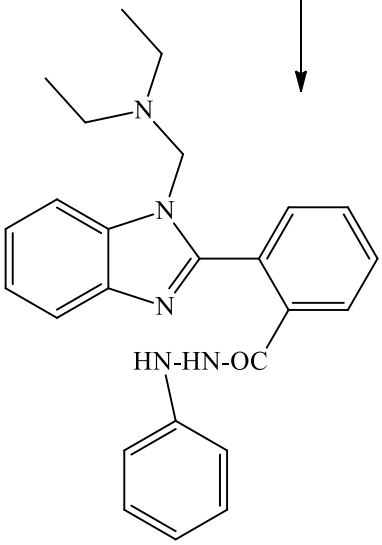
R_2-Dimethylamine

R_2-Diethylamine



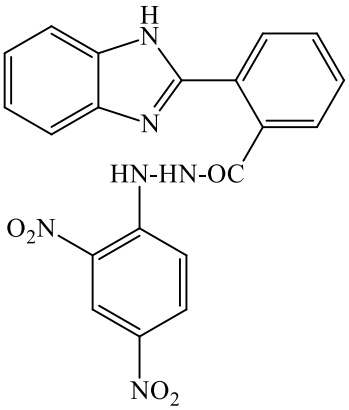
2-(1-((dimethylamino)methyl)-
1*H*-benzo[*d*]imidazol-2-yl)-
N'-phenylbenzohydrazide

(Compound-1b$_1$)



2-(1-((diethylamino)methyl)-
1*H*-benzo[*d*]imidazol-2-yl)-
N'-phenylbenzohydrazide

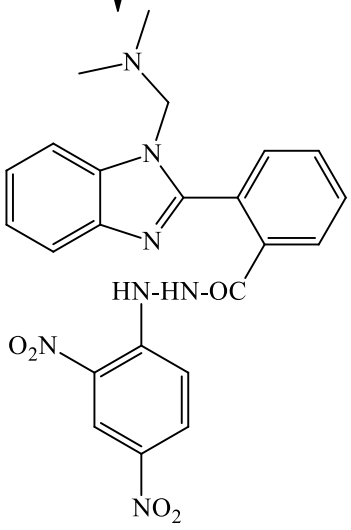
(Compound-1b$_2$)



(Compound-1c)

R_2 + HCHO + C_2H_5OH — stirring with reflux for 10hrs

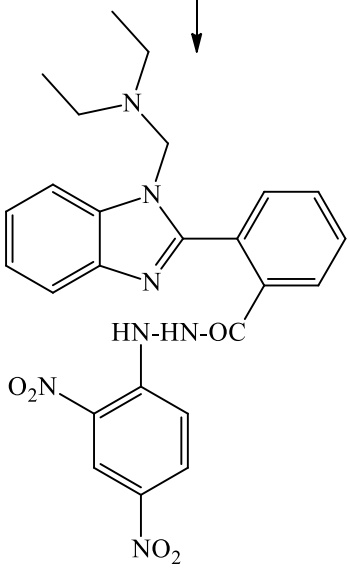
R_2-Dimethylamine



2-(1-((dimethylamino)methyl)-1*H*-benzo[*d*]imidazol-2-yl)-*N*'-(2,4-dinitrophenyl) benzohydrazide

(Compound-1c$_1$)

R_2-Diethylamine



2-(1-((diethylamino)methyl)-1*H*-benzo[*d*]imidazol-2-yl)-*N*'-(2,4-dinitrophenyl) benzohydrazide

(Compound-1c$_2$)

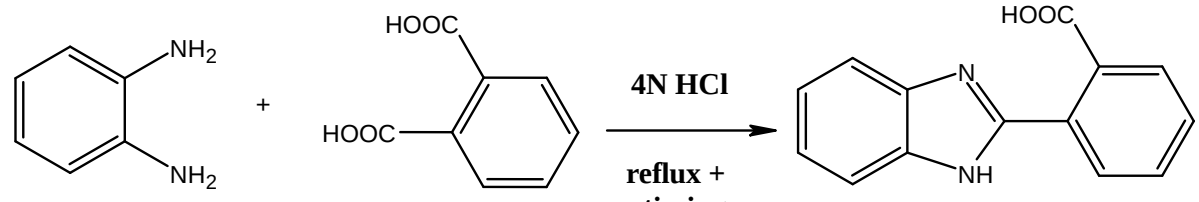
EXPERIMENTS

EXPERIMENTS

SYNTHESIS OF BENZIMIDAZOLE AND ITS DERIVATIVES:

Compound 1:2-(1H-benzimidazole-2-yl) benzoic acid:

4.20g of *O*-phenylenediamine and 3.32g of phthalic acid was weighed and refluxed with subsequent stirring in a 50ml of 4N HCl solution for 4hrs. Then the resulting solution was poured into 100ml of ice water and it was adjusted to pH 9 using ammonia solution. The resulting solid was filtered, washed, dried and recrystallized from ethanol.[40]



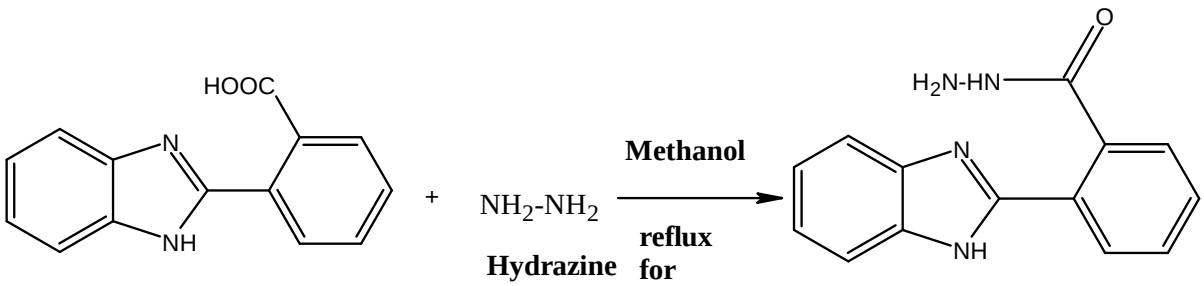
Benzene-1,2-diamine **Phthalic acid** **2-(1*H*-benzo[*d*]imidazol-2-yl) benzoic acid**

(COMPOUND-1)

SYNTHESIS OF BENZIMIDAZOLE DERIVATIVES:

Compound 1A: 2-(1H-benzimidazol-2-yl) benzoydrazide

4.76g (0.02 mole) of compound 1 (synthesized benzimidazole) and 1.052ml (0.0217 mole) of hydrazine hydrate was refluxed in methanol for 5hrs. Then the resulting mixture was poured into crushed ice with constant stirring. The solid obtained was filtered, dried and recrystallized from ethanol.

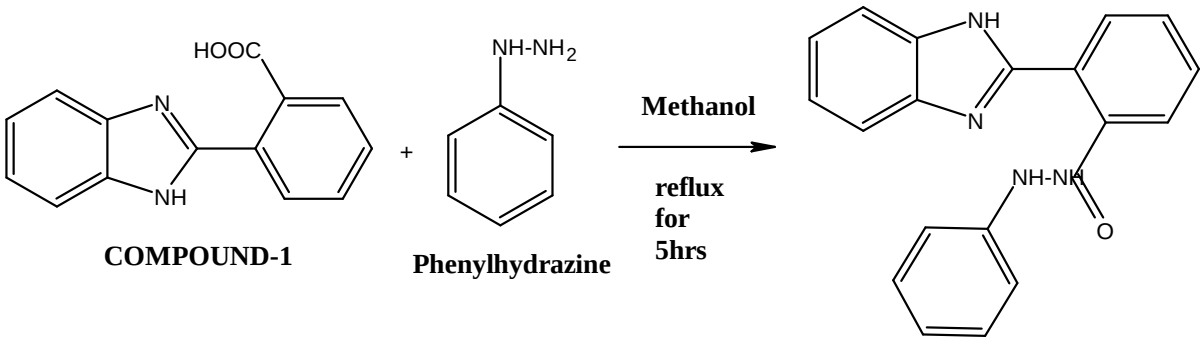


COMPOUND-1 **Hydrazine** 2-(1*H*-benzo[*d*]imidazol-2-yl)benzohydrazide

(COMPOUND-1A)

Compound 1B: 2-(1-H-benzimidazol-2-yl)-N`-phenylbenzohydrazide

4.76g (0.02 mole) of compound 1 (synthesized benzimidazole) and 2.34g (0.0217 mole) of phenylhydrazine was refluxed in methanol for 5hrs. Then the resulting mixture was poured into crushed ice with constant stirring. The solid obtained was filtered, dried and recrystallized from ethanol.



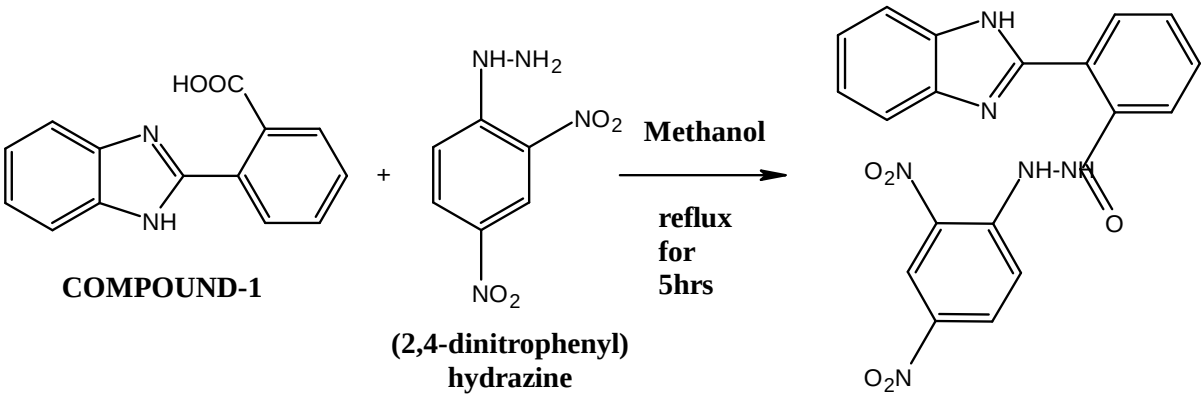
COMPOUND-1 **Phenylhydrazine**

2-(1*H*-benzo[*d*]imidazol-2-yl)-*N*'-phenylbenzohydrazide

(COMPOUND-1B)

Compound 1C: 2-(1-H-benzimidazol-2-yl)-N`-(2, 4 dinitrophenyl) benzohydrazide

4.76g (0.02 mole) of compound 1 (synthesized benzimidazole) and 4.29g (0.0217 mole) of 2, 4 dinitro phenylhydrazine was refluxed in methanol for 5hrs. Then the resulting mixture was poured into crushed ice with constant stirring. The solid obtained was filtered, dried and recrystallized from ethanol.[13]



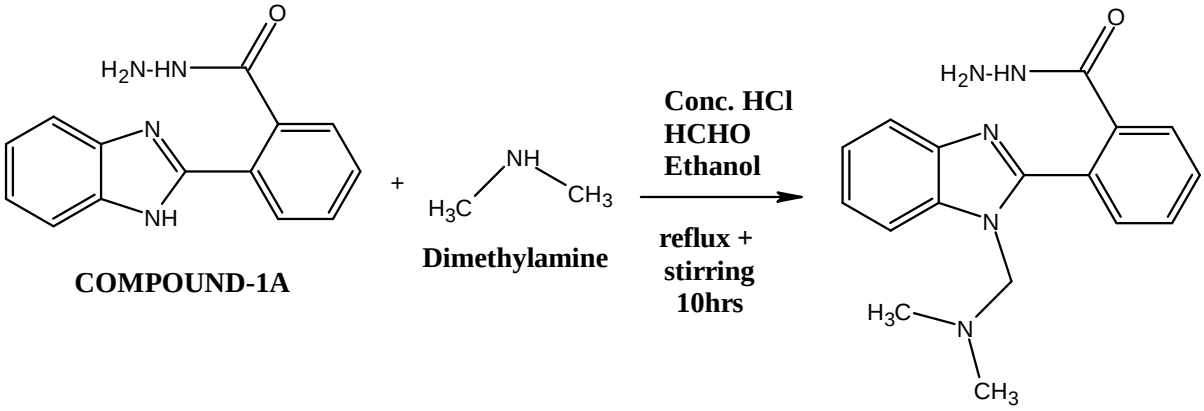
COMPOUND-1 **(2,4-dinitrophenyl) hydrazine**

2-(1*H*-benzo[*d*]imidazol-2-yl)-*N*'-(2,4-dinitrophenyl) benzohydrazide

(COMPOUND-1C)

Compound 1A$_1$: 2-(1-[(dimethylamino) methyl]-1H-benzimidazol-2-yl)benzohydrazide

0.01 mole of compound 1A was dissolved in ethanol of 15ml followed by the addition of 0.01 mole of dimethylamine and 0.015 mole of formaldehyde solution (40%w/v) was added to undergo mannich reaction. Then the reaction mixture was refluxed with continous stirring for about 6-10hrs at 70-75℃. Reaction completion was checked by TLC using a solvent system of chloroform: methanol (9:1). After completion of the reaction, the resulting mixture was poured into crushed ice and kept in a refrigerator about overnight. The precipitated product obtained was filtered, dried and recrystallized from ethanol.



COMPOUND-1A

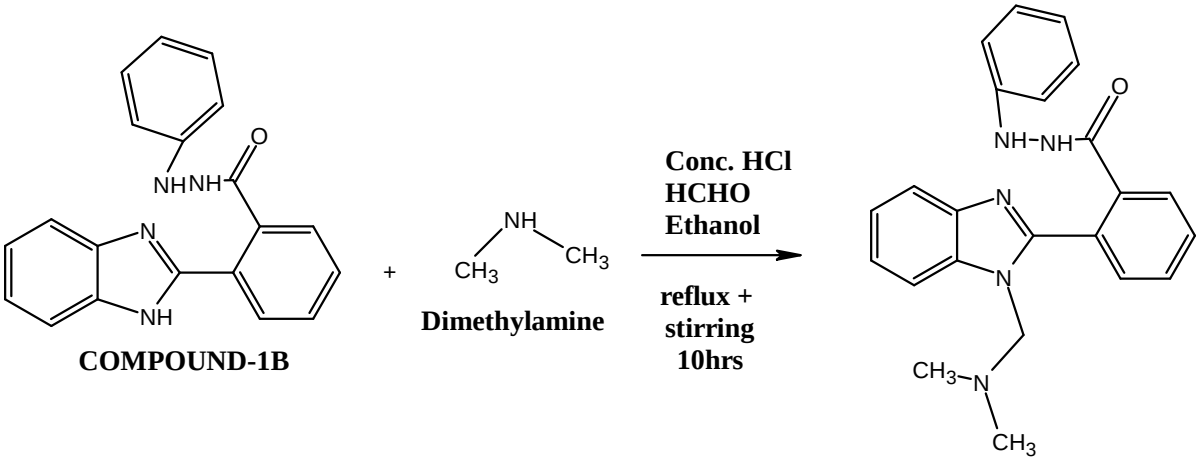
Dimethylamine

2-(1-((dimethylamino)methyl)-1*H*-benzo[*d*]imidazol-2-yl) benzohydrazide

(COMPOUND-1A$_1$)

COMPOUND 1B$_1$: 2-(1-[(dimethylamino) methyl]-1H-benzimidazol-2-yl)-N`-phenylbenzohydrazide

0.01 mole of compound 1B was dissolved in 15ml of ethanol followed by the addition of 0.01 mole of dimethylamine and 0.015 mole of formaldehyde solution (40%w/v) was added to undergo mannich reaction. Then the reaction mixture was refluxed with continous stirring for about 6-10hrs at 70-75℃. Reaction completion was checked by TLC using a solvent system of chloroform: methanol (9:1). After completion of the reaction, the resulting mixture was poured into crushed ice and kept in a refrigerator about overnight. The precipitated product obtained was filtered, dried and recrystallized from ethanol.

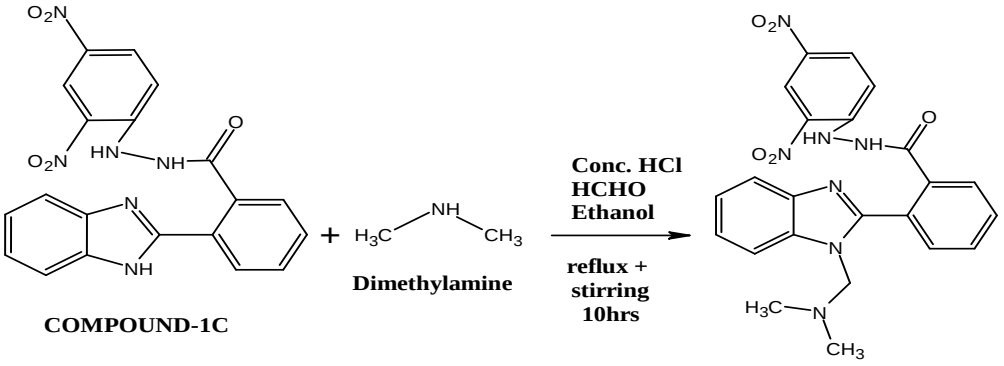


2-(1-((dimethylamino)methyl)-1*H*-benzo[*d*]imidazol-2-yl)-*N*'-phenylbenzohydrazide

(COMPOUND-1B$_1$)

Compound 1C$_1$: 2-(1-[(dimethylamino) methyl]-1H-benzimidazol-2-yl)-N`-(2, 4 dinitrophenyl) benzohydrazide

0.01 mole of compound 1C was dissolved in 15ml of ethanol followed by the addition of 0.01 mole of dimethyl amine and 0.015 mole of formaldehyde solution (40%w/v) was added to undergo mannich reaction. Then the reaction mixture was refluxed with continous stirring for about 6-10hrs at 70-75℃. Reaction completion was checked by TLC using a solvent system of chloroform: methanol (9:1). After completion of the reaction, the resulting mixture was poured into crushed ice and kept in a refrigerator about overnight. The precipitated product obtained was filtered, dried and recrystallized from ethanol.

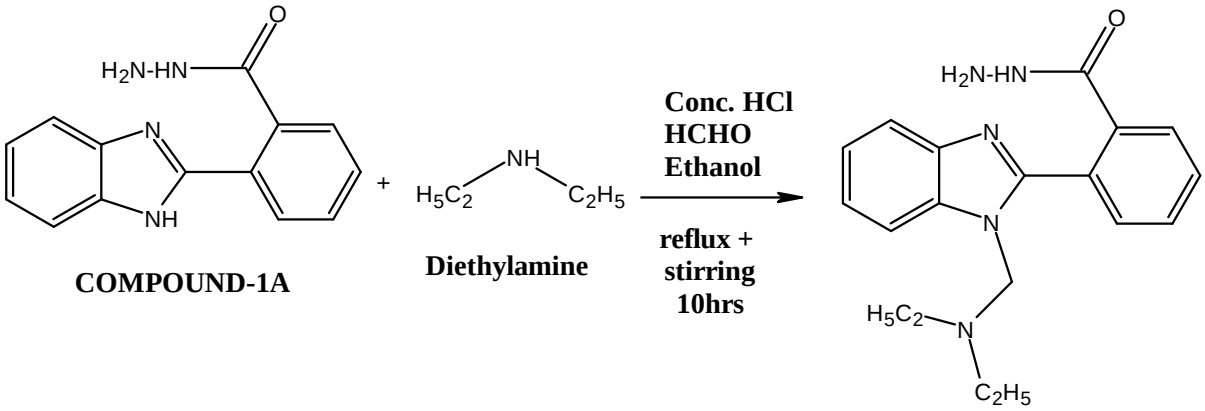


2-(1-((dimethylamino)methyl)-1*H*-benzo[*d*]imidazol-2-yl)-*N*'-(2,4-dinitrophenyl) benzohydrazide

(COMPOUND-1C$_1$)

COMPOUND 1A$_2$: 2-(1-[(diethylamino) methyl]-1H-benzimidazol-2-yl) benzohydrazide

0.01 mole of compound 1A was dissolved in 15ml of ethanol followed by the addition of 0.01 mole of diethylamine and 0.015mole of formaldehyde solution (40%w/v) was added to undergo mannich reaction. Then the reaction mixture was refluxed with continous stirring for about 6-10hrs at 70-75℃. Reaction completion was checked by TLC using a solvent system of chloroform: methanol (9:1). After completion of the reaction, the resulting mixture was poured into crushed ice and kept in a refrigerator about overnight. The precipitated product obtained was filtered, dried and recrystallized from ethanol.



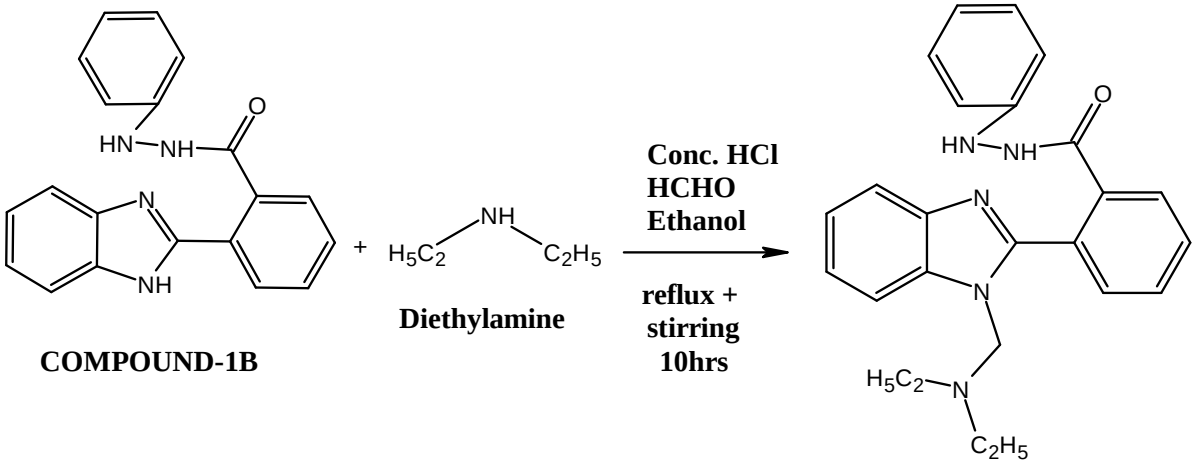
COMPOUND-1A **Diethylamine**

2-(1-((diethylamino)methyl)-1*H*-benzo[*d*]imidazol-2-yl) benzohydrazide

(COMPOUND-1A$_2$)

COMPOUND 1B$_2$: 2-(1-[(diethylamino) methyl]-1H-benzimidazol-2-yl)-N`-phenylbenzohydrazide

0.01 mole of compound 1B was dissolved in 15ml of ethanol followed by the addition of 0.01 mole of diethylamine and 0.015 mole of formaldehyde solution (40%w/v) was added to undergo mannich reaction. Then the reaction mixture was refluxed with continous stirring for about 6-10hrs at 70-75℃. Reaction completion was checked by TLC using a solvent system of chloroform: methanol (9:1). After completion of the reaction, the resulting mixture was poured into crushed ice and kept in a refrigerator about overnight. The precipitated product obtained was filtered, dried and recrystallized from ethanol.



COMPOUND-1B

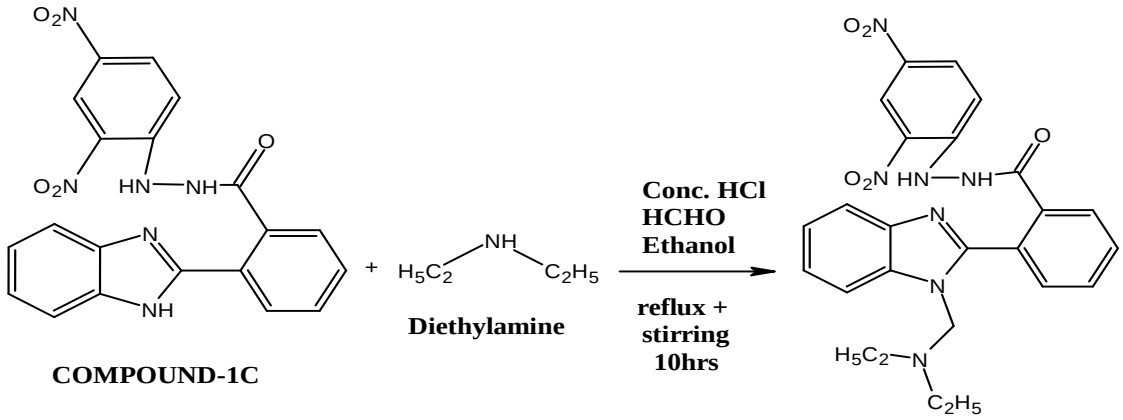
Diethylamine

2-(1-((diethylamino)methyl)-1*H*-benzo[*d*]imidazol-2-yl)-*N*'-phenylbenzohydrazide

(COMPOUND-1B$_2$)

COMPOUND 1C$_2$: 2-(1-[(diethylamino) methyl]-1H-benzimidazol-2-yl)-N`-(2,4dinitrophenyl) benzohydrazide

0.01 mole of compound 1C was dissolved in 15ml of ethanol followed by the addition of 0.01 mole of diethylamine and 0.015 mole of formaldehyde solution (40%w/v) was added to undergo mannich reaction. Then the reaction mixture was refluxed with continous stirring for about 6-10hrs at 70-75℃. Reaction completion was checked by TLC using a solvent system of chloroform: methanol (9:1). After completion of the reaction, the resulting mixture was poured into crushed ice and kept in a refrigerator about overnight. The precipitated product obtained was filtered, dried and recrystallized from ethanol.[41]



COMPOUND-1C

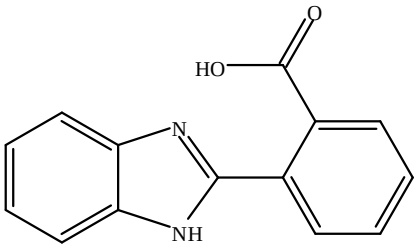
Diethylamine

2-(1-((diethylamino)methyl)-1*H*-benzo[*d*]imidazol-2-yl)-*N*'-(2,4-dinitrophenyl) benzohydrazide

(COMPOUND-1C$_2$)

SPECTRAL CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS

COMPOUND-1: 2-(1H-benzimidazole-2-yl) benzoic acid



2-(1*H*-benzimidazol-2-yl)benzoic acid

PHYSICAL CHARACTERIZATION

Molecular weight : 238.24 g/mol

Melting point : 158-165 ℃

CHEMICAL CHARACTERIZATION

Thin layer chromatography : Solvent system - Hexane: Ethyl acetate (3:7)

Visualizing agent - Iodine vapor

R_f value -0.62

UV-Vis wavelength (λ max) **:** Solvent -Methanol

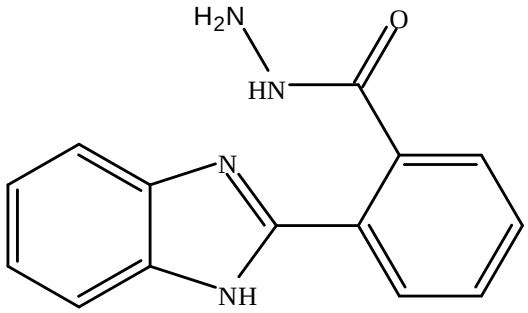
λ max -297.40 nm

IR (KBr) cm^{-1} **:** 1599.84 (Ar C=C Stretch), 3209.33, 3387.73, 3471.83(Hetero Ar N-H), 1646.13 (Ar C=O), 2917.13, 3159.18 (OH Stretch).

^{1}H NMR δ : 7.2-8.1 (Ar CH), 6.4 (Ar NH), 9.7 (-COOH).

Mass m/z value : (m+2) 240, 221,193, 167, (m+2) 156.

COMPOUND-1A: 2-(1H-benzimidazol-2-yl) benzohydrazide



2-(1*H*-benzimidazol-2-yl)benzohydrazide

PHYSICAL CHARACTERIZATION

Molecular weight : 252.27 g/mol

Melting point : 218-220 ℃

CHEMICAL CHARACTERIZATION

Thin layer chromatography : Solvent system -Benzene: Ethyl acetate (4:1)

Visualizing agent - Iodine vapour

R_f value -0.61

UV-Vis wavelength (λ max) **:** Solvent -Methanol

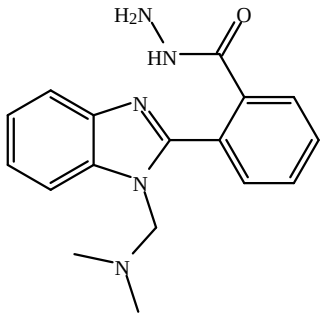
λ max - 293.00nm

IR (KBr) cm^{-1} **:** 1456.16(Ar C=C Stretch), 3375.20(Hetero Ar N-H), 1652.05 (Ar amide C=O), 3178.47(amide N-HStretch), 3461.99 (N-N Stretch).

^{1}H NMR δ : 7.2-8.0(Ar CH), 5.8 (Ar NH), 8.0(amide NH), 2.5(NH_2).

Mass m/z value **:** 252, 221, 193, 168, 153

COMPOUND-$1A_1$: 2-(1-[(dimethylamino) methyl]-1H-benzimidazol-2-yl) benzohydrazide



2-(1-((dimethylamino)methyl)-
1*H*-benzimidazol-2-yl)benzohydrazide

PHYSICAL CHARACTERIZATION

Molecular weight **:** 309.37 g/mol

Melting point **:** 149-152 ℃

CHEMICAL CHARACTERIZATION

Thin layer chromatography **:** Solvent system - chloroform: methanol (9: 1)

Visualizing agent - Iodine vapor

R_f value - 0.50

UV-Vis wavelength (λ max) **:** Solvent - Methanol

λ max -282.40nm

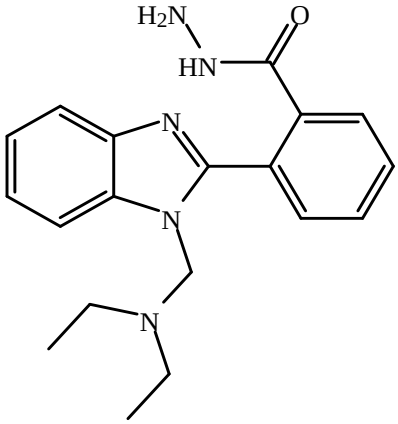
IR (KBr) cm^{-1} **:** 1507.27(Ar C=C Stretch), 3069.50(Ar C-H Stretch), 3335.66(Hetero Ar N-H), 1652.88 (Ar amide C=O), 3195.83(amide N-H Stretch), 3307.67 (N-N Stretch), 2927.74 (Ali C-H), 1250.75, 1408.98 (C-N Stretch), 1458.08(-CH_2-), 1458.0 (-CH_3).

^{1}H NMR δ : 7.2-7.9(Ar CH), 8.1 (amide NH), 5.8 (Het ArNH)

2.0 (-NH_2), 3.3(-CH_2-), 2.6(-CH_3).

Mass m/z value **:** 309, 278, 220, 192, 167, 153

COMPOUND-1A$_2$: 2-(1-((diethylamino) methyl)-1H-benzimidazol-2-yl) benzohydrazide



2-(1-((diethylamino)methyl)-
1*H*-benzimidazol-2-yl)benzohydrazide

PHYSICAL CHARACTERIZATION

Molecular weight : 337.42 g/mol

Melting point : 127-130 ℃

CHEMICAL CHARACTERIZATION

Thin layer chromatography : Solvent system - chloroform: methanol (9:1)

Visualizing agent - Iodine vapor

R_f value - 0.69

UV-Vis wavelength (λ max) : Solvent - Methanol

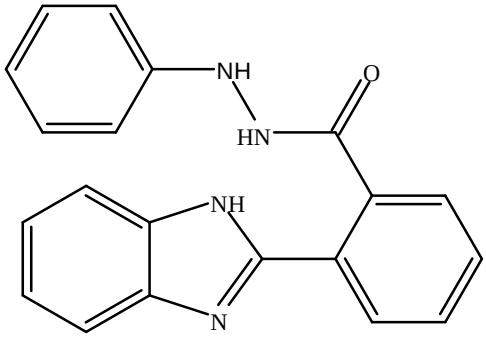
λ max -285.60nm

IR (KBr) cm^{-1} : 1650.95(Ar C=C Stretch), 3062.75(Ar C-H Stretch), 3385.80(Hetero Ar N-H), 1698.21 (Ar amide C=O), 3235.37(amide N-H Stretch), 3479.34, 3500.56(N-N Stretch), 2931.60 (Ali C-H), 1109.95(C-N Stretch), 1456.16(-CH_2-).

^{1}H NMR δ : 7.2-7.8 (Ar CH), 8.2(amide NH), 2.5 (-NH_2), 3.3(-CH_2-), 1.2(-CH_2CH_3).

Mass m/z value : 337, 306, 220, 192, 178, 153

COMPOUND-1B: 2-(1-H-benzimidazol-2-yl)-N`-phenylbenzohydrazide



2-(1*H*-benzimidazol-2-yl)-
N'-phenylbenzohydrazide

PHYSICAL CHARACTERIZATION

Molecular weight : 328.37 g/mol

Melting point **:** 229-331℃

CHEMICAL CHARACTERIZATION

Thin layer chromatography : Solvent system -Benzene: Ethyl acetate (4:1)

Visualizing agent - Iodine vapour

R_f value - 0.49

UV-Vis wavelength (λ max) **:** Solvent -Methanol

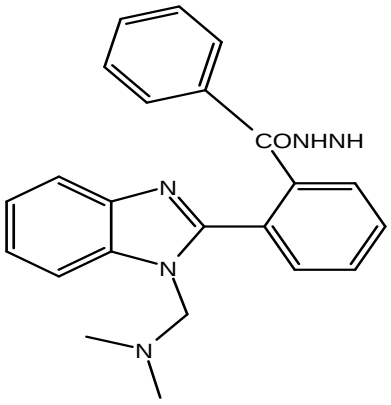
λ max -286.00nm

IR (KBr) cm^{-1} **:** 1507.27, 1576.70(Ar C=C Stretch), 3067.57 (Ar C-H Stretch), 3452.34(Hetero Ar N-H), 1653.85(Ar amide c=o), 3266.22(amide N-H Stretch), 3404.13 (N-N Stretch).

^{1}H NMR δ : 7.2-8.0(Ar CH), 6.7(Hetero Ar NH), 8.1(amide NH), 3.4(Ar NH).

Mass m/z value : 328, 276, 236, 221, 193, 167

COMPOUND-1B$_1$:2-(1-((dimethylamino) methyl)-1H-benzimidazol-2-yl)-N`-phenylbenzohydrazide



2-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)-*N*'-phenylbenzohydrazide

PHYSICAL CHARACTERIZATION

Molecular weight **:** 385.46 g/mol

Melting point **:** 199-210℃

CHEMICAL CHARACTERIZATION

Thin layer chromatography : Solvent system - chloroform: methanol (9:1)

Visualizing agent -Iodine vapour

R_f value - 0.61

UV-Vis wavelength (λ max) **:** Solvent -Methanol

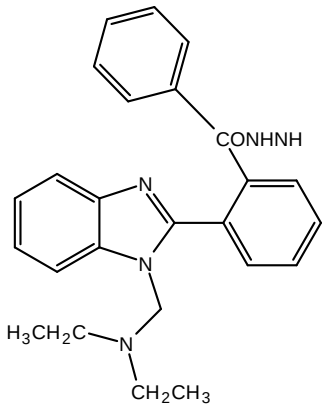
λ max -283.60nm

IR (KBr) cm^{-1} **:** 1593.09(Ar C=C Stretch), 3025.14(Ar C-H Stretch), 3176.25(Hetero Ar N-H), 1646.13(Ar amide C=O), 3157.25(amide N-H Stretch), 3419.56 (N-N Stretch), 2848.67, 2917.13 (Ali C-H), 1220.86 (C-N Stretch), 1440.73(-CH_2-), 1440.73 (-CH_3).

^{1}H NMR δ : 7.0-7.9(Ar CH), 8.0 (amide NH), 3.4(-CH_2), 2.5(-CH_3).

Mass m/z value : 385, 308, 250, 220, 192, 178, 127

COMPOUND-1B$_2$:2-(1-((diethylamino) methyl)-1H-benzimidazol-2-yl)-N`-phenylbenzohydrazide



2-(1-((diethylamino)methyl)-1*H*-benzimidazol-2-yl)-*N*'-phenylbenzohydrazide

PHYSICAL CHARACTERIZATION

Molecular weight : 413.51 g/mol

Melting point : 188-190℃

CHEMICAL CHARACTERIZATION

Thin layer chromatography : Solvent system -Chloroform: Methanol (9:1)

Visualizing agent -Iodine vapour

R_f value -0.59

UV-Vis wavelength (λ max) **:** Solvent -Methanol

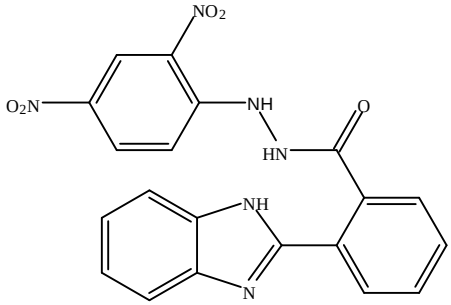
λ max -297.40nm

IR (KBr) cm^{-1} **:** 1598.88(Ar C=C Stretch), 3029.00(Ar C-H Stretch), 3194.86(Hetero Ar N-H), 1650.95(Ar amide C=O), 3305.76(amide N-H Stretch), 3399.30 (N-N Stretch), 2872.77 (Ali C-H), 1219.89(C-N Stretch), 1455.19(-CH_2-).

^{1}H NMR δ : 7.1-7.9(Ar CH), 8.1 (amide NH), 3.4(-CH_2), 1.2(-CH_2CH_3).

Mass *m/z* value : 413, 336, 250, 221, 192, 178, 127

COMPOUND-1C: 2-(1-H-benzimidazol-2-yl)-N`-(2, 4 dinitrophenyl) benzohydrazide



2-(1*H*-benzimidazol-2-yl)-
N'-(2,4-dinitrophenyl)benzohydrazide

PHYSICAL CHARACTERIZATION

Molecular weight : 418.36 g/mol

Melting point : 235-237°C

CHEMICAL CHARACTERIZATION

Thin layer chromatography **:** Solvent system - Benzene: Ethyl acetate (4:1)

Visualizing agent -Iodine vapour

R_f value - 0.53

UV-Vis wavelength (λ max) **:** Solvent -Methanol

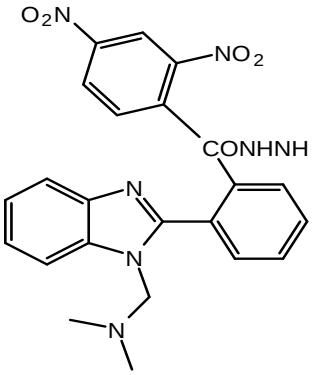
λ max -356.80nm

IR (KBr) cm^{-1} **:** 1519.80, 1574.77(Ar C=C Stretch), 3086.86 (Ar C-H Stretch), 3373.27(Hetero Ar N-H), 1646.13 (Ar amide C=O), 3196.79(amide N-H Stretch), 3326.01 (N-N Stretch), 1318.25(-NO_2).

^{1}H NMR δ : 7.2-8.8(Ar CH), 5.0 (Het Ar NH), 7.8 (amide NH), 3.4 (-NH-NH-).

Mass m/z value : 418, 327, 302, 251, 236, 221, 193, 178, 153, 127

COMPOUND-1C$_1$:2-(1-((dimethyl amino) methyl)-1H-benzimidazol-2-yl)-N`-(2, 4 dinitrophenyl) benzohydrazide



2-(1-((dimethylamino)methyl)-
1*H*-benzimidazol-2-yl)-
N'-(2,4-dinitrophenyl)benzohydrazide

PHYSICAL CHARACTERIZATION

Molecular weight : 475.46 g/mol

Melting point : 217-219℃

CHEMICAL CHARACTERIZATION

Thin layer chromatography : Solvent system - Chloroform: Methanol (9:1)

Visualizing gent -Iodine vapour

R_f value - 0.57

UV-Vis wavelength (λ max) **:** Solvent - Methanol

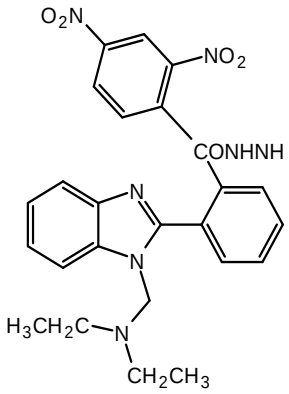
λ max -282.40nm

IR (KBr) cm^{-1} **:** 1506.30 (Ar C=C Stretch), 3055.03 (Ar C-H Stretch), 3227.93(Hetero Ar N-H), 1616.24 (Ar amide C=O), 3209.33(amide N-H Stretch), 3360.73, 3381.93 (N-N Stretch), 2848.67(Ali C-H Stretch), 1417.36 (Ali C-N Stretch), 1456.16 (-CH_2-), 1331.76(-NO_2).

^{1}H NMR δ : 7.2-9.0(Ar CH), 8.0 (amide NH), 3.3(-CH_2-), 2.1(-CH_3).

Mass m/z value : 475, 383, 325, 311, 236, 206, 178, 127

COMPOUND-1C_2:2-(1-((diethylamino) methyl)-1H-benzimidazol-2-yl)-N`-(2, 4 dinitrophenyl) benzohydrazide



2-(1-((diethylamino)methyl)-
1*H*-benzimidazol-2-yl)-*N*'-(2,4-dinitrophenyl)benzohydrazide

PHYSICAL CHARACTERIZATION

Molecular weight **:** 503.51 g/mol

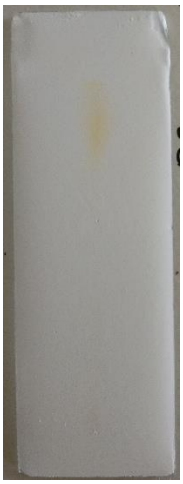
Melting point : 199-210℃

CHEMICAL CHARACTERIZATION

Thin layer chromatography : Solvent system - Chloroform: Methanol (9:1)

Visualizing agent -Iodine vapour

R_f value - 0.67



UV-Vis wavelength (λ max) **:** Solvent - Methanol

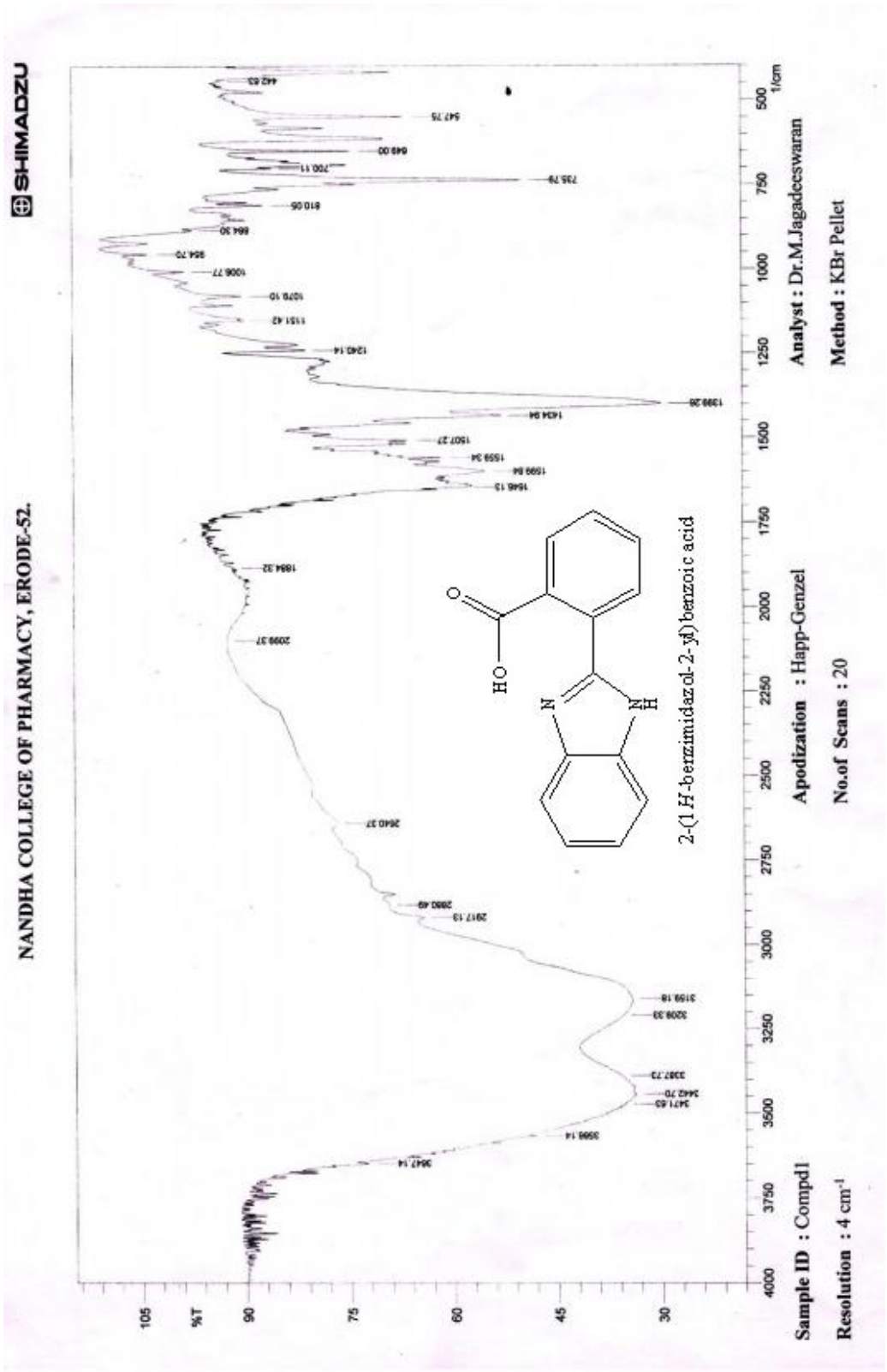
λ max -285.60nm

IR (KBr) cm^{-1} **:**1593.09 (Ar C=C Stretch), 3054.07 (Ar C-H Stretch), 3176.84(Hetero Ar N-H), 1615.27 (Ar amide C=O), 3339.54(amide N-H Stretch), 3464.84 (N-N Stretch), 2848.67(Ali C-H Stretch), 1219.89(Ali C-N Stretch), 1417.58 ($-CH_2-$), 1332.72($Ar-NO_2$).

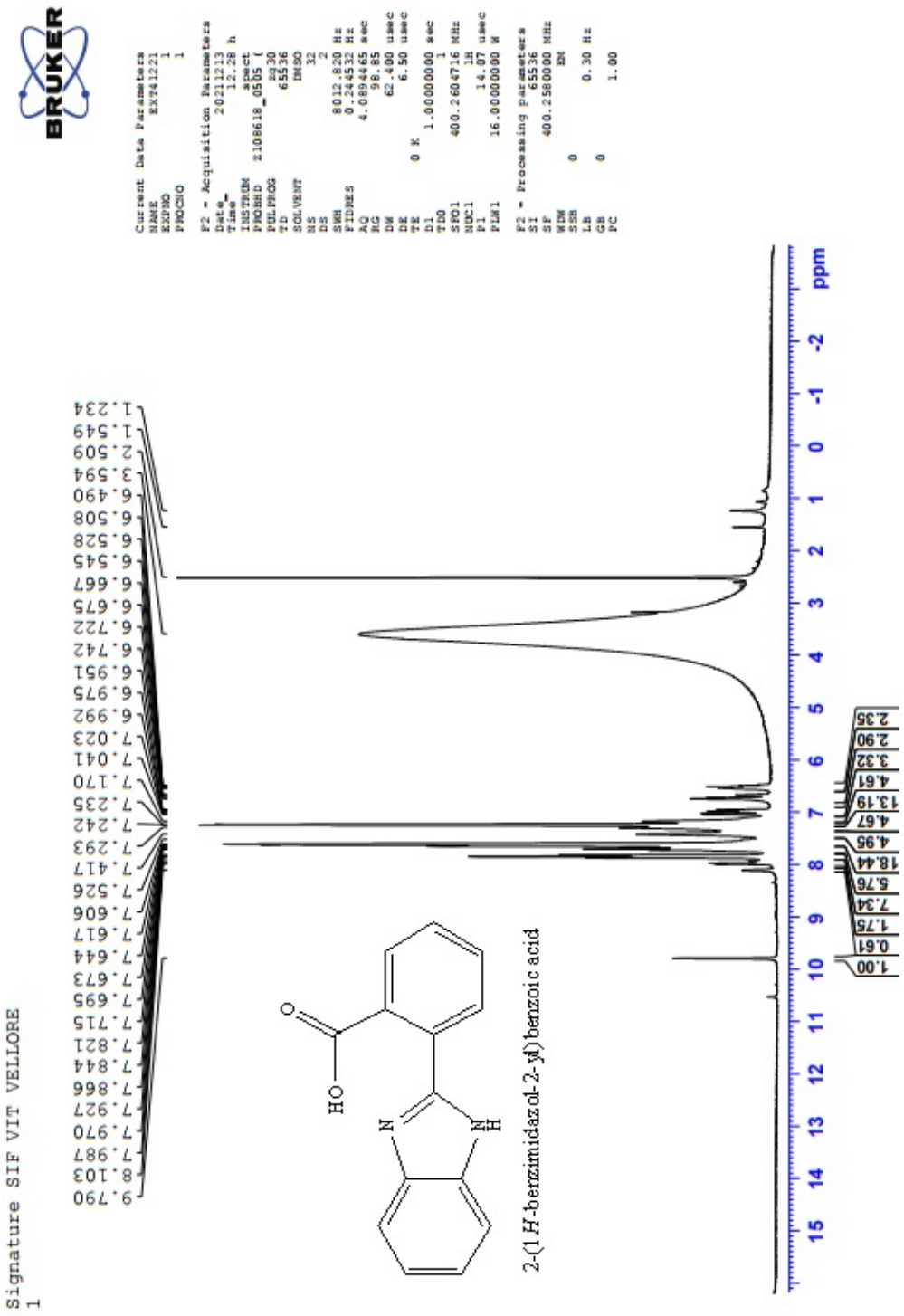
^{1}H NMR δ : 7.2-8.8(Ar CH), 8.1 (amide NH), 3.3($-CH_2-$), 2.1 ($-CH_2CH_3$).

Mass m/z value : 503, 473, 381, 325, 286, 236, 206, 178, 127

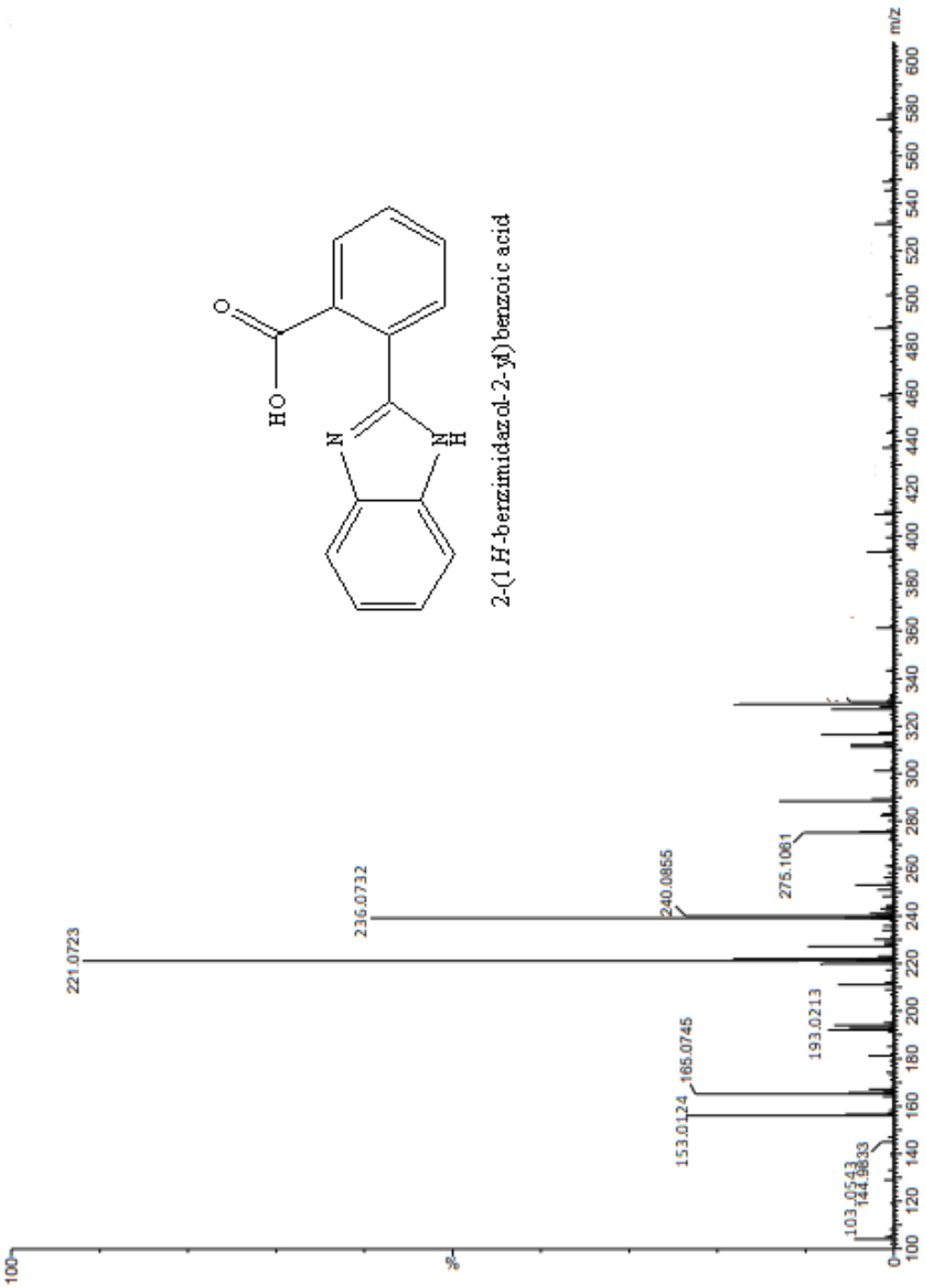
Figure 1 Spectral interpretation of synthesized compounds (1-1C$_2$) by IR, NMR and Mass



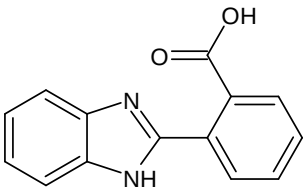
IR SPECTRUM OF COMPOUND- 1



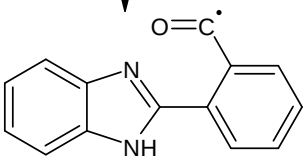
NMR SPECTRRUM OF COMPOUND-1



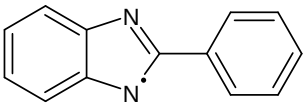
MASS SPECTRUM OF COMPOUND-1



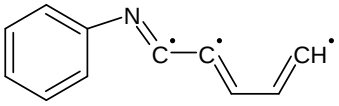
m/z Value: 238



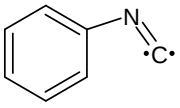
m/z Value: 221



m/z Value: 193

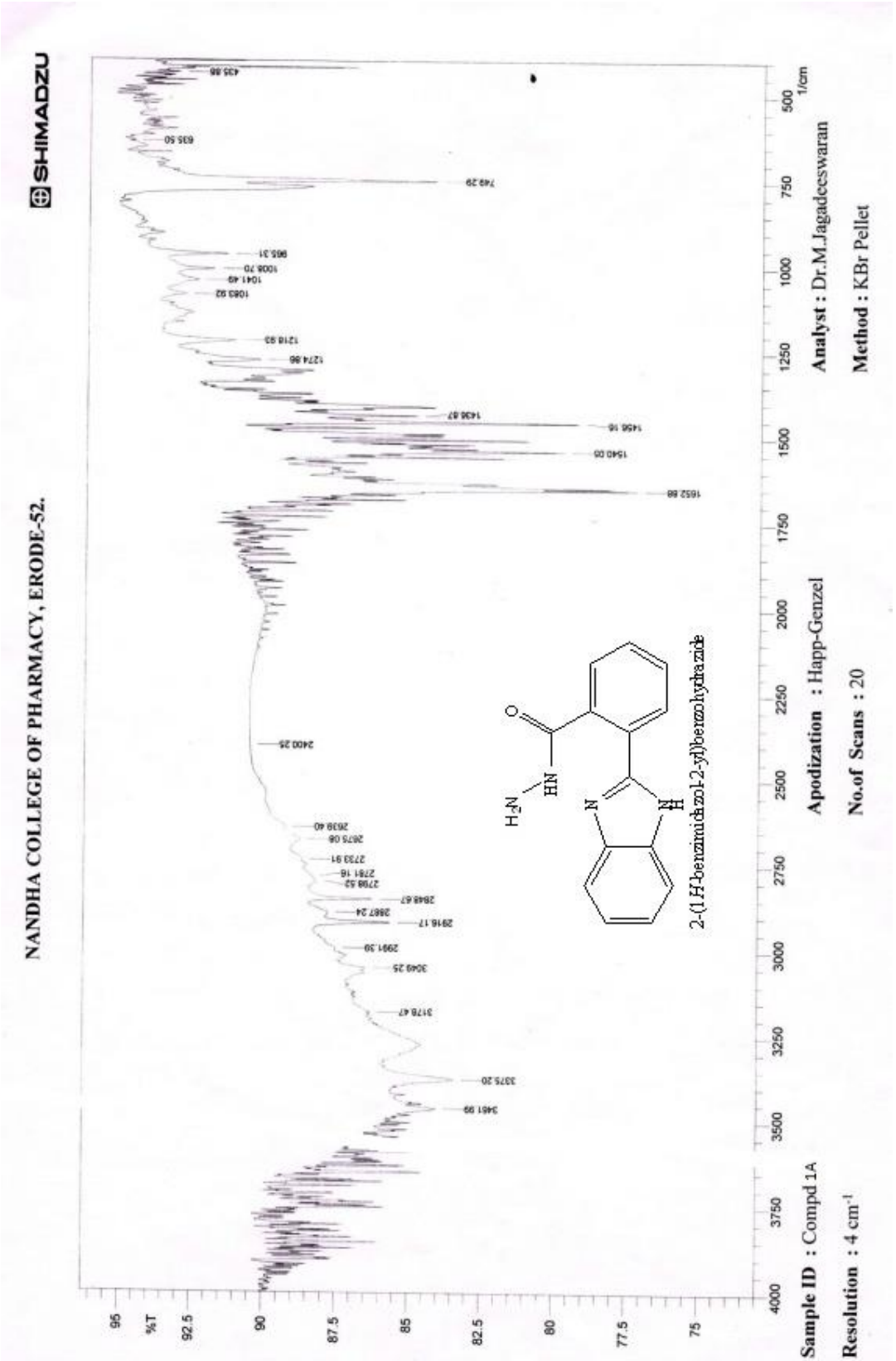


m/z Value: 154

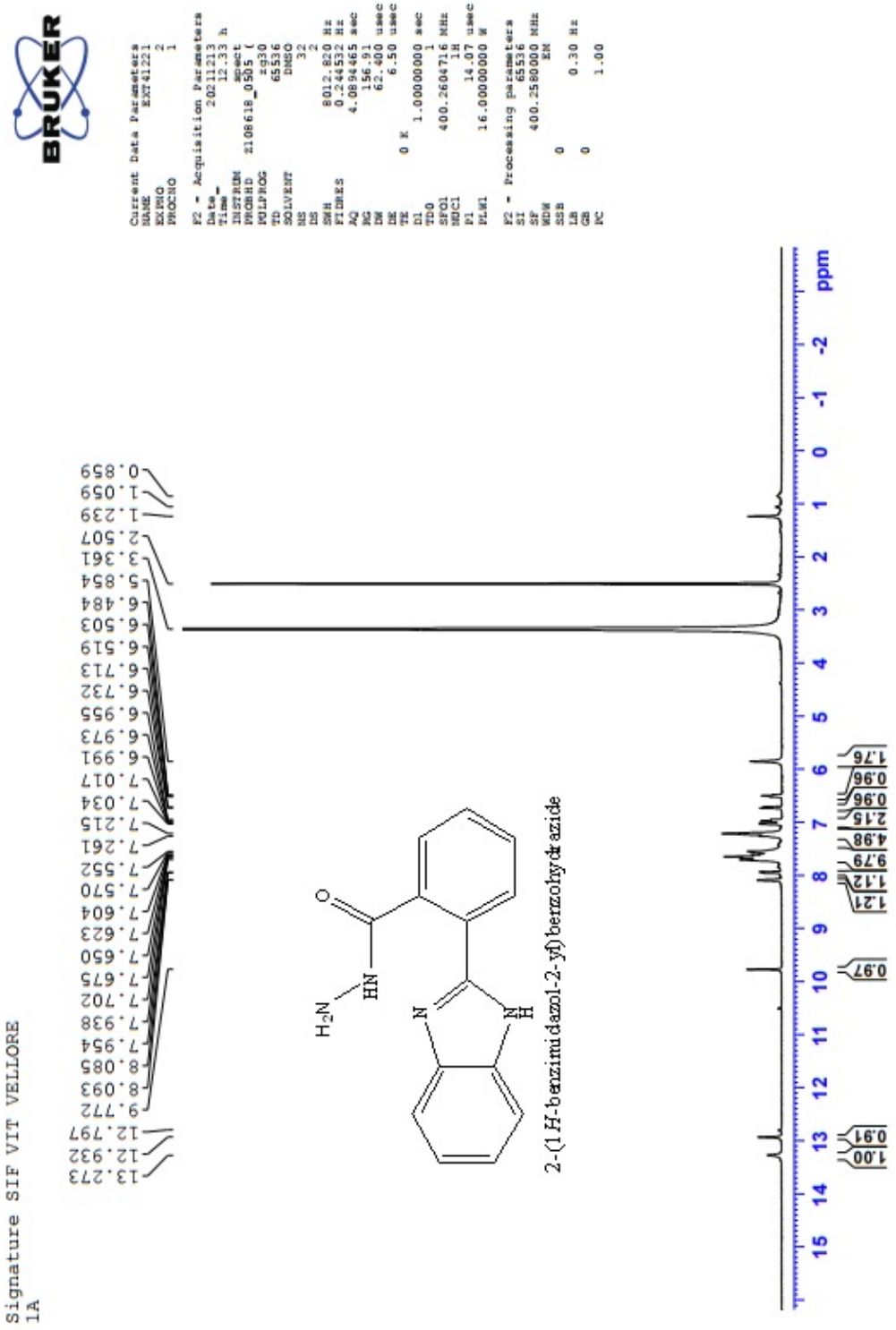


m/z Value: 103

MASS FRAGMENTATION OF COMPOUND-1

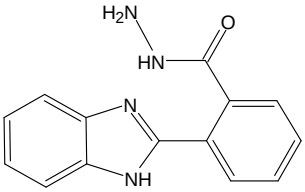


IR SPECTRUM OF COMPOUND-1A

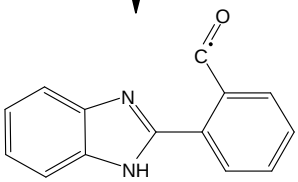


NMR SPECTRUM OF COMPOUND-1A

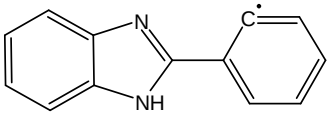
FIGURE 2.3 MASS SPECTRUM OF COMPOUND-1A



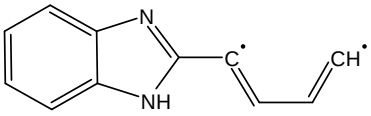
m/z value: 252



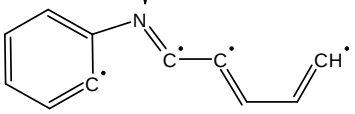
m/z value: 221



m/z value: 193

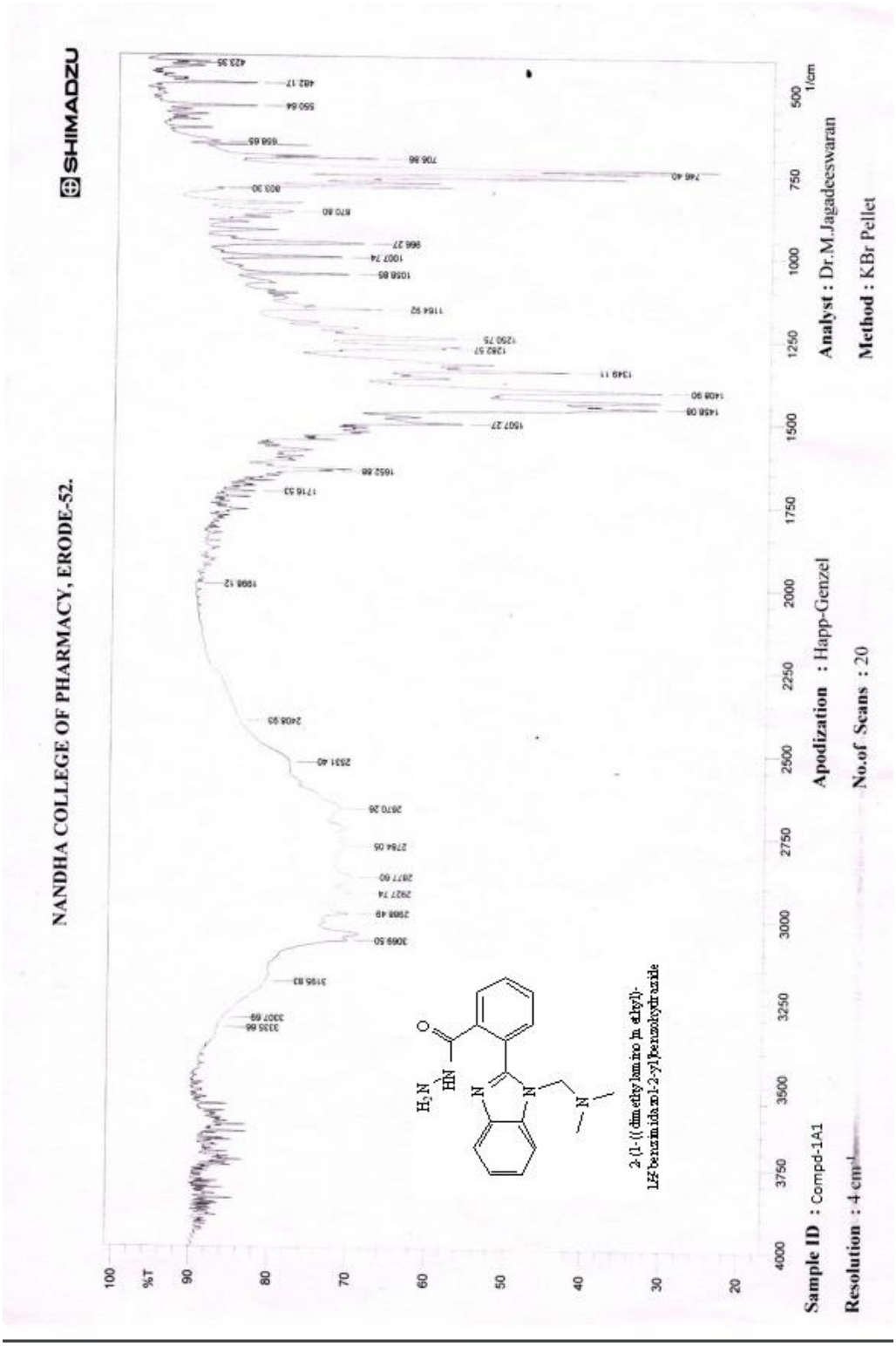


m/z value: 168

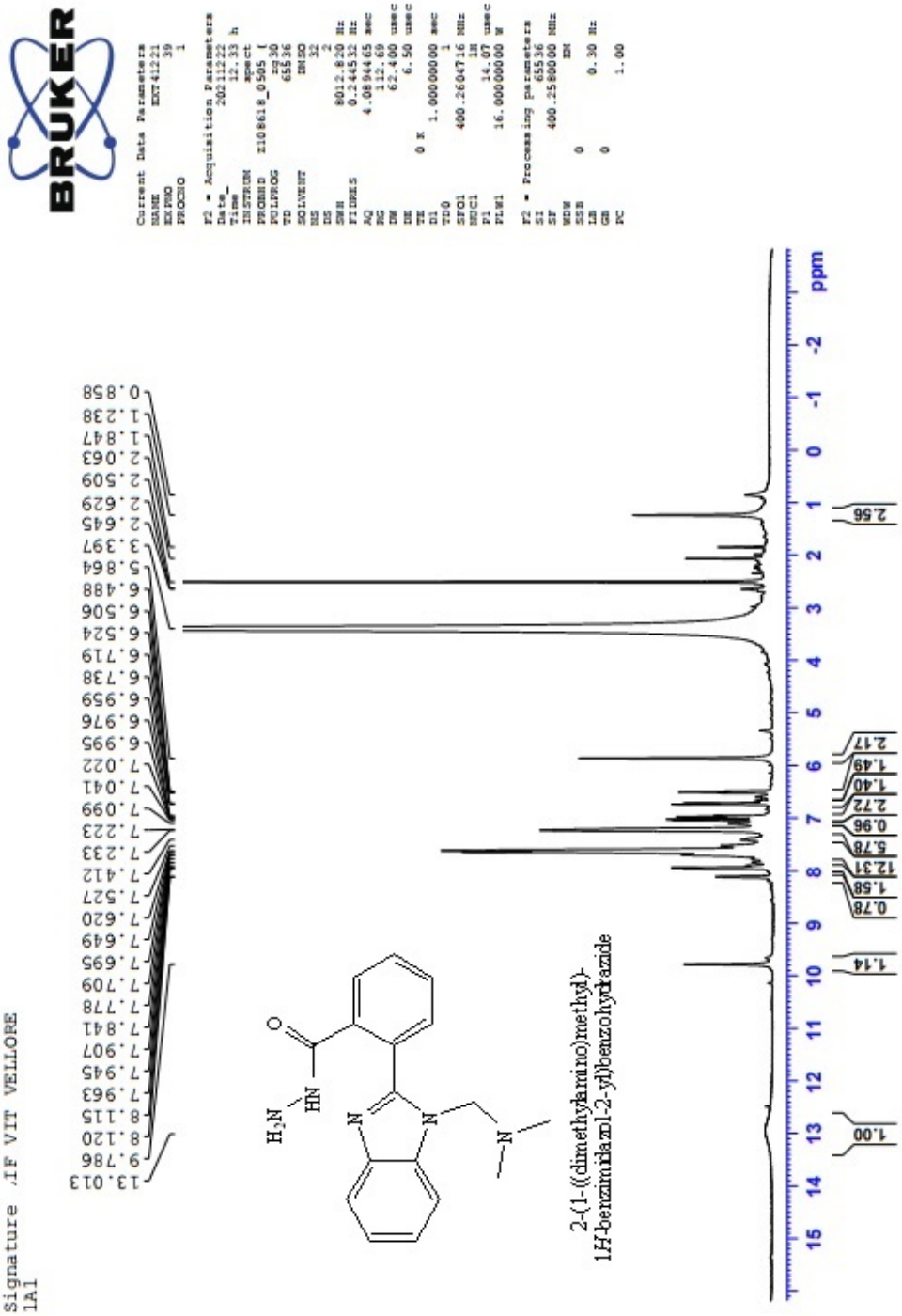


m/z value: 103

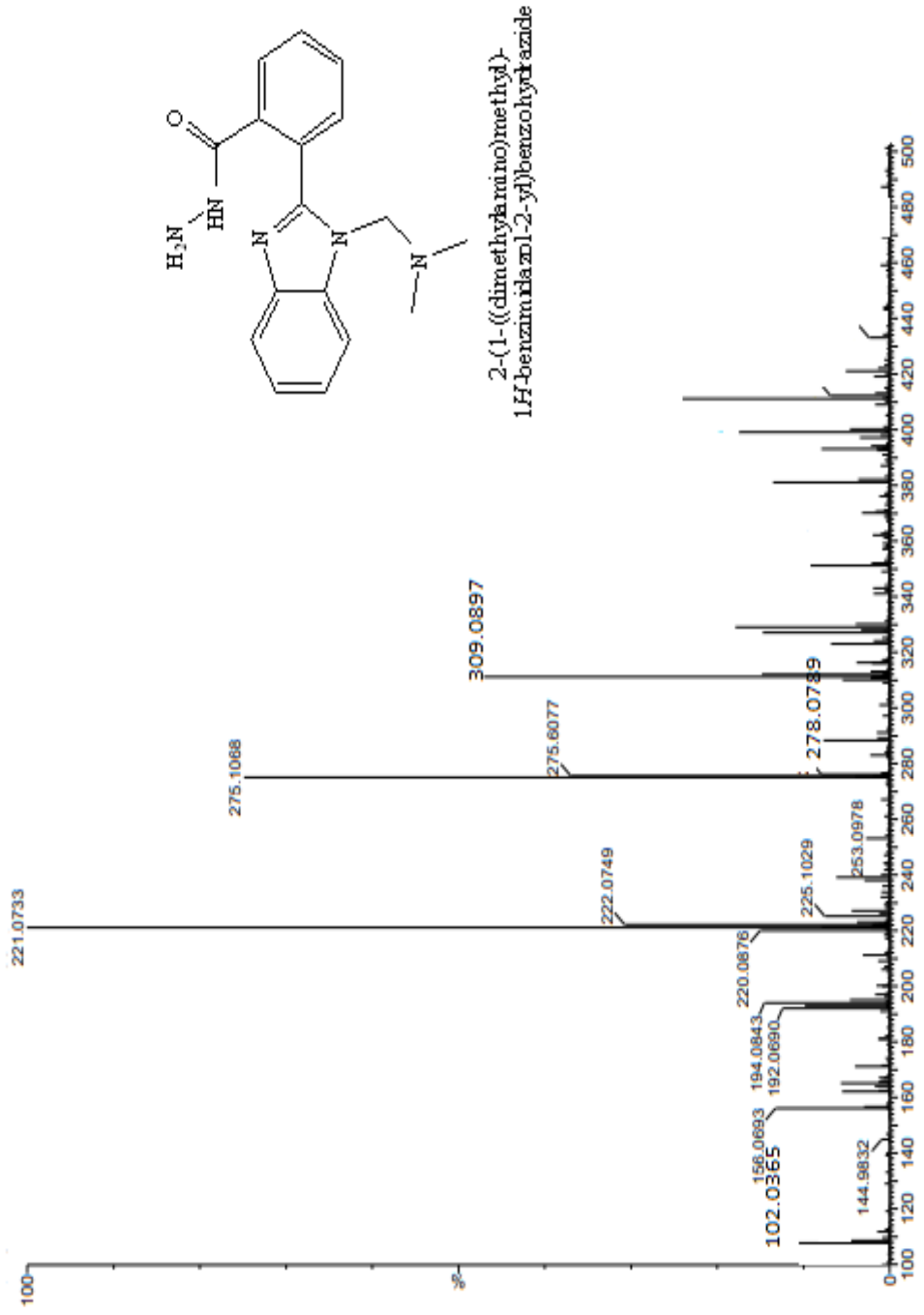
MASS FRAGMENTATION OF COMPOUND-1A



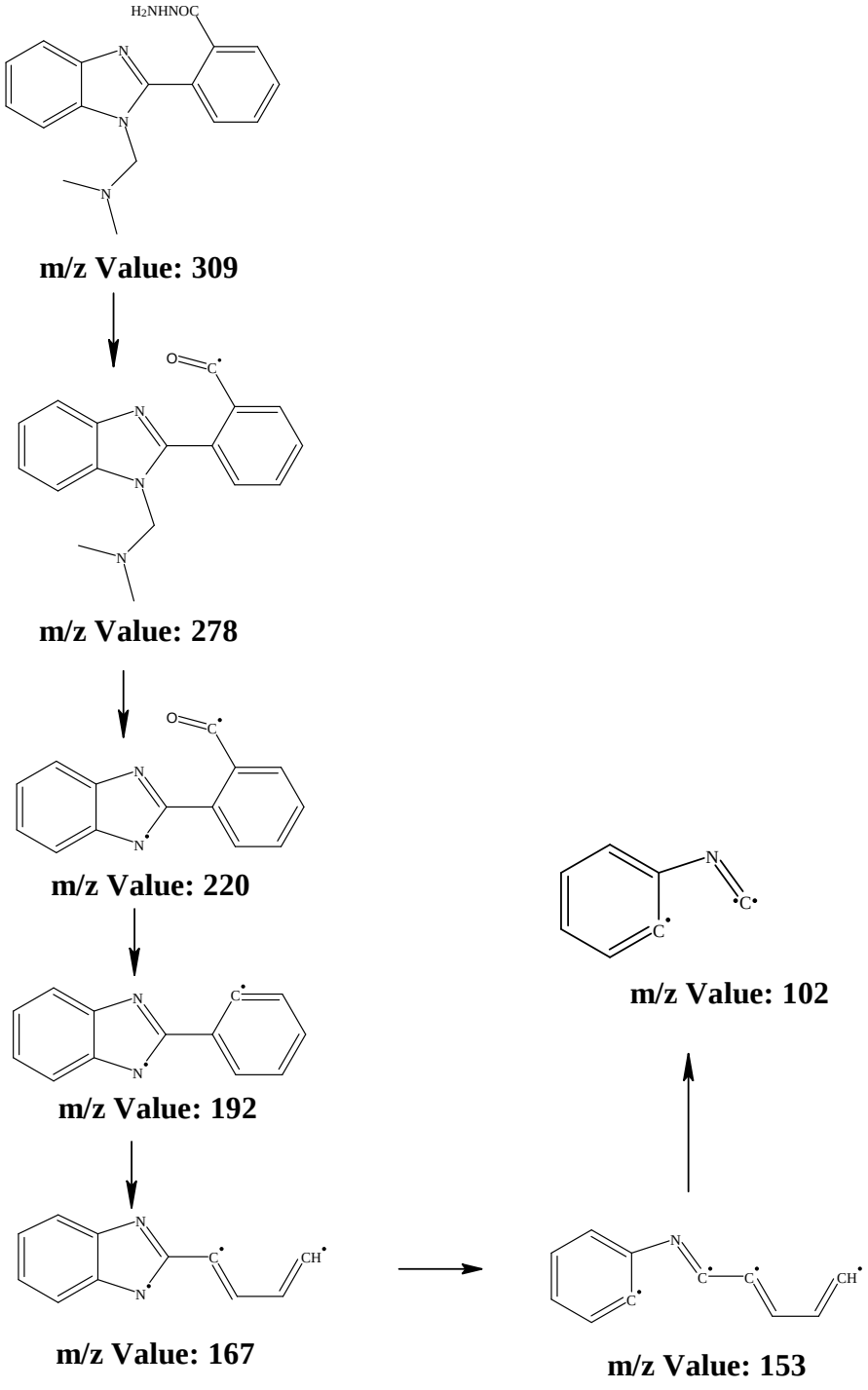
IR SPECTRUM OF COMPOUND-1A$_1$



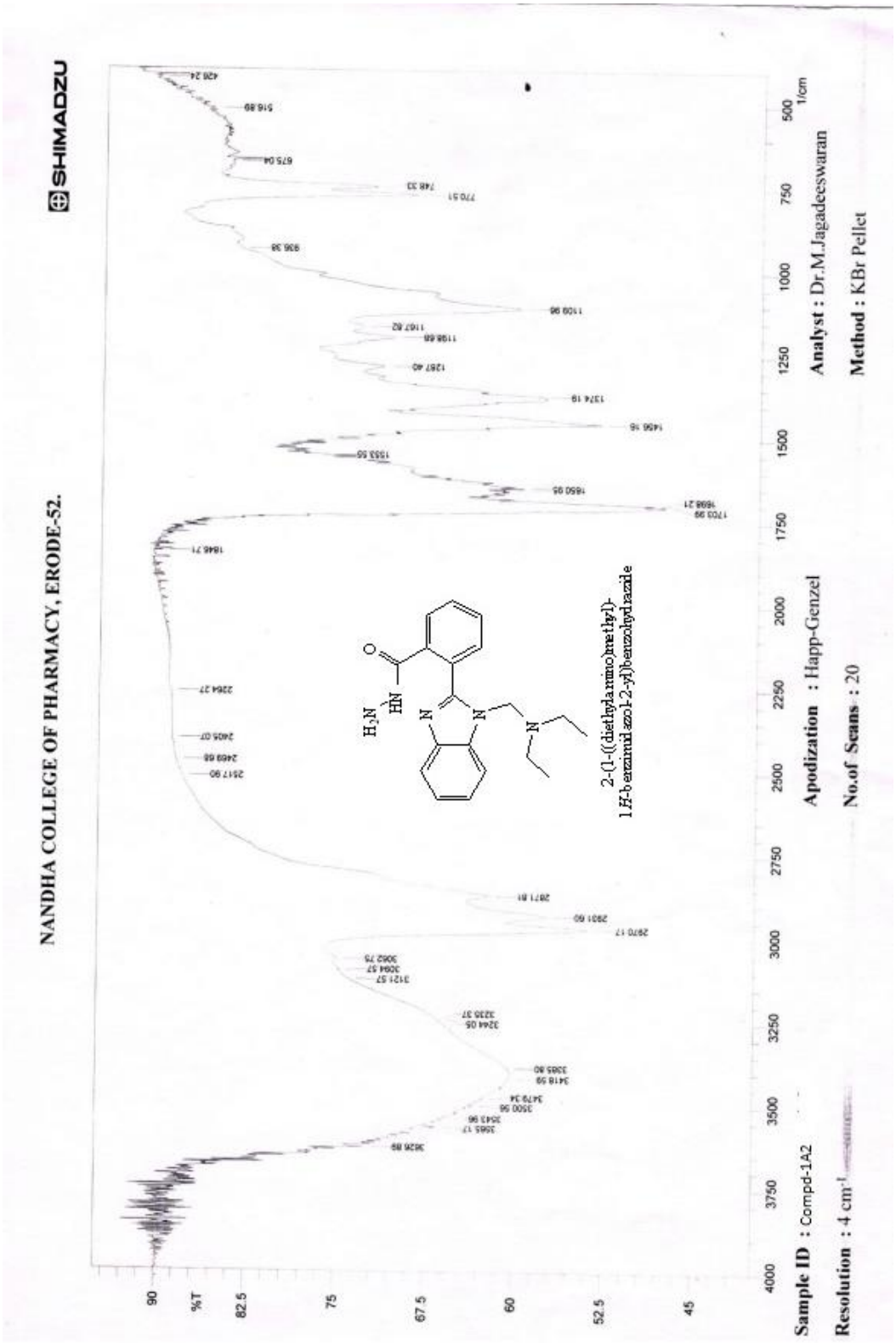
NMR SPECTRUM OF COMPOUND-1A$_1$



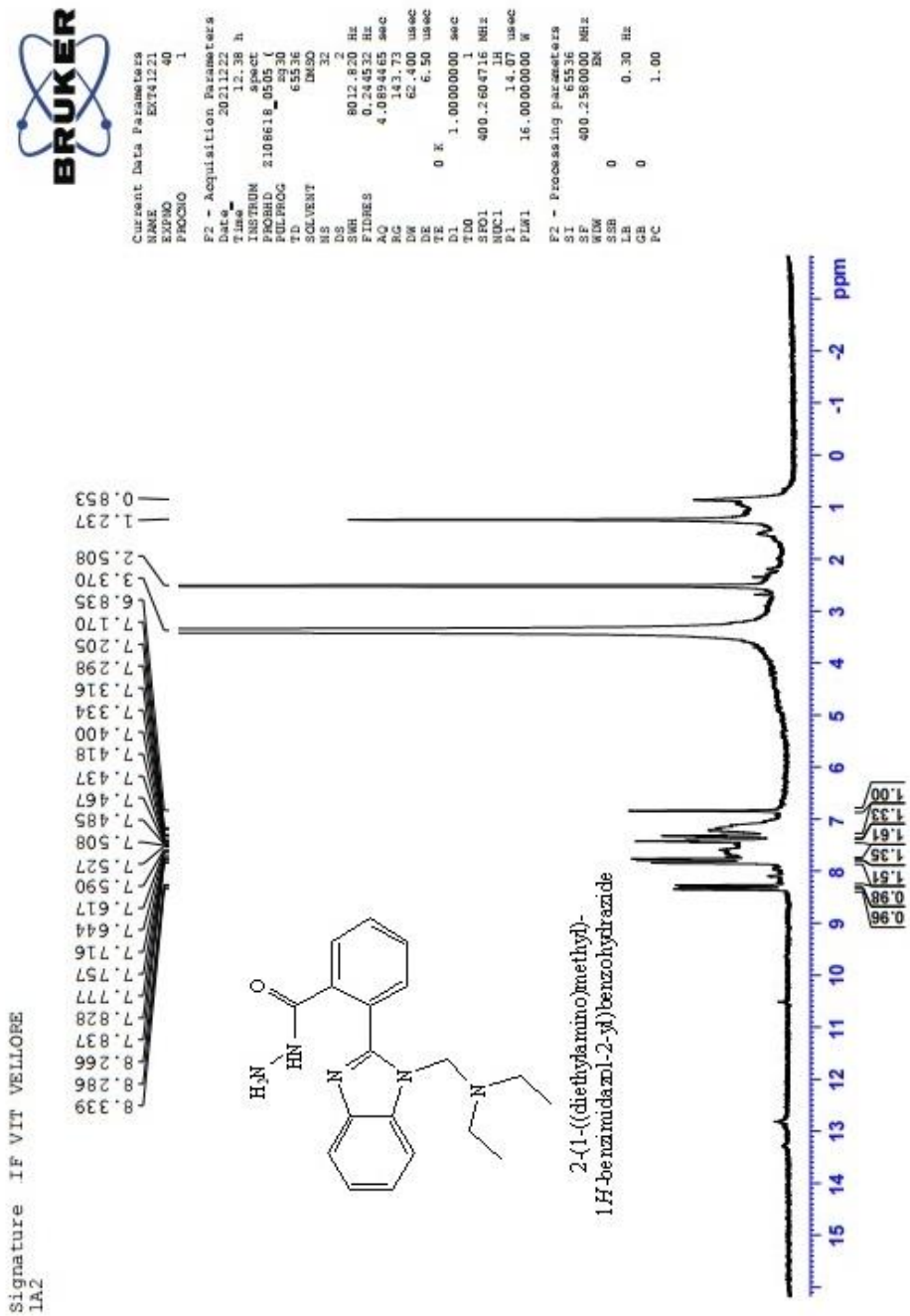
MASS SPECTRUM OF COMPOUND-1A$_1$



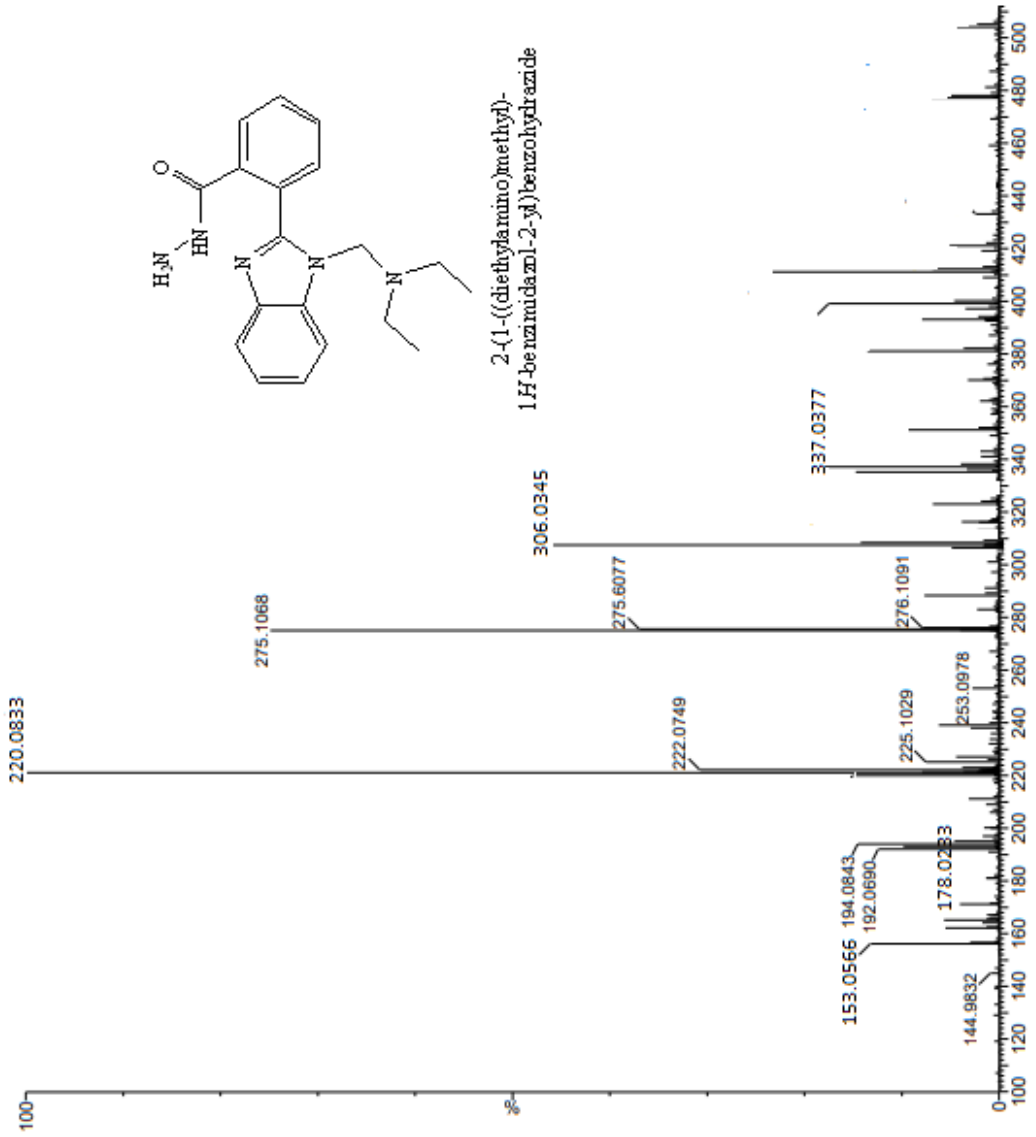
MASS FRAGMENTATION OF COMPOUND-1A$_1$



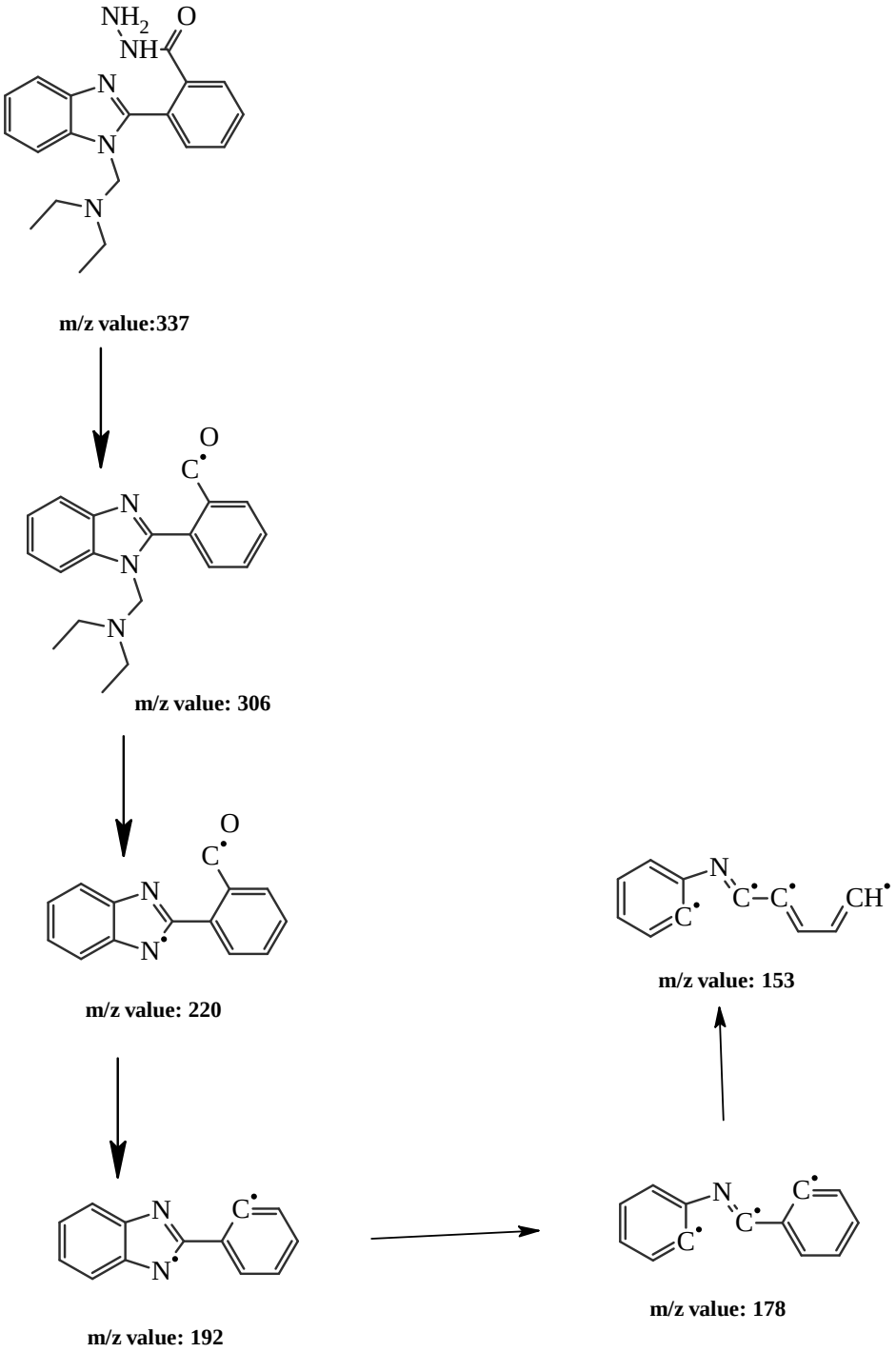
IR SPECTRUM OF COMPOUND-1A$_2$



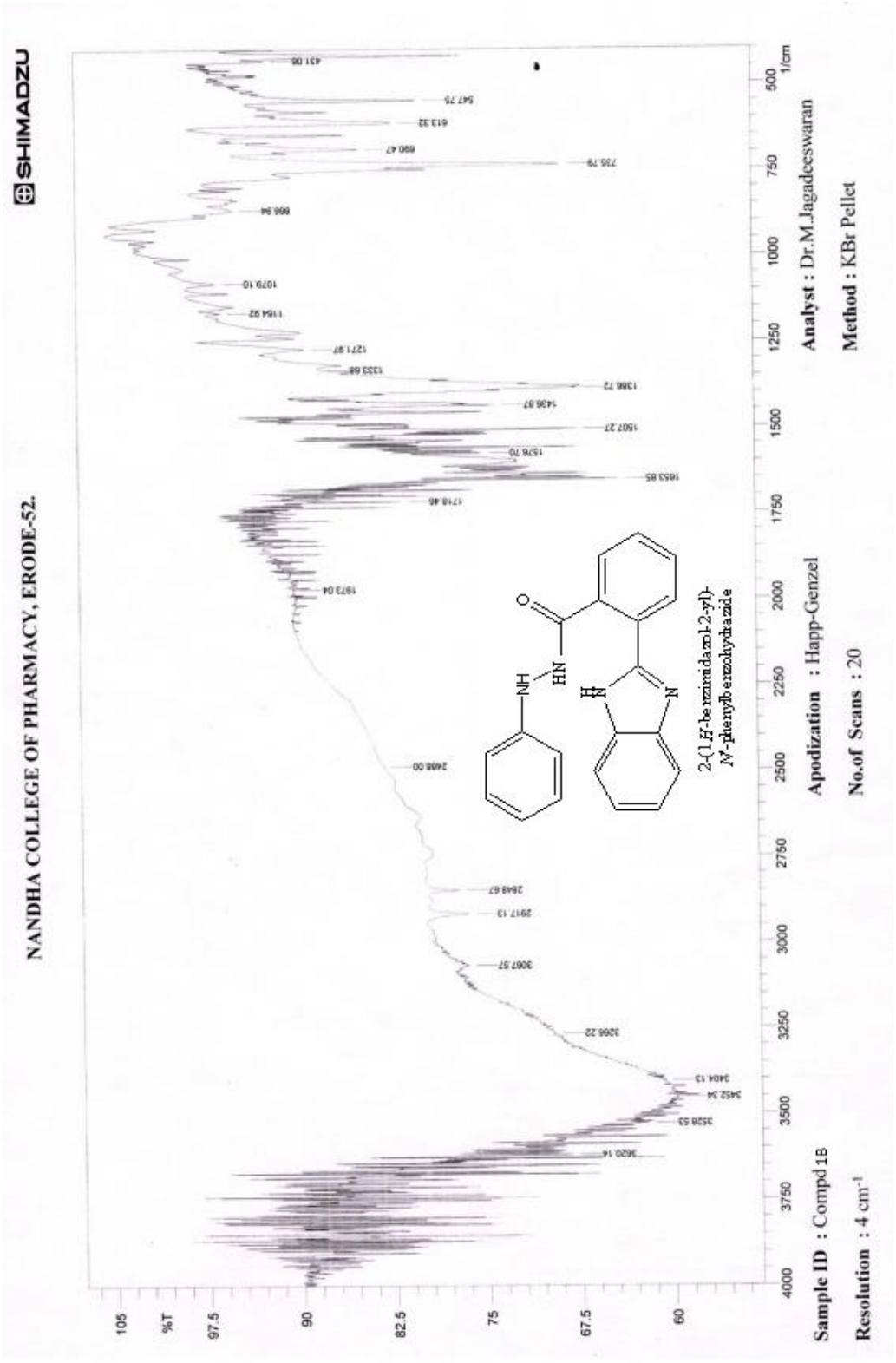
NMR SPECTRUM OF COMPOUND-1A$_2$



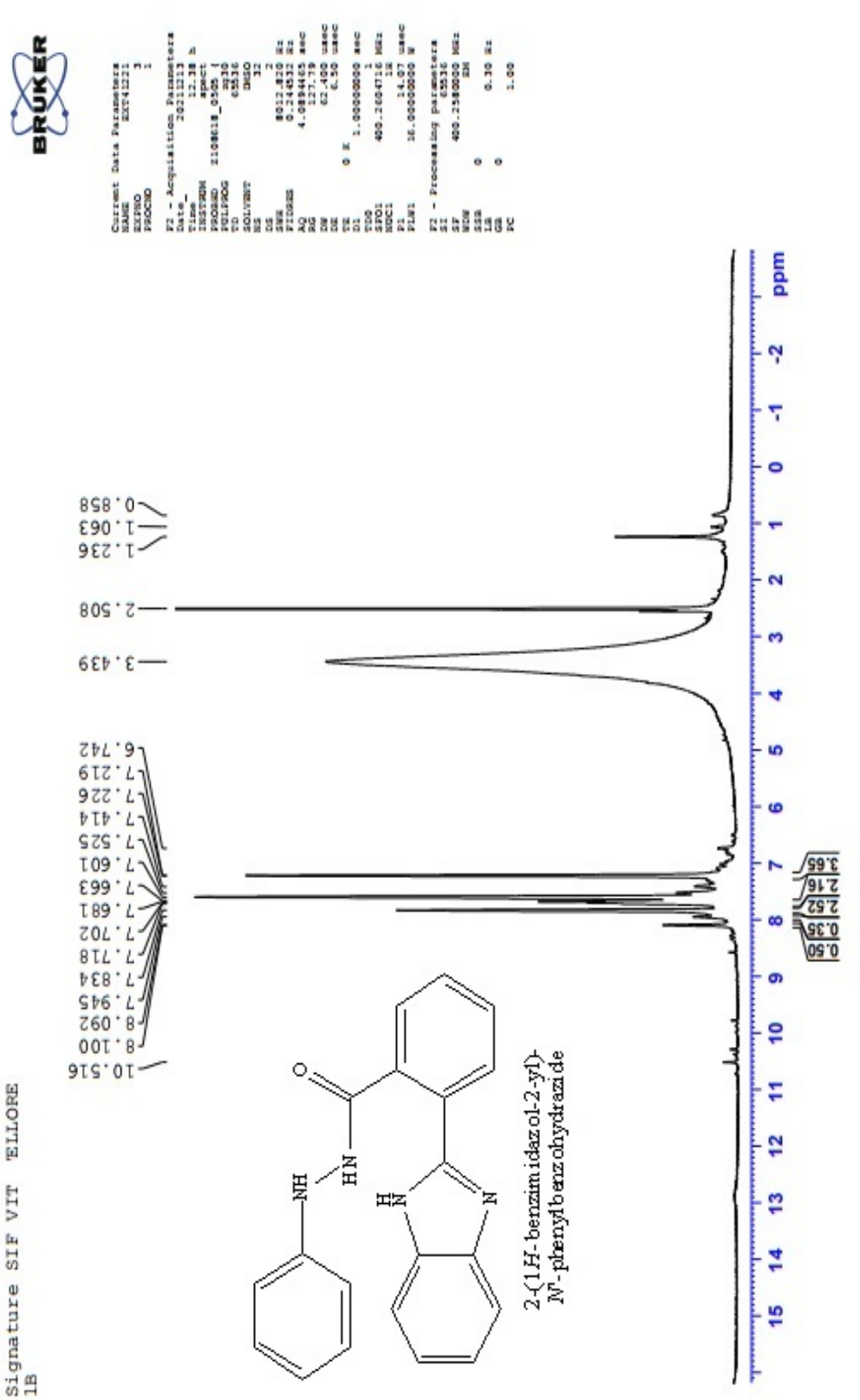
MASS SPECTRUM OF COMPOUND-1A$_2$



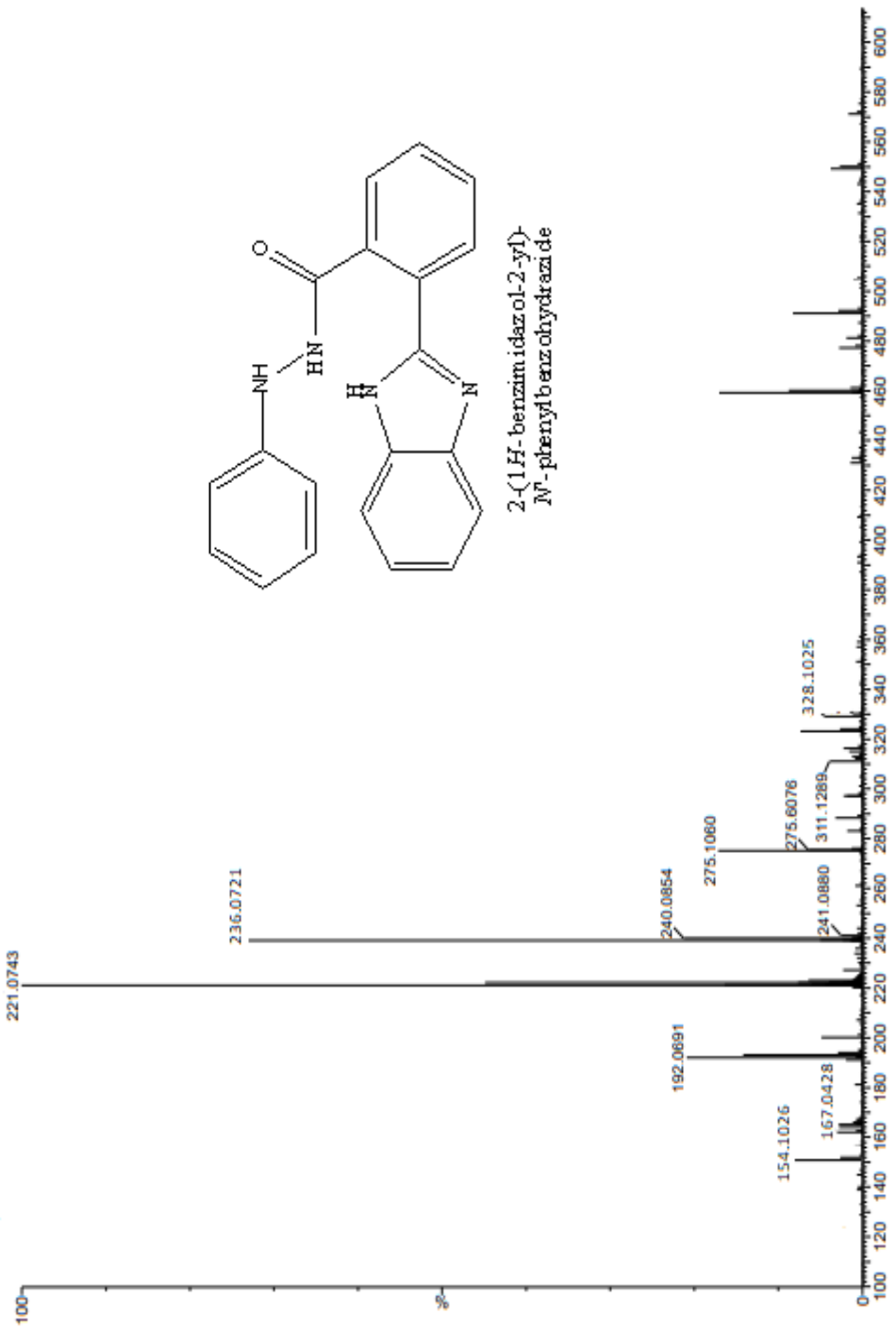
MASS FRAGMENTATION OF COMPOUND-1A$_2$



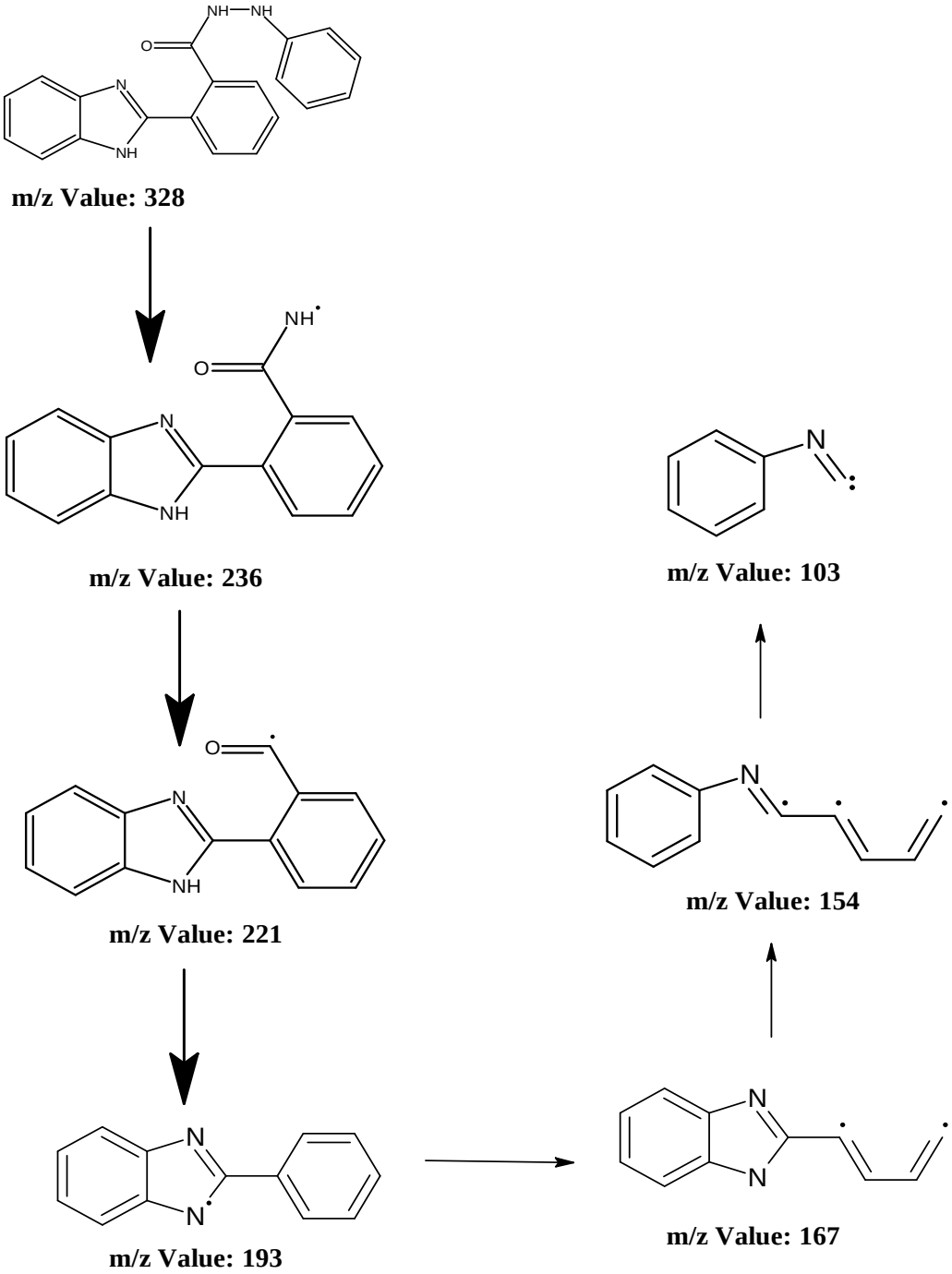
IR SPECTRUM OF COMPOUND-1B



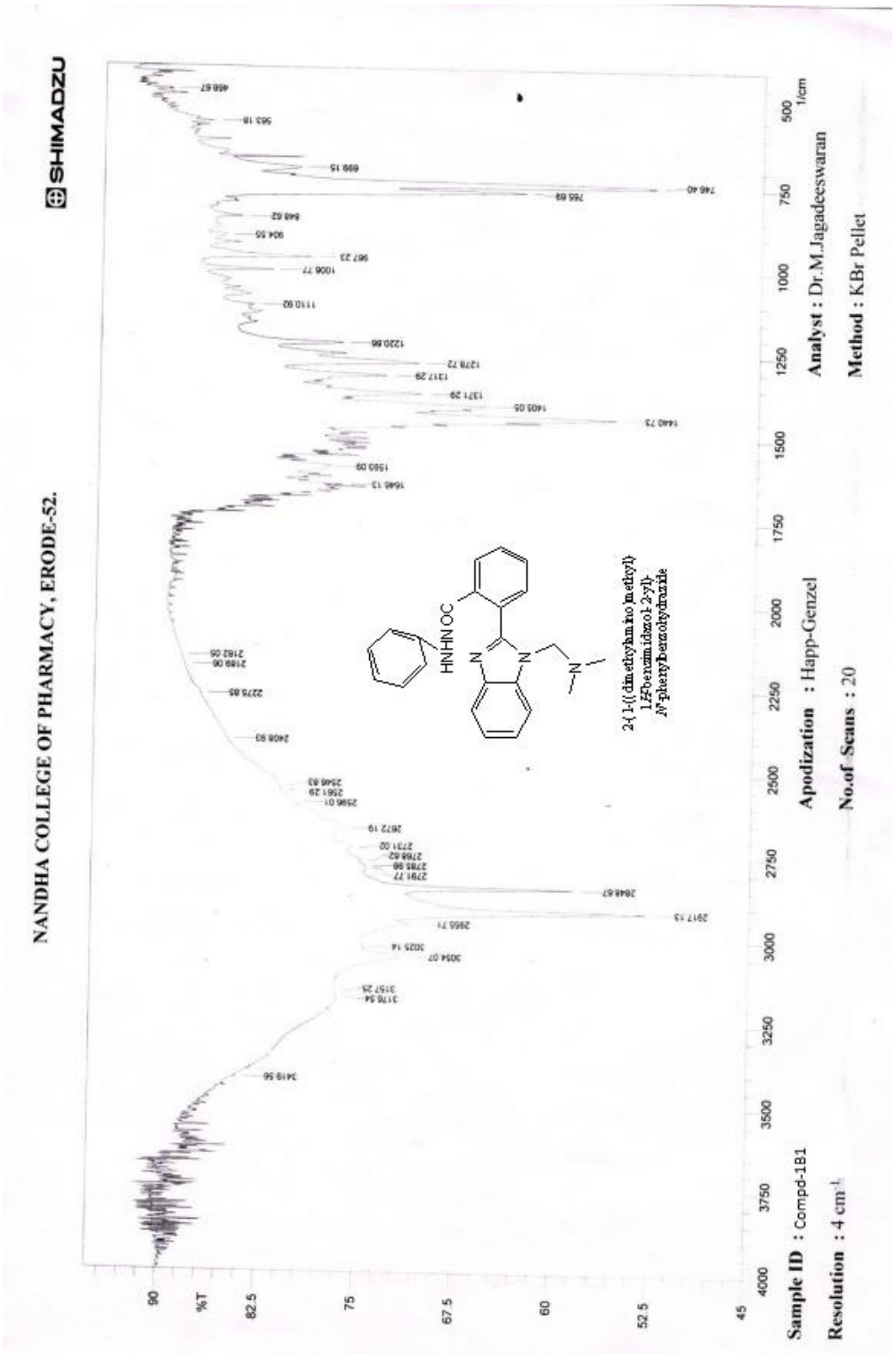
NMR SPECTRUIM OF COMPOUND-1B



MASS SPECTRUM OF COMPOUND-1B

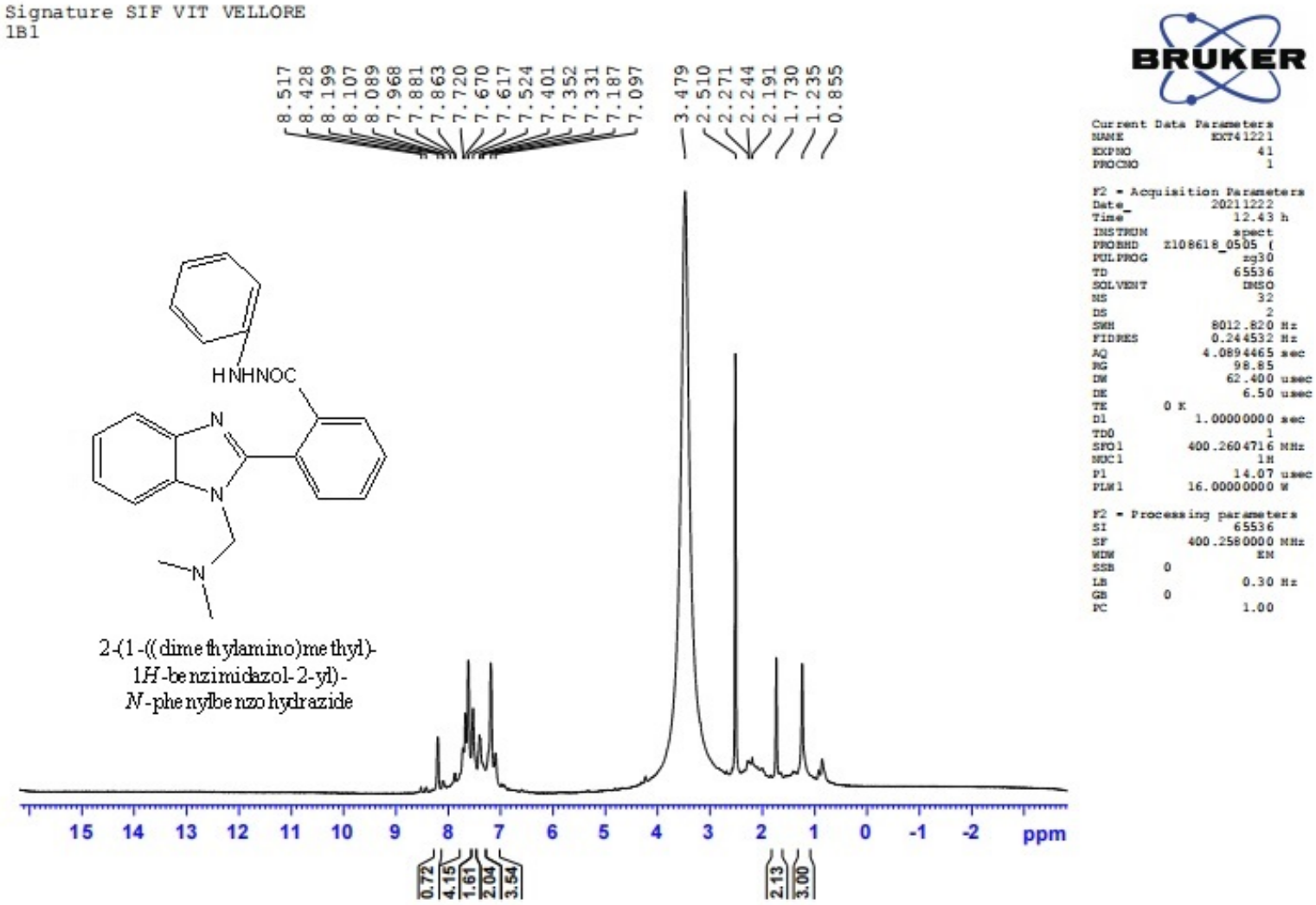


MASS FRAGMENTATION OF COMPOUND-1B



IR SPECTRUM OF COMPOUND-1B$_1$

NMR SPECTRUM OF COMPOUND-1B1



BRUKER

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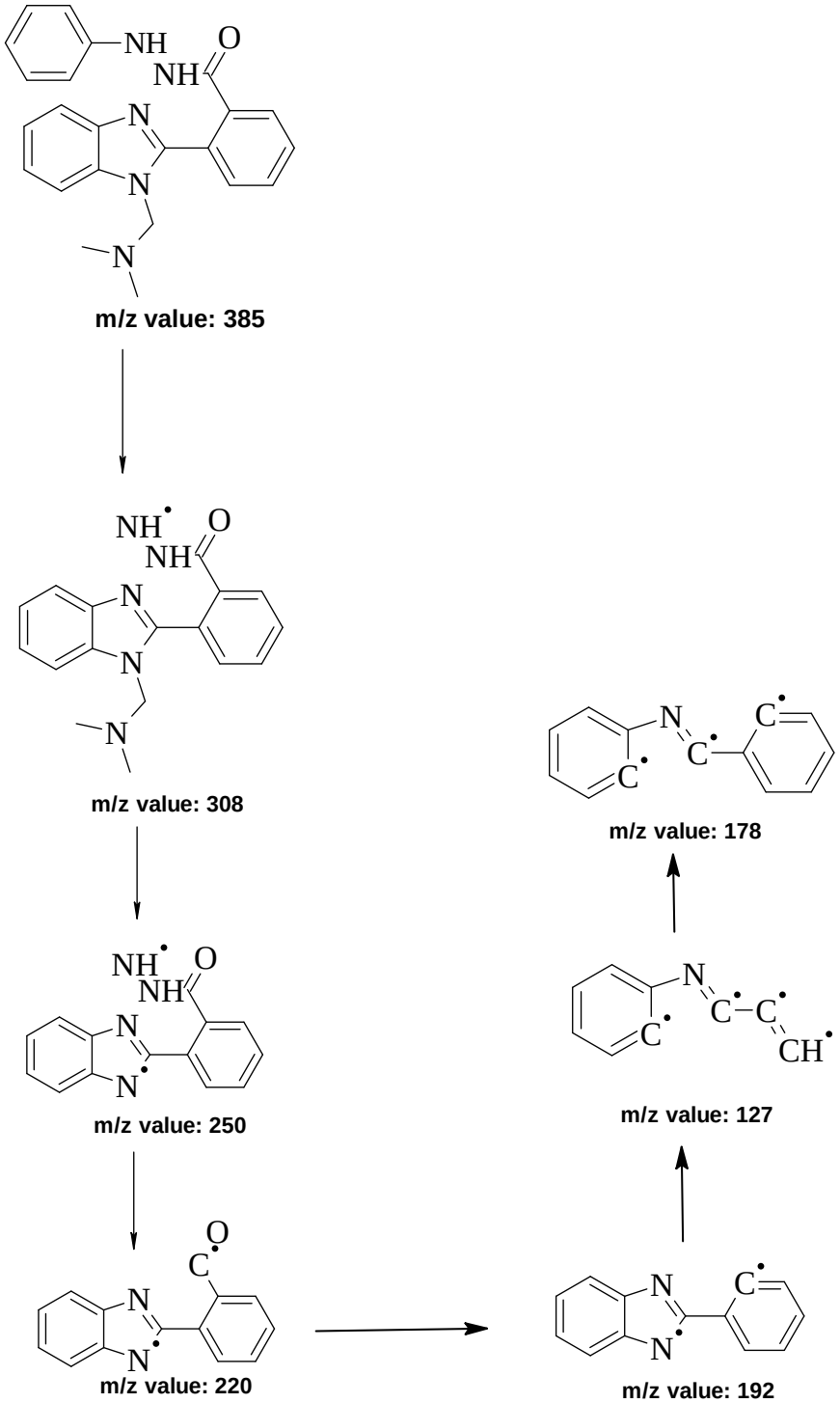

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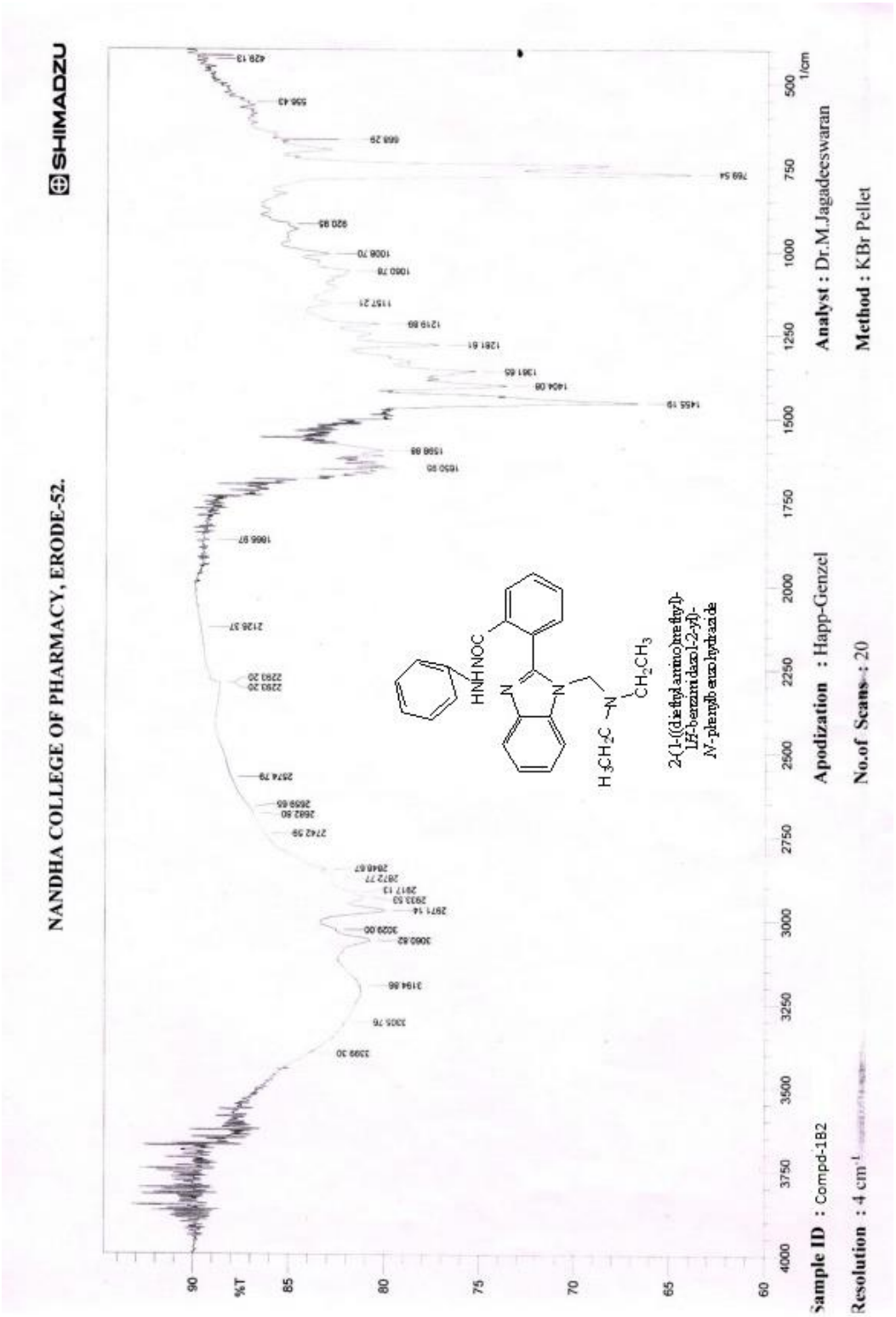
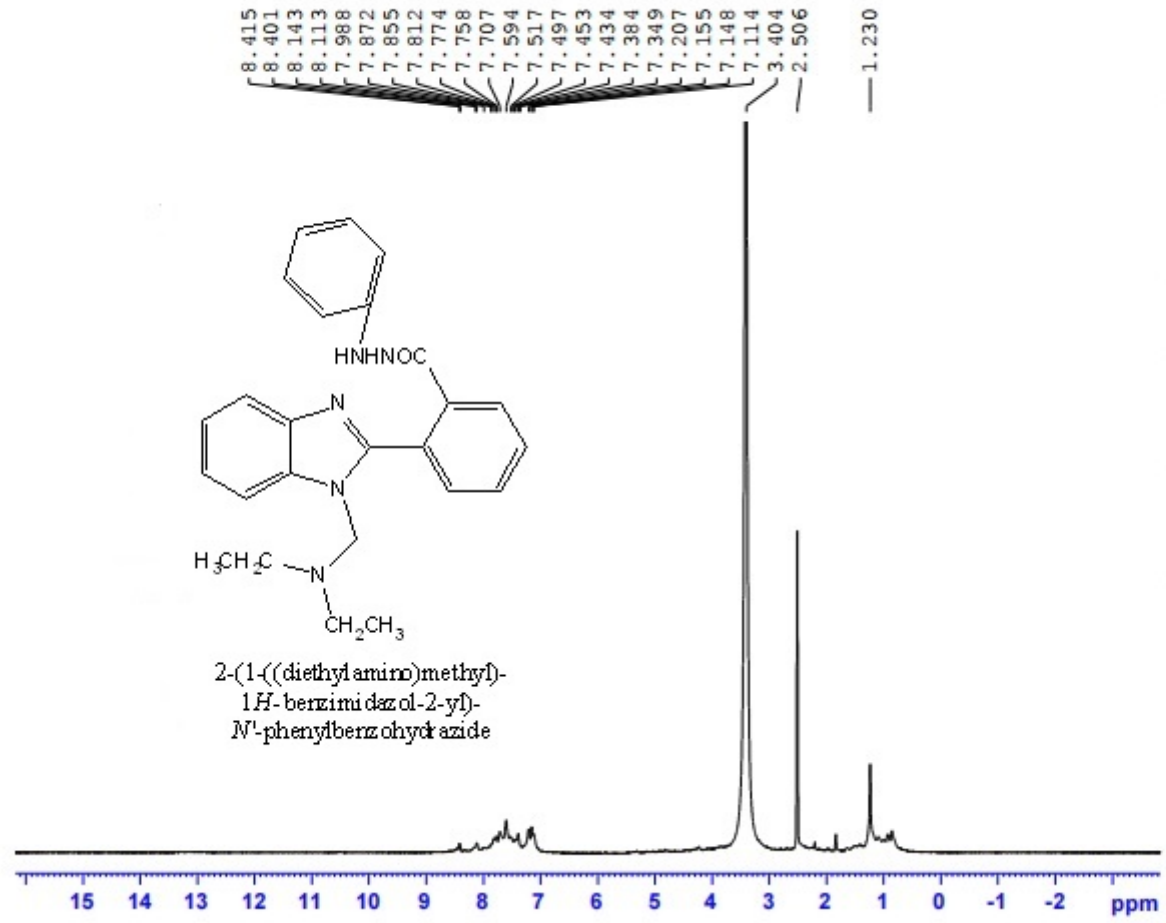


IR SPECTRUM OF COMPOUND-1B$_2$

NMR SPECTRUM OF COMPOUND-1B$_2$

Signature SIF VIT VELLORE
1B2



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BRUKER

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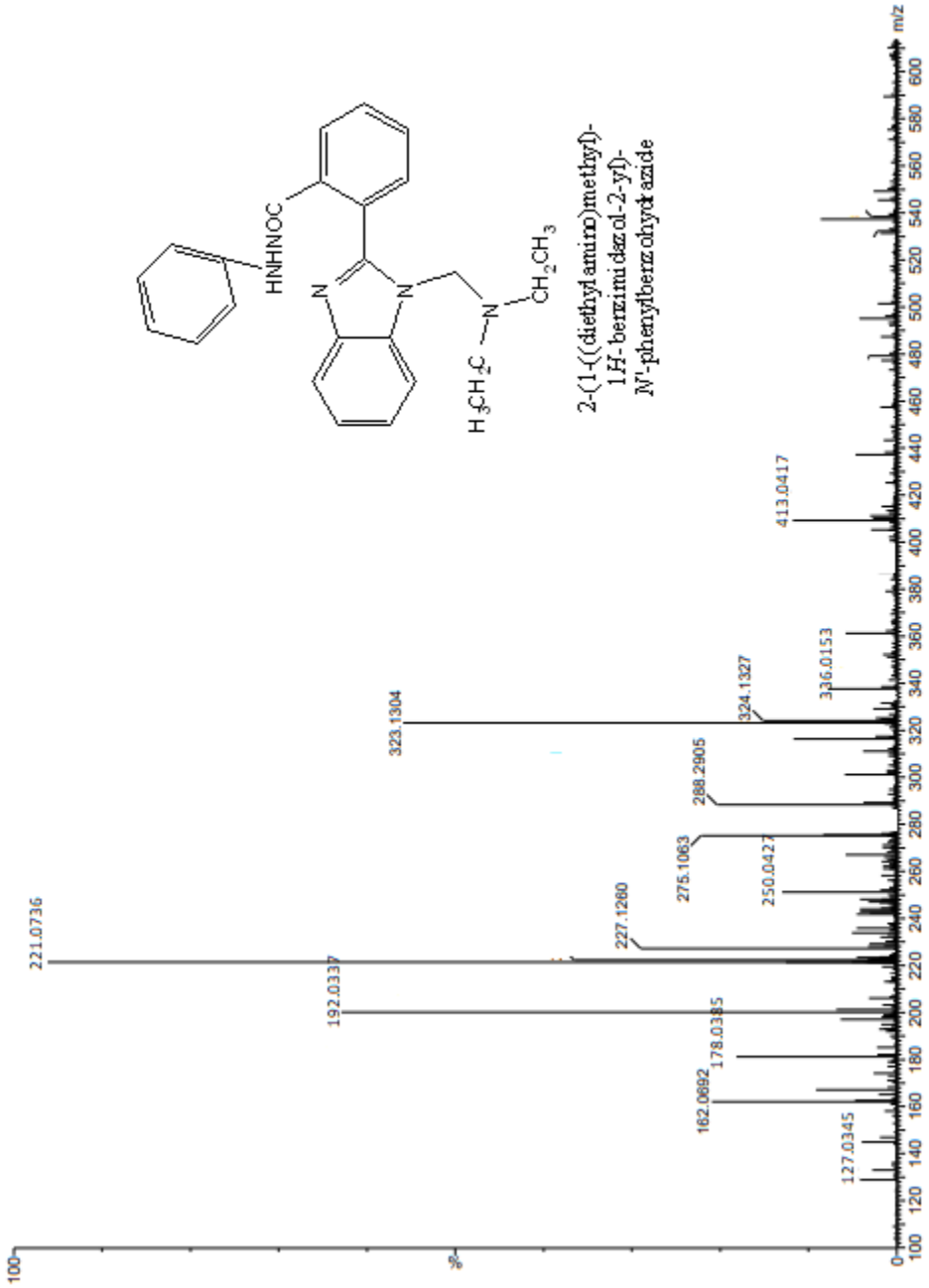

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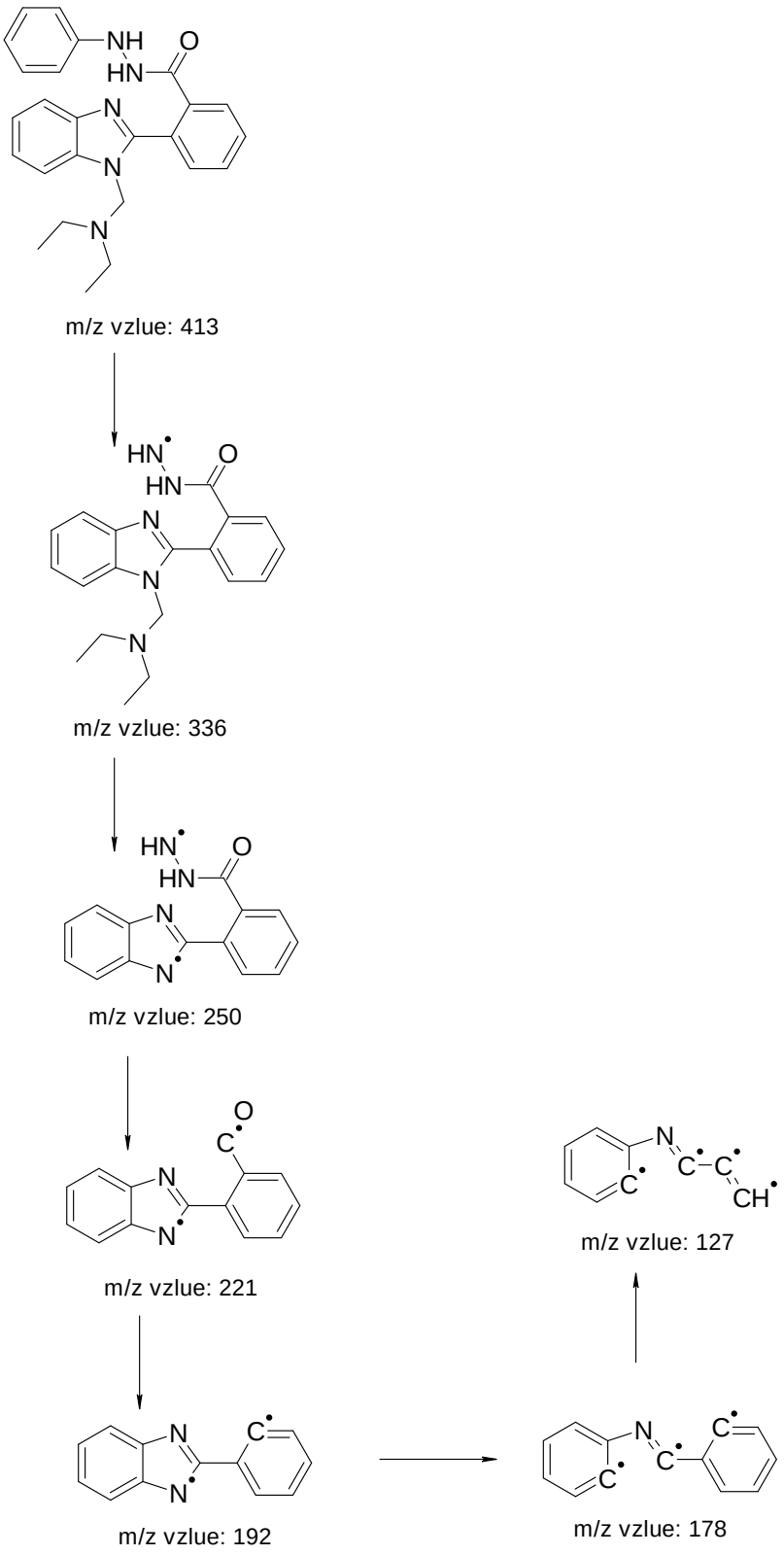

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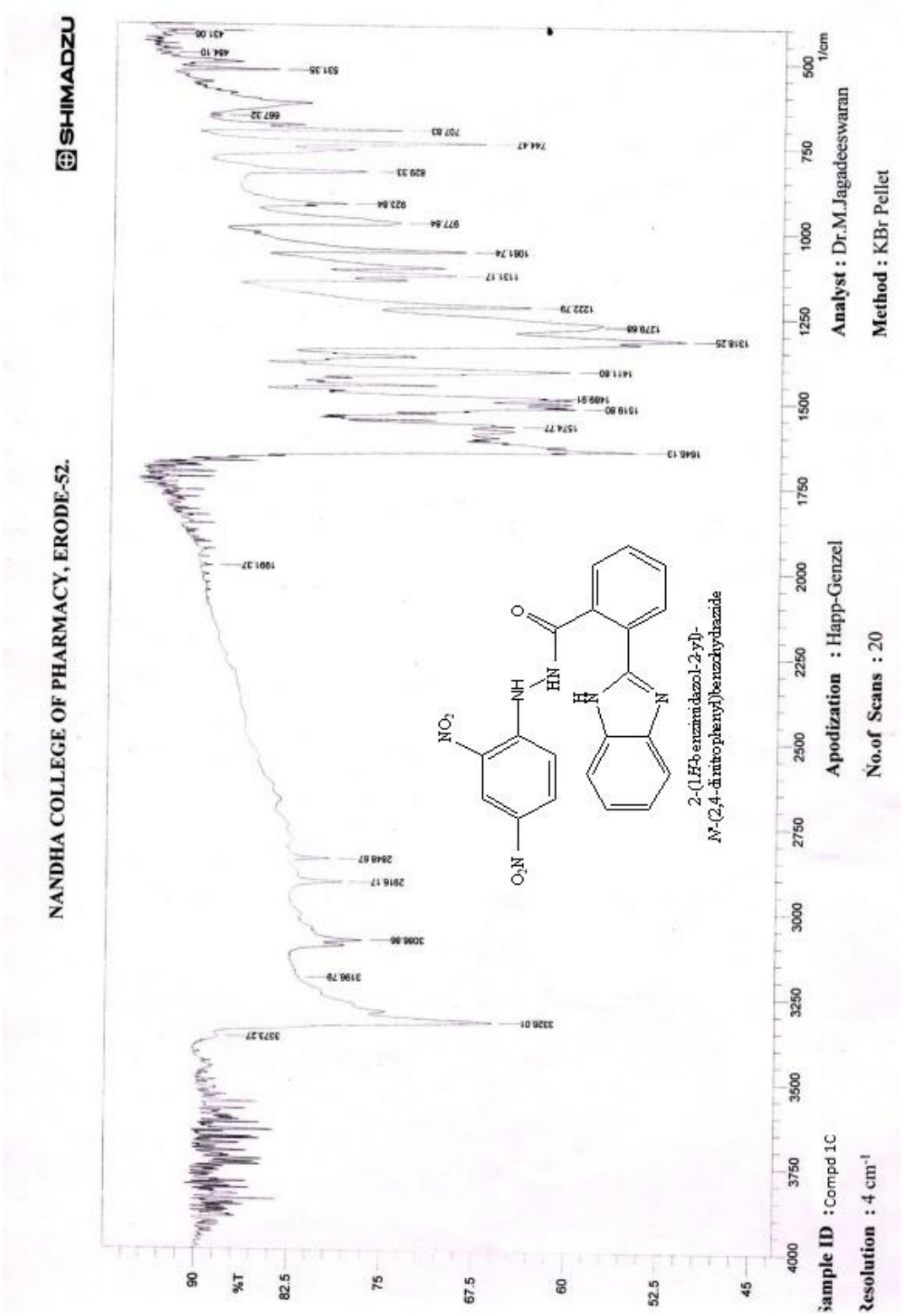

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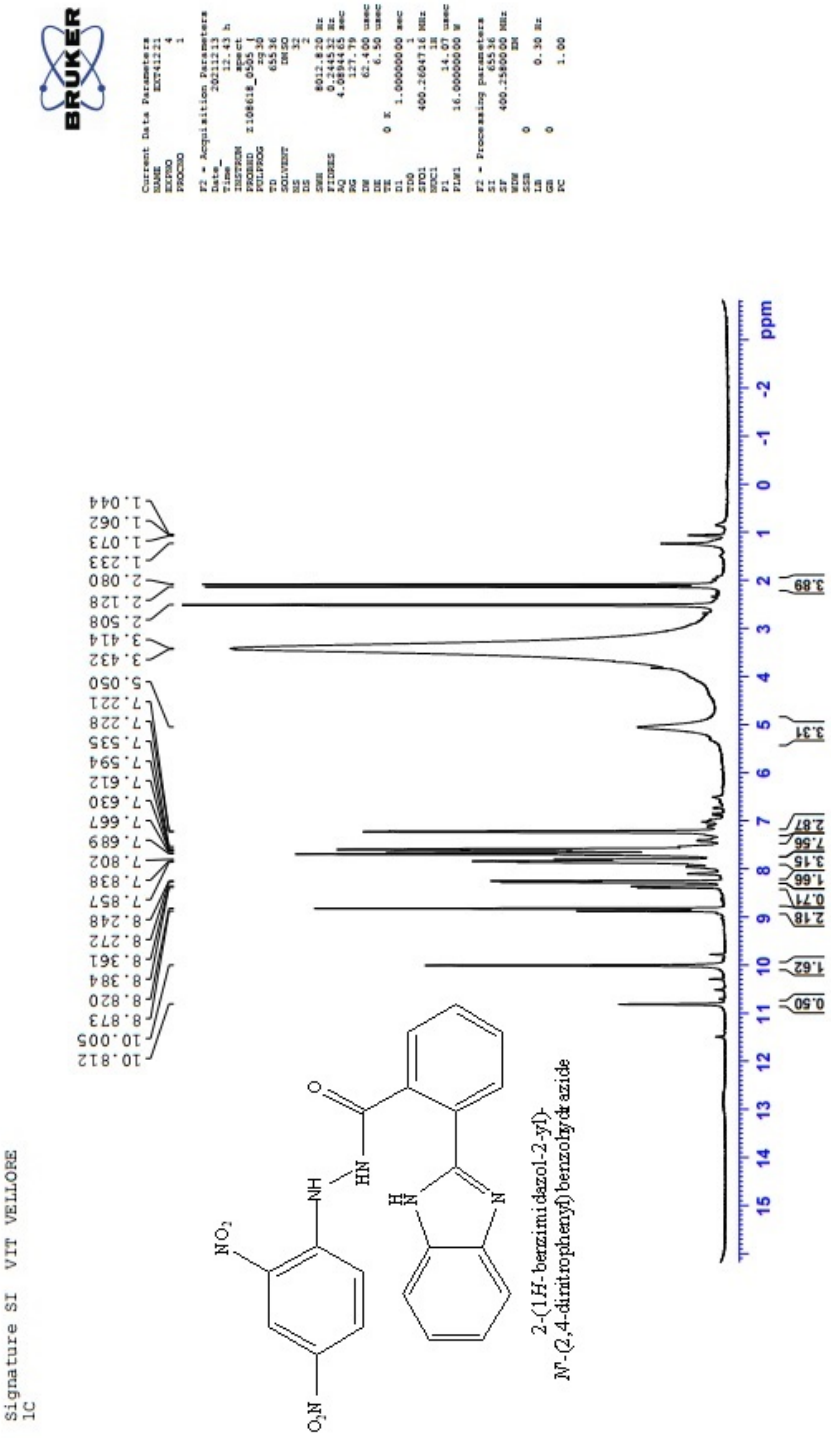

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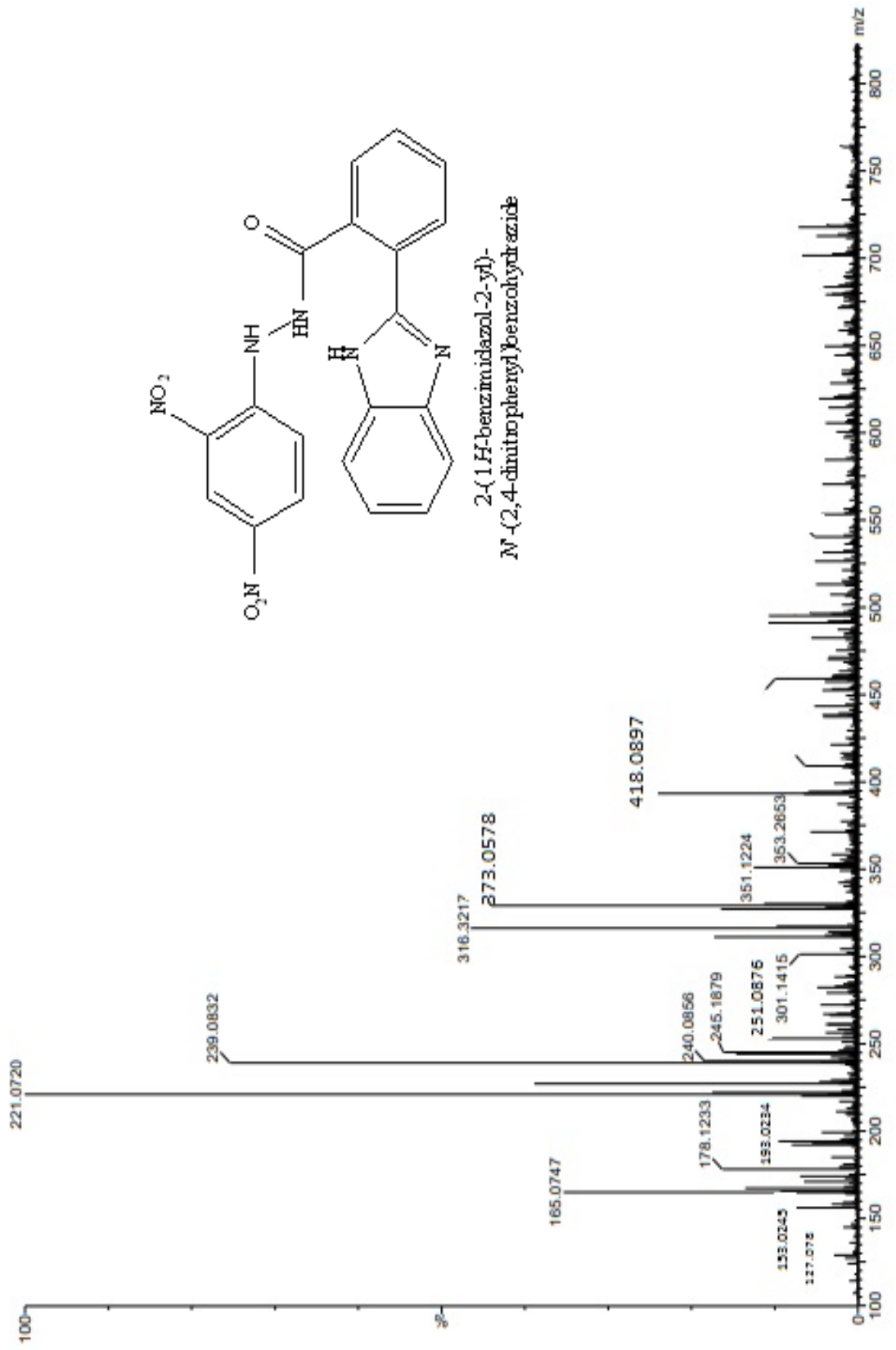

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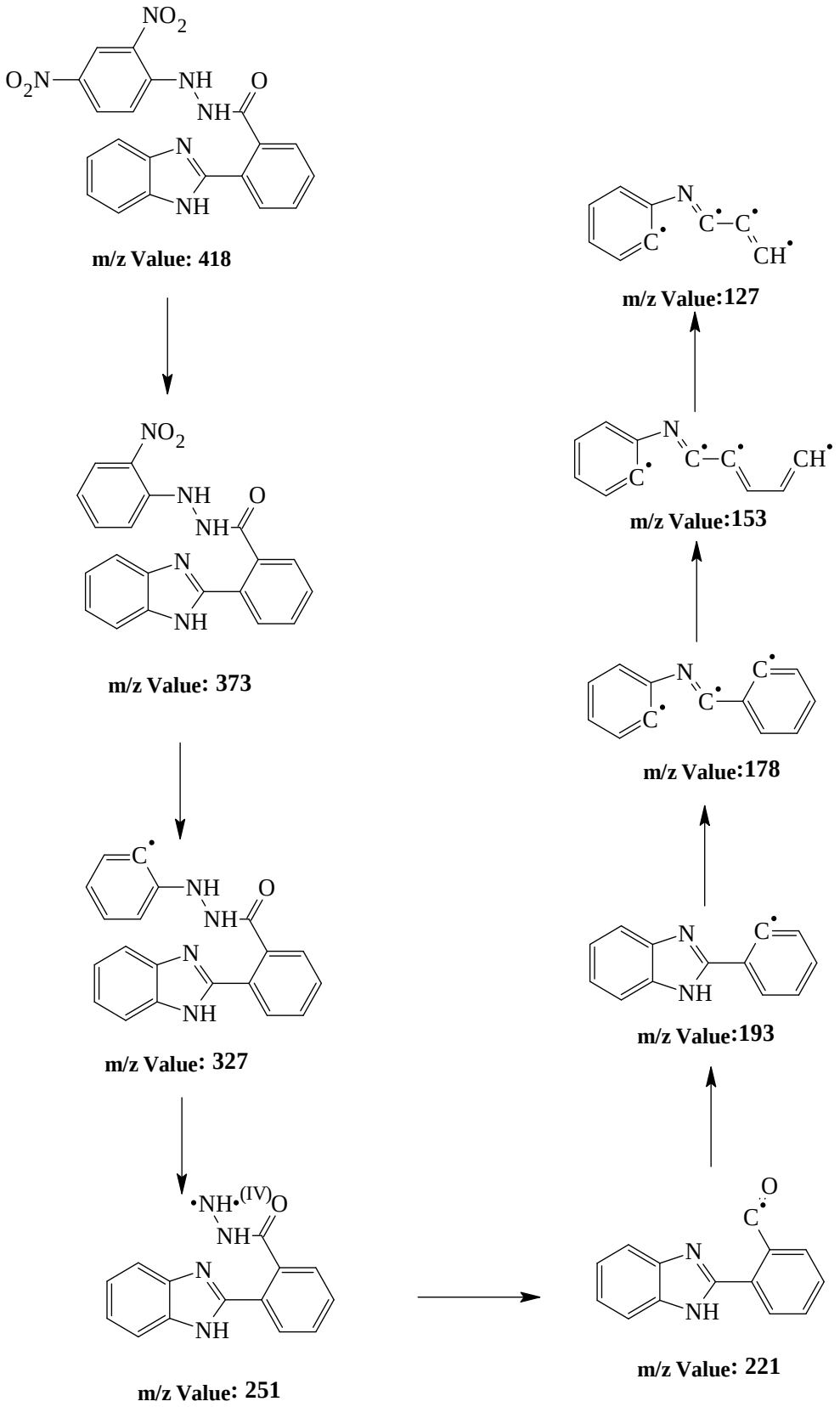
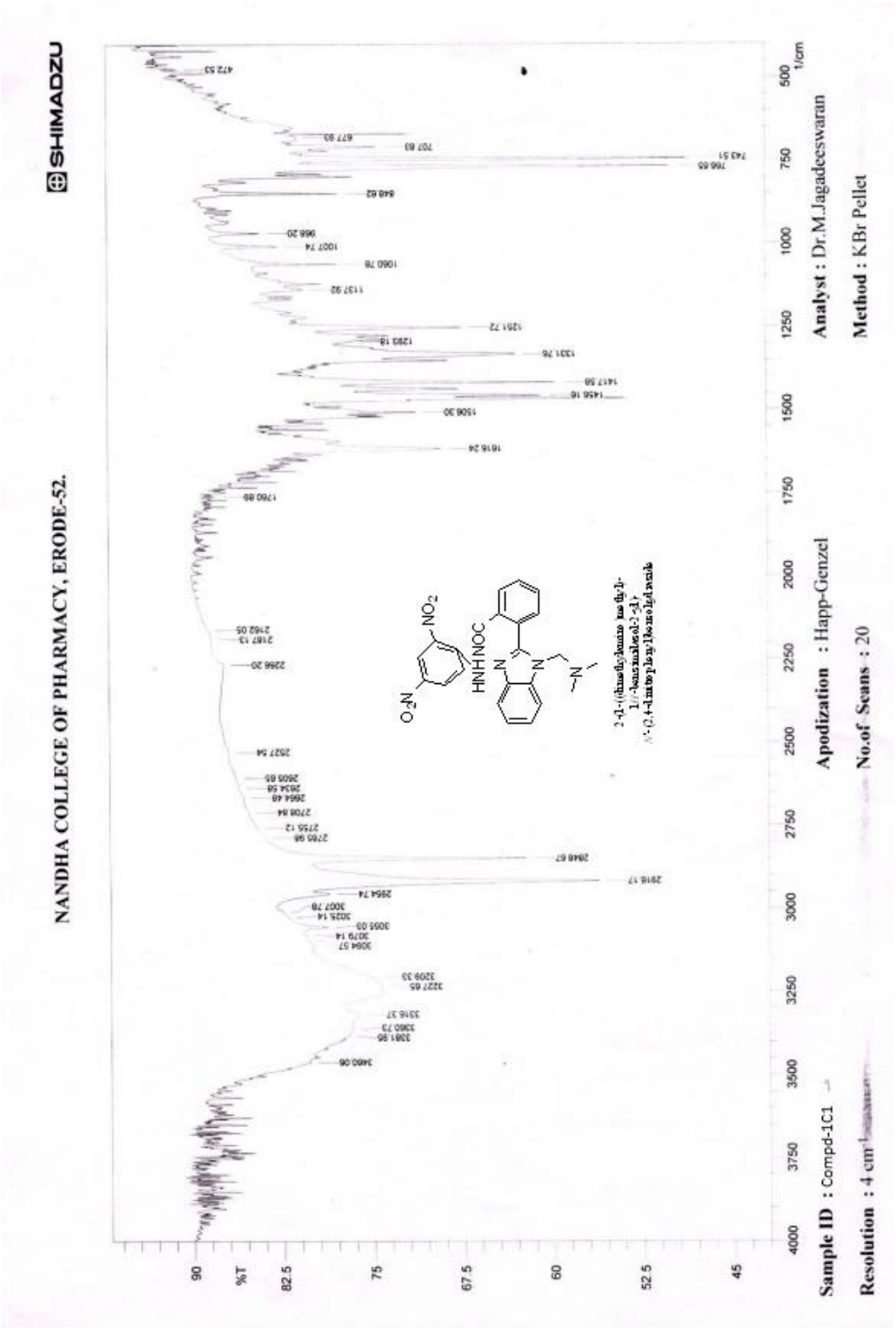


MASS FRAGMENTATION OF COMPOUND-1C



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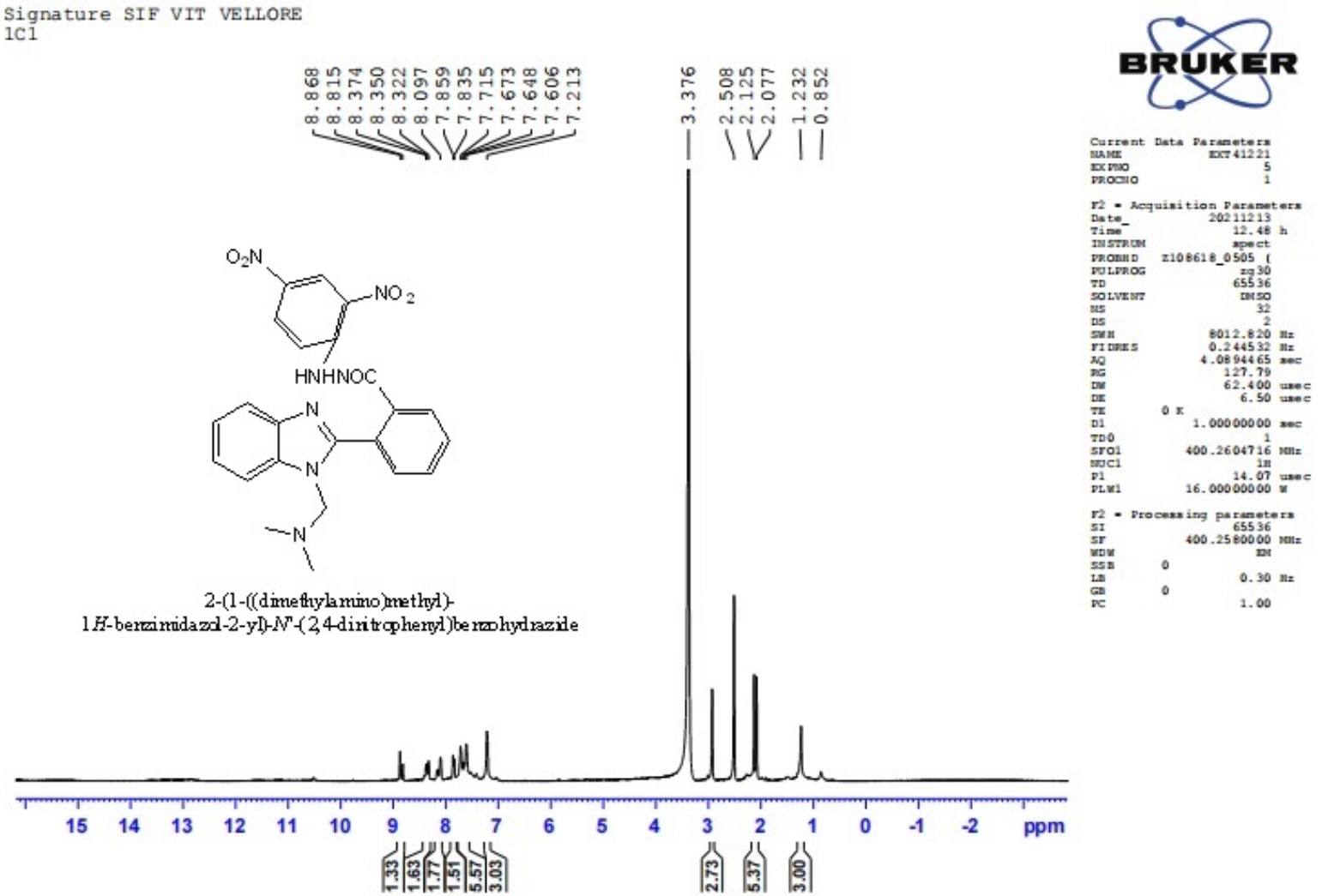
NMR SPECTRUM OF COMPOUND-1C$_1$



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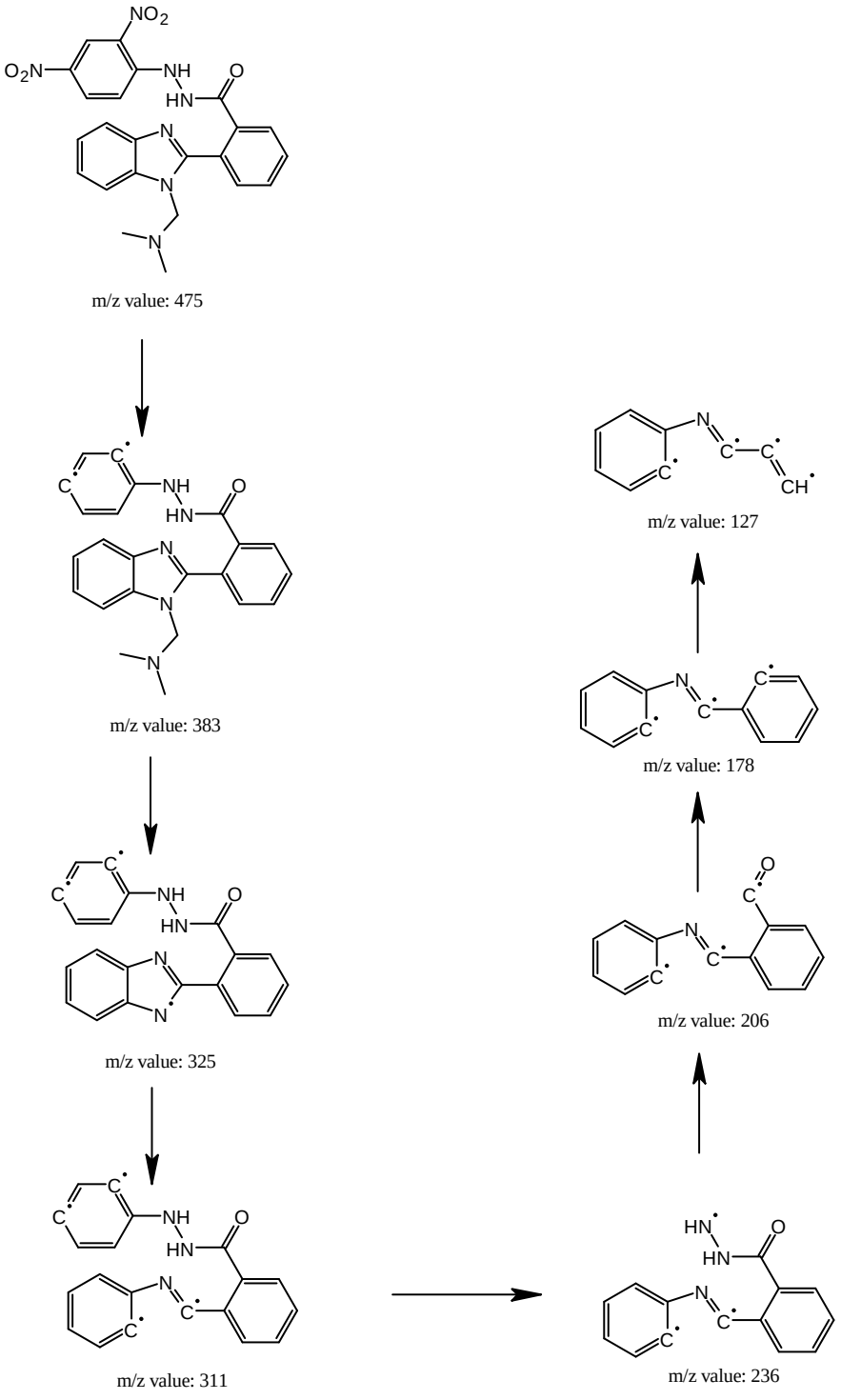

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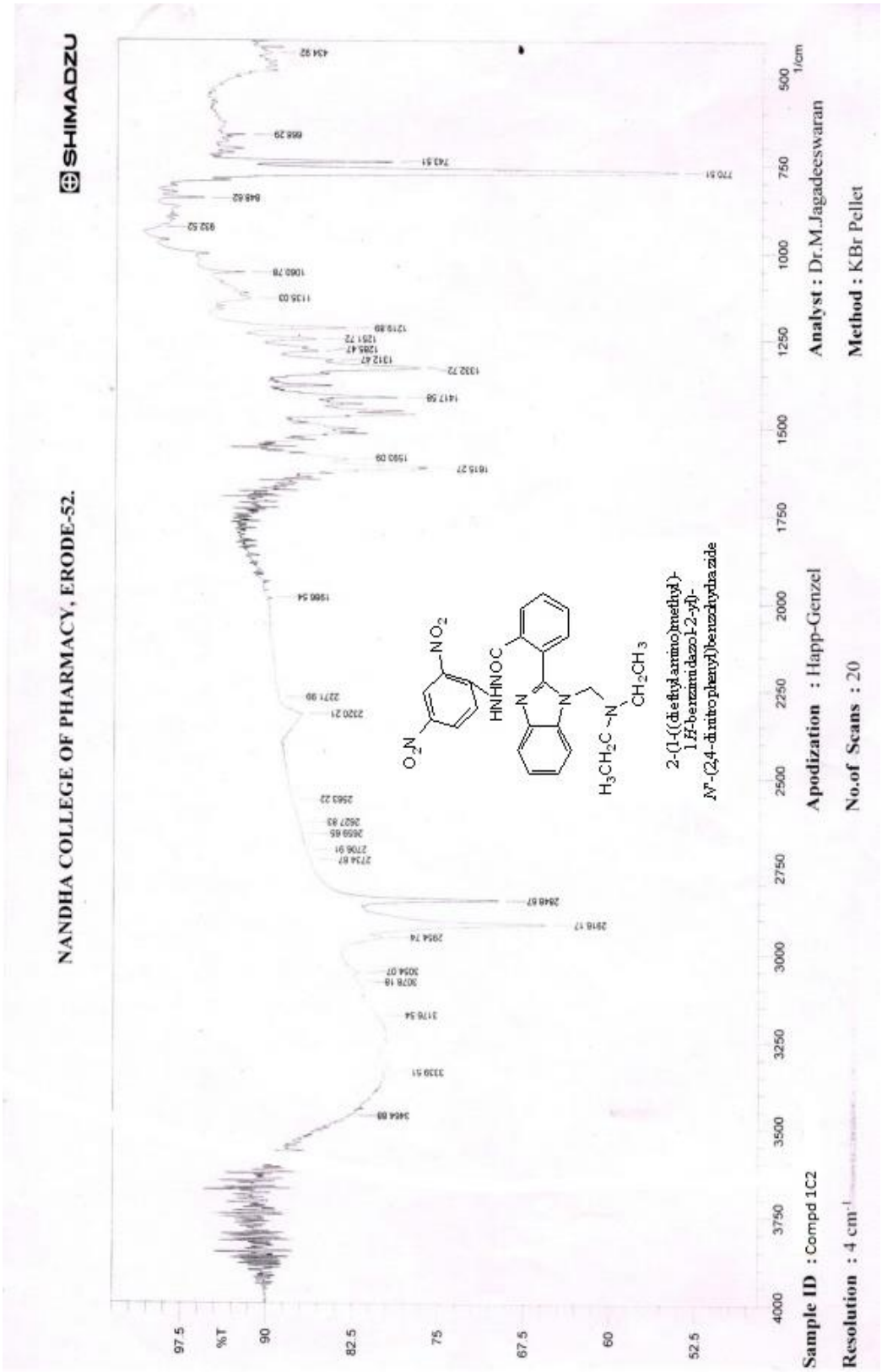

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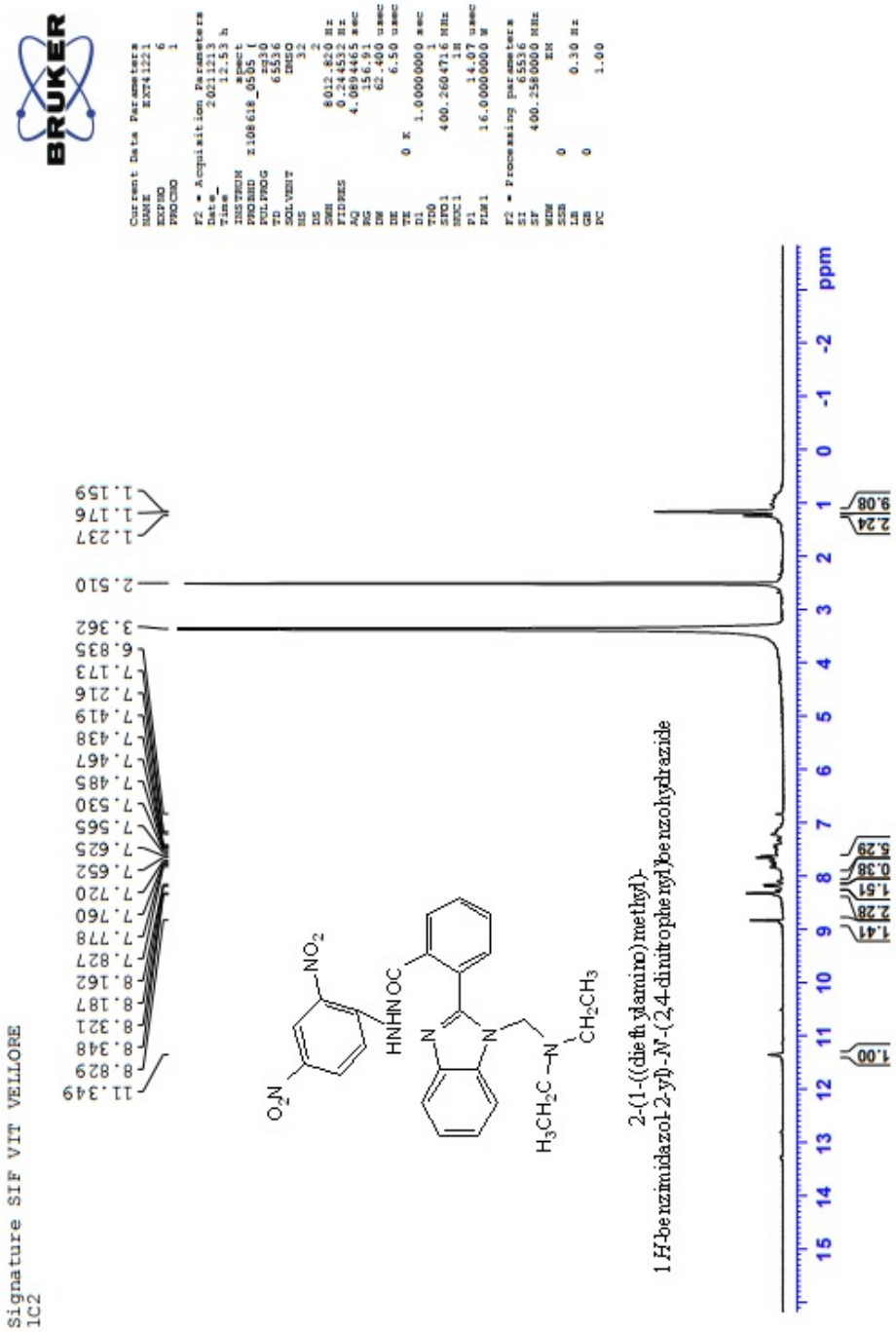
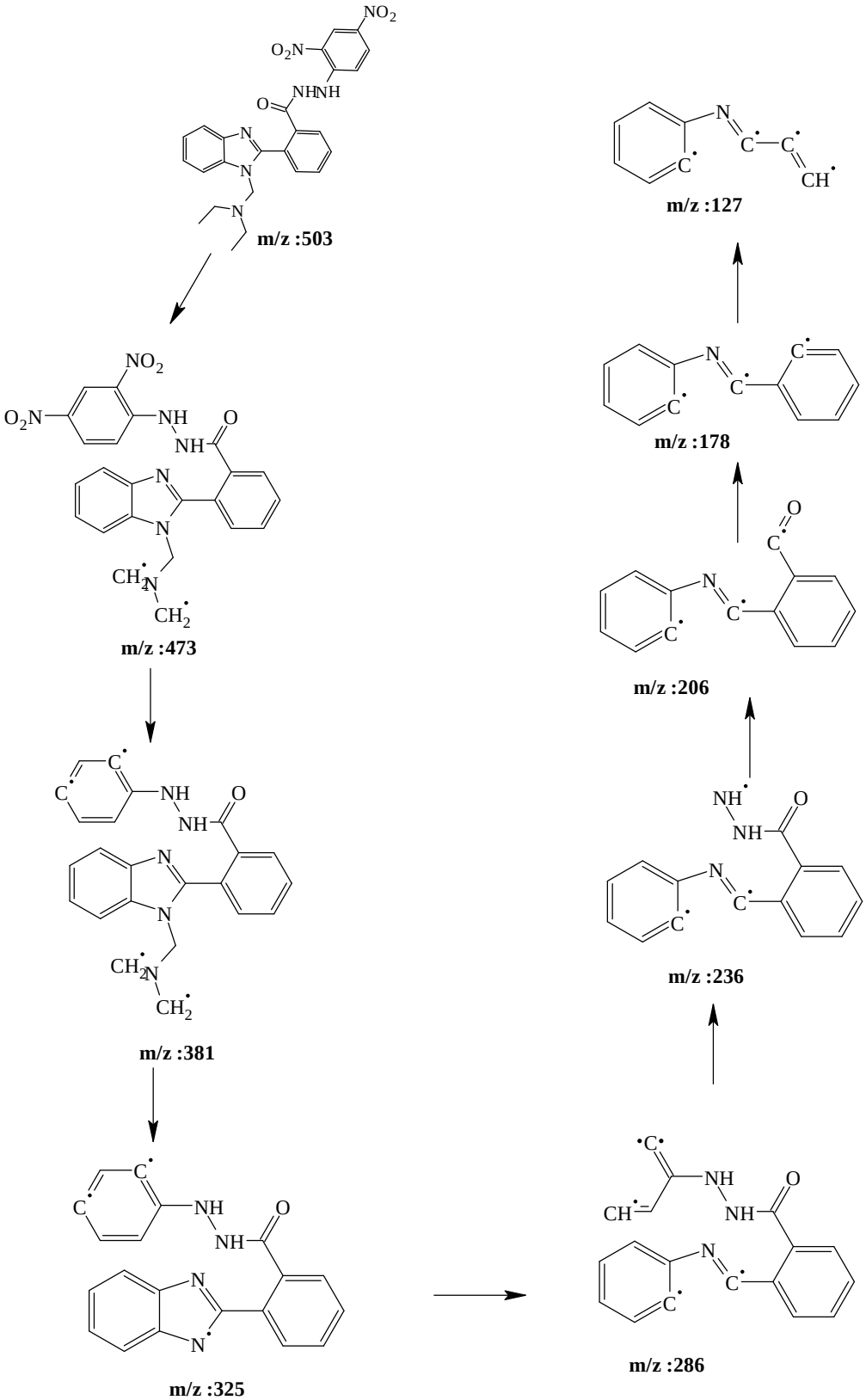


NMR SPECTRUM OF COMPOUND-1C$_2$

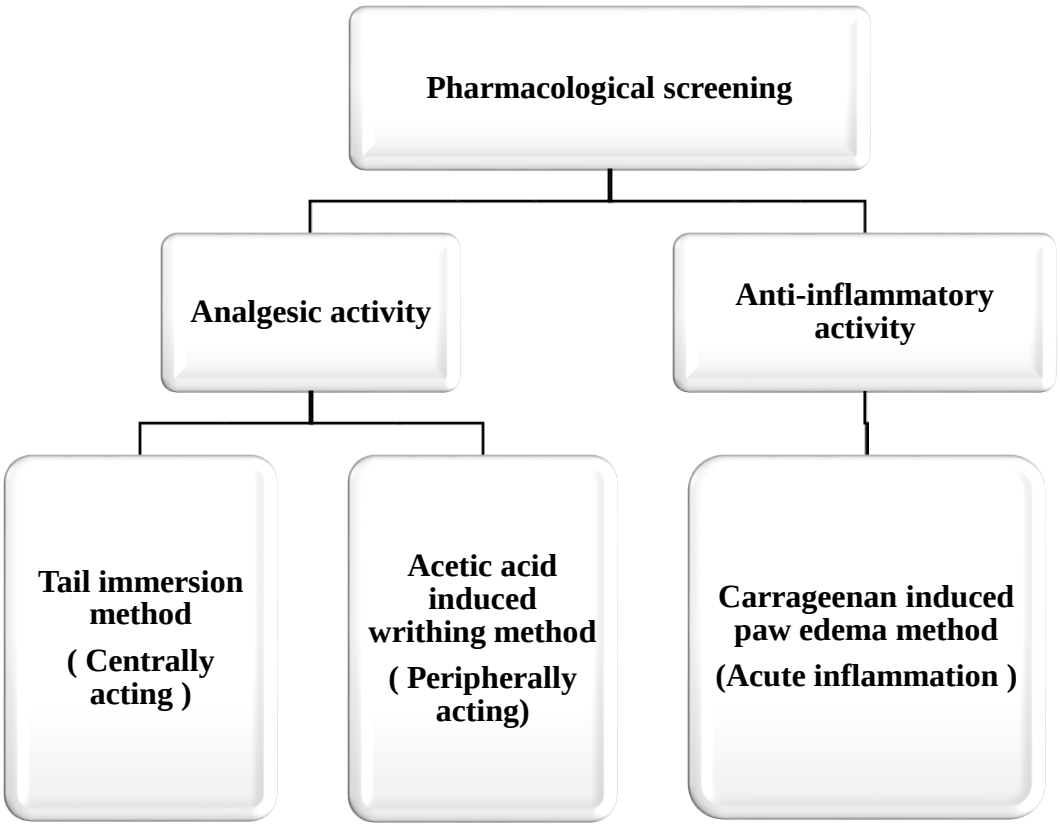
MASS SPECTRUM OF COMPOUND-1C$_2$



MASS FRAGMENTATION OF COMPOUND-1C2

PHARMACOLOGICAL SCREENING

PHARMACOLOGICAL SCREENING



ACUTE TOXICITY STUDY

Acute toxicity studies were performed according to OECD-423 (Organization of economic and cooperation development) guideline. Swiss albino mice selected by random sampling technique were employed in this study. The animals were fasted for four hours with free access to water. Various derivative of Benzimidazole compounds (1-1C_2) were administrated orally at a dose of 5 mg/kg initially and mortality if any was absorbed for first 24 hours and after 72 hrs. if mortality was absorbed in two out of three animals, then the dose administrated was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals, then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300, 1000 and 2000 mg/kg) doses of test compounds were employed for further toxicity studied. The general behavior like Sedative, Hypnosis, Convulsion, Ptosis, Analgesia, Stupar reaction, Motor activity, Muscle relaxant, Pilo erection, change in skin color, Lacrimal secretion and Stool consistency were observed during the acute toxicity study.

ANALGESIC ACTIVITY

Tail immersion method:

Mice fasted overnight will be divided into twelve groups of three animals each. Group I will be given distilled water (10 ml/kg); Group II will be administered Pentazocine (20 mg/kg).Group III to XII receive different test drugs at dose of 50 mg/kg through oral route. The lower 5cm portion of the tail was marked and this part of tail was immersed in a cap of water having temperature 55℃. Reaction time was recorded before and after the administration of drug. The pain threshold (time required for removal of tail from hot water) was measured at 0, 30 min and 1hr

Acetic acid induced writhing method:

Mice fasted overnight will be divided into twelve groups of three animals each. Group I will be administered acetic acid (i.p) alone. Group II will be administered acetic acid with Diclofenac sodium (50mg/kg). Group III to XII receive different test drugs at dose of 50 mg/kg through oral route. Sixty minutes after treatment was carried out, mice were administered with acetic acid (0.6%, v/v in saline, 10 ml/kg, i.p.). The number of writhes (characterized by contraction of the abdominal musculature and extension of the hind limbs) was then counted for 30 min at 5 min interval.

$$\text{Inhibition (\%)} = \frac{\text{Number of Writhes [Control]} - \text{Number of Writhes [Treatment]}}{\text{Number of Writhes [Control]}} \times 100$$

Statistical analysis:

Statistical significant was determined by One way Analysis Of Variance (ANOVA) followed by Dunnet`s t-test.

ACUTE ANTI-INFLAMMATORY ACTIVITY:

Carrageenan induced paw edema method:

The Wister rats were divided into twelve groups each consisting of three animals, where Group I receives negative control carrageenan, Group II receives a positive control of Indomethacin (10 mg/kg), and Group III to XII receives the synthesized benzimidazole derivatives at the dose 50 mg/kg respectively. After one hour the paw edema were induced on left hind paw of the rats into the sub plantar tissues by injecting 1% W/V of carrageenan (1ml) in saline solution. After carrageenan induction, the paw perimeters of the rats were measured at hourly intervals for 4hrs by using Vernier calipers. The right hind paw of the rats were measured as normal which was not inflamed, the paw perimeter were compared with the standard group (Indomethacin) for evaluation of anti-inflammatory activity. The percentage inhibition of anti-inflammatory activity was calculated by the following formula,

$$\% \text{ Inhibition} = T_c\text{-}T_t/T_c \times 100$$

Where,

T_c- Thickness of paw perimeter in Control

T_t- Thickness of paw perimeter in Test

Statistical analysis:

Statistical significant was determined by One way Analysis Of Variance (ANOVA) followed by Dunnet`s t-test

RESULT AND DISCUSSION

RESULT AND DISCUSSION

Benzimidazole was synthesized from the *O*-phenylenediamine and phthalic acid then followed by the synthesis of its derivatives as per the procedure given in experimental work. The synthesized compounds were identified by its physical characterization like molecular weight, melting point (Table 1) and its spectral interpretation of IR, NMR & Mass spectral values (Figure 1).

Condensation of *O*-phenylenediamine and phthalic acid gives 2-substituted Benzimidazole then substitution of hydrazine containing compounds at 2nd position of benzoic acid present in benzimidazole nucleus and 1st position of benzimidazole undergoes Mannich reaction, a three component organic reaction involves the amino alkylation of acidic proton next to a carbonyl functional group by a formaldehyde and a primary/ secondary amine or ammonia. The final product is a β amino carbonyl compound called as mannich base.[42]

Table 1 Physical Characterization of Synthesized Benzimidazole and its derivatives

S. no	Code	Molecular formula	Molecular weight (g)	Melting point(℃)	R_f value	Wavelength λmax (nm)	% yield (w/w)
1	1	$C_{14}H_{10}N_2O_2$	238.24	158-166	0.62	297.40	60.49%
2	1A	$C_{14}H_{12}N_4O$	252.27	218-220	0.61	293.00	39.08%
3	$1A_1$	$C_{17}H_{19}N_5O$	309.37	149-152	0.50	282.40	43.71%
4	$1A_2$	$C_{17}H_{23}N_5O$	337.42	127-130	0.69	285.60	38.92%
5	1B	$C_{20}H_{16}N_4O$	328.37	229-231	0.49	286.00	40.9%
6	$1B_1$	$C_{23}H_{23}N_5O$	385.46	199-210	0.59	279.20	61.53%
7	$1B_2$	$C_{25}H_{27}N_5O$	413.51	188-190	0.61	283.60	58.33%
8	1C	$C_{20}H_{14}N_6O_5$	418.36	235-237	0.53	356.80	67.85%
9	$1C_1$	$C_{23}H_{21}N_7O_5$	475.46	217-219	0.57	282.40	65.51%
10	$1C_2$	$C_{25}H_{25}N_7O_5$	503.51	199-210	0.67	285.60	61.72%

Table 2 Solubility of synthesized Benzimidazole and its derivatives

Codes	Hexane	Ethyl Acetate	DMSO	Chloroform	Acetone	Ethanol	Methanol	Water
1	+	++	+++	++	++	++	++	++
1A	+	++	+++	++	+++	++	++	++
$1A_1$	+	++	+++	++	++	++	+++	++
$1A_2$	+	++	+++	++	++	++	++	++
1B	+	++	+++	++	+++	++	++	++
$1B_1$	+	+++	+++	++	++	++	+++	++
$1B_2$	+	+++	+++	++	++	++	+++	++
1C	+	++	+++	++	+++	++	++	++
$1C_1$	+	++	+++	++	+	++	++	++
$1C_2$	+	++	+++	++	+	++	++	++

+ - Insoluble ++-partially soluble +++- soluble

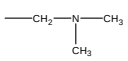
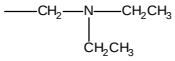
SPECTRAL INTERPRETATION

Identification of compound-1 from the IR spectrum by the appearance of peaks at 3381.73 cm^{-1} for hetero aromatic N-H Stretching and aromatic carboxylic acid peaks were obtained at 1646.13cm^{-1} of C=O Stretching & OH Stretching peak at 3159.18 cm^{-1} and from its NMR spectrum, the appearance of multiplet peak at δ 7.0-7.9 due to Aromatic CH proton

of benzene, singlet peak at δ 6.4 may due to hetero aromatic N-H proton and δ 9.7 is due to the proton of –COOH group.

IR spectrum of compounds – 1A, 1B, 1C, were identified as 1H benzimidazole benzohydrazide by the absence of aromatic acid (OH Stretching) at the range of 2500-3000 cm^{-1} & the formation of benzohydrazide peaks at 1646.13-1653.85 cm^{-1} of amide (C=O Stretching), 3178-3196 cm^{-1} of amide (N-H Stretching) & N-N Stretching at 3326-3461 cm^{-1} and from the NMR spectrum, the peaks of multiplet of phenyl ring protons, two singlet for –NH-NH- proton of benzohydrazide group and singlet for hetero aromatic NH proton.

	1A	1B	1C
Aromatic CH proton (phenyl ring) δ	7.0-7.9	7.2-7.9	7.2-7.8
-NH-NH- proton (benzohydrazide) δ	8.0, 2.5	8.0, 3.4	8.2, 3.4
Hetero Ar NH proton (benzimidazole) δ	5.8	6.7	5.0

IR Spectrum of compounds-$1A_1$, $1A_2$, $1B_1$, $1B_2$, $1C_1$ & $1C_2$, were identified as dimethylamino -2-benzohydrazide benzimidazole($1A_1$, $1B_1$ & $1C_1$) and diethylamino -2-benzohydrazide benzimidazole ($1A_2$, $1B_2$ & $1C_2$), the appearance of  &  peaks at the range of 1417-1458 cm^{-1} for (-CH_2- Stretching), 1109.95- 1417 cm^{-1} for C-N stretching and from its NMR spectrum, the peaks multiplet of phenyl ring protons, two singlet peaks for –NH-NH- group protons of benzohydrazide. In these compounds, hetero aromatic NH group proton peaks were replaced by -CH_2 group proton peak of mannich base and in addition to those peaks -CH_3 proton peaks for $1A_1$, $1B_1$ & $1C_1$ and for $1A_2$, $1B_2$ & $1C_2$, the peaks multiplet of phenyl ring protons, two singlet peaks for –NH-NH- group protons of benzohydrazide. In these compounds, hetero aromatic NH group proton peaks were replaced by -CH_2 group proton peak of mannich base and in addition to those peaks -CH_2 CH_3 proton peaks

	$1A_1$	$1B_1$	$1C_1$
Aromatic CH proton (phenyl ring) δ	7.2-7.9	7.0-7.9	7.2-7.9
-NH-NH- proton (benzohydrazide) δ	8.1	8.0	8.0
-CH_2 proton (mannich base) δ	3.3	3.4	3.3

-CH_3 proton (dimethyl amine) δ	2.6	2.5	2.1
	$1A_2$	$1B_2$	$1C_2$
Aromatic CH proton (phenyl ring) δ	7.2-7.8	7.1-7.9	7.2-8.8
-NH-NH- proton (benzohydrazide) δ	8.2	8.1	8.1
-CH_2 proton (mannich base) δ	3.3	3.4	3.3

Mass spectrum was taken for all the synthesized compounds (1-$1C_2$) and their respective molecular weights were obtained from their molecular ion peak. All the compounds shares the common fragmentation peaks of 220, 192, 178, 153, and 127 as shown in mass fragmentation of Figure 1.

PHARMACOLOGICAL SCREENING [43]

Acute toxicity studies:

Acute toxicity study was done. The general behaviour was observed during the acute toxicity study results of acute toxicity of test compounds where shown on the Table 3.

Table 3 Oral Acute toxicity study of synthesized Benzimidazole and its derivatives

S. No	General Behaviour	Compounds									
		1	**1A**	**1B**	**1C**	**$1A_1$**	**$1A_2$**	**$1B_1$**	**$1B_2$**	**$1C_1$**	**$1C_2$**
1	Sedation	-	-	-	-	-	-	-	-	-	-
2	Hypnosis	-	-	-	-	-	-	-	-	-	-
3	Convulsion	-	-	-	-	-	-	-	-	-	-
4	Ptosis	-	+	+	+	-	-	-	-	+	-
5	Analgesia	+	+	+	+	+	+	+	+	+	+
6	Stupar reaction	-	-	-	-	-	-	-	-	-	-
7	Motor activity	-	-	-	-	-	-	-	-	-	-
8	Muscle relaxant	-	-	-	+	-	+	-	-	+	-
9	CNS stimulant	-	-	-	-	-	-	-	-	-	-
10	CNS depressant	-	-	-	-	-	-	-	-	-	-
11	Pilo excretion	-	-	-	-	-	-	-	-	-	-
12	Skin colour	-	-	-	-	-	-	-	-	-	-
13	Lacrimation	-	-	-	-	-	-	-	-	-	-
14	Stool Consistancy	-	-	-	-	-	-	-	-	-	-

+ Present **- Absent**

The test compounds produce only analgesic and muscle relaxant behaviour, it does not produce any other general behaviours in acute toxicity studies, mortality was not also observed during and at the end of the study, which shows that the test compounds upto 2000 mg/kg were safe after single oral administration in mice.

Analgesic activity

The synthesized benzimidazole derivatives were evaluated for analgesic activity of centrally acting mechanism by tail immersion method and peripherally acting mechanism by acetic acid induced writhing method.

Tail immersion method:

Central pain mechanism involves the major organs brain and spinal cord. The targets of the pain mediators (substance P, endogenous opioids, somatostatine, inhibitory hormones are located at the dorsal part of the spinal cord. The established methods for central analgesic effects are tail flick, tail immersion models through opioid receptors.

The compounds were evaluated for centrally acting mechanism by tail immersion mechanism which was performed on mice by marking the lower portion (5cm) of the tail and the marked portion of the tail was immersed in a cap of water having a temperature of 55°C and the compounds were administered orally at the dose of 50 mg/kg.The pain threshold were measured at 0, 30 min and 1hr which was summarized in the Table 4.

Table 4 Effect of Analgesic activity by Tail Immersion method

Grouping	Initial	After 30min	After One Hour
Control(water)	5.55 ± 0.61	5.8 ± 0 .98	5.75 ± 1.02
Standard-Pentazocine sodium(20 mg/kg)	5.00 ± 0.93	5.75 ± 0.64	10 ± 0.57
Compound-1 (50 mg/kg)	5.83 ± 0.74	7 ± 0.63	17.33*** ± 1.20
Compound-1A (50 mg/kg)	5.66 ± 0.66	13.66*** ± 1.22	20.05*** ± 1.25
Compound-1B (50 mg/kg)	5.89 ± 0.87	12.5*** ± 0.88	20.66*** ± 1.20
Compound- 1C (50 mg/kg)	7.66 ± 1.05	13.5***± 1.33	22.33*** ± 1.05
Compound-$1A_1$ (50 mg/kg)	6.33 ± 0.55	9.83 ± 0.79	12.16 ± 0.60
Compound-$1A_2$ (50 mg/kg)	6.33 ± 0.55	11.33** ± 0.8	18** ± 0.68
Compound-$1B_1$ (50 mg/kg)	5.83 ± 0.80	11.33** ± 0.80	12.33* ± 0.76
Compound-$1B_2$ (50 mg/kg)	6.5 ± 1.04	10.03* ± 1.06	12.14* ± 0.82
Compound-$1C_1$ (50 mg/kg)	6.53 ± 0.49	11.5* ± 0.76	14.5* ± 1.08
Compound-$1C_2$ (50 mg/kg)	5.66 ± 0.49	11.16* ± 1.30	15.66** ± 0.66

All the values are mean ± SEM n=3 One way analysis of variance followed Dunnet`s t- test was performed .As a significance level of *$p<0.05$, **$p<0.01$, ***$p<0.001$ when compare with the standard.

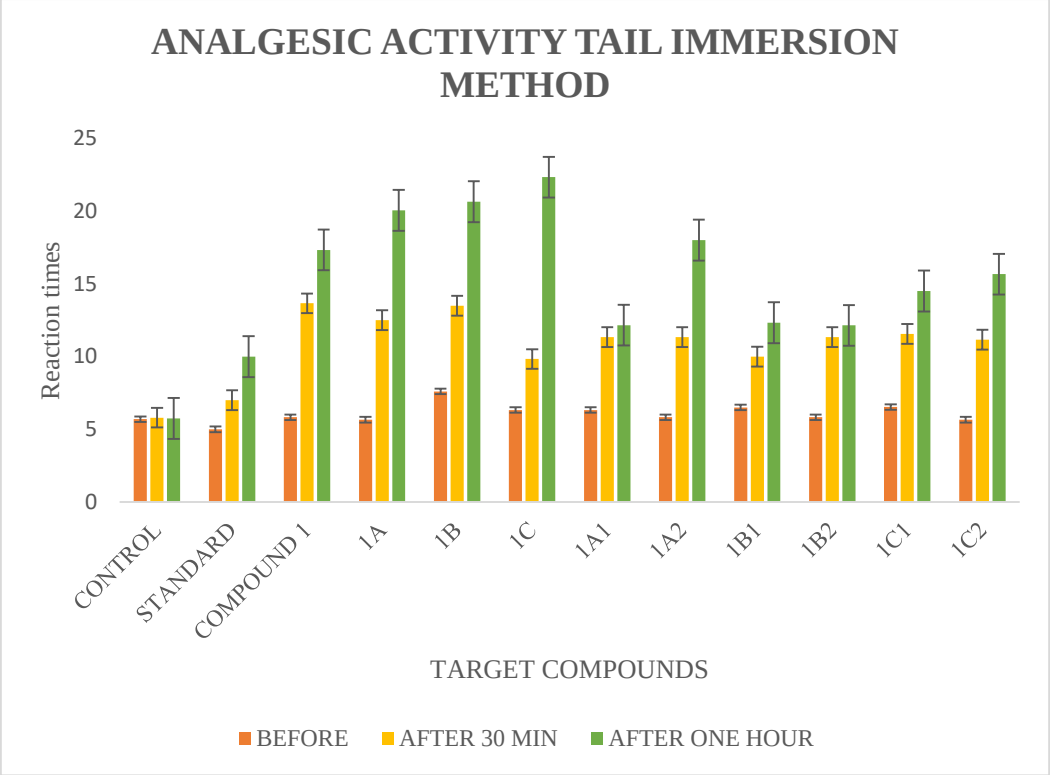


Figure 2 Effect of Analgesic activity by Tail immersion method

Compounds 1A, 1B, 1C showed the better activity, Compounds 1, $1A_2$ and $1C_2$ showed the good activity, Compounds $1A_1$,$1B_1$, $1B_2$ and $1C_1$ showed the moderate activity when compare with the standard.

Acetic acid induced writhing method:

The compounds were subjected to peripherally acting analgesic activity by acetic acid induced writhing performed on mice by administering the inducing agent through intraperitonial route and the compounds were given at the dose of 50 mg/kg through oral route. The number of writhes were counted and compared with the standard writhes for 30 min at 5min interval which was summarized in Table 5.

Table 5 Effect of Analgesic activity by Acetic acid induced writhing method

Grouping	Standard mean error	Percentage of inhibition
Control (Acetic acid 0.6% V/V)	26.33 ± 0.76	
Standard-Diclofenac sodium (20 mg/kg)	10.33 ± 1.38***	60.76%
Compound- 1 (50 mg/kg)	8.33 ± 0.49***	68.36%
Compound-1A (50 mg/kg)	14.00 ± 1.36**	46.82%
Compound-1B (50 mg/kg)	10.66 ± 0.98***	59.51%
Compound-1C (50 mg/kg)	9.5 ± 0.76***	63.9%
Compound-$1A_1$ (50 mg/kg)	18.33 ± 1.15*	30.38%
Compound-$1A_2$ (50 mg/kg)	14.33 ± 0.98**	45.57%
Compound-$1B_1$ (50 mg/kg)	16.83 ± 1.01*	36.08%
Compound-$1B_2$ (50 mg/kg)	17.33 ± 1.11*	34.18%
Compound-$1C_1$ (50 mg/kg)	15.33 ± 0.71**	41.70%
Compound-$1C_2$ (50 mg/kg)	14.5 ± 0.84**	44.90%

All the values are mean ±SEM n=3 One way analysis of variance followed the Dunnet`s t -test was performed. As a significance level of *p<.05, **p<0.01, ***p<0.001 when compare with the standard.

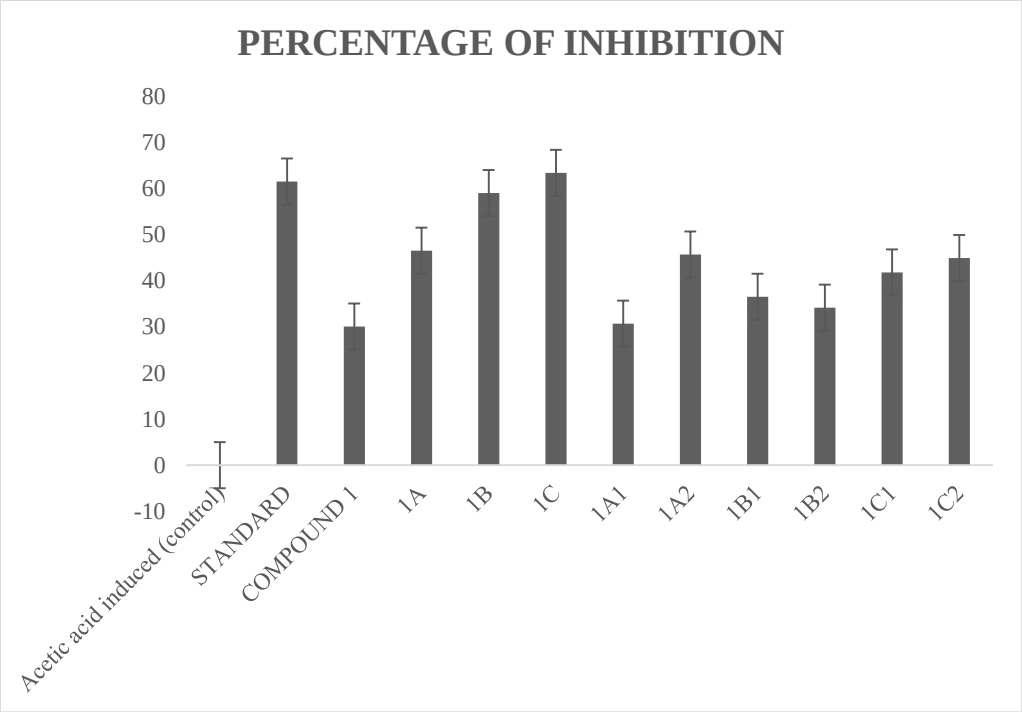


Figure 3 Effect of Analgesic activity by Acetic induced writhing method

Intraperitoneal administration of acetic acid produced both peripheral and central nociception action due to the release of endogenous mediators and blocked by non-steroidal anti-inflammatory drugs.

Compounds-1, 1B and 1C showed better reduction in writhing and stretching induced by acetic acid , Compounds- 1A, $1A_2$, $1C_1$ and $1C_2$ showed good reduction in writhing and stretching induced by acetic acid , Compounds- $1A_1$, $1B_1$ and $1C_1$ showed moderate reduction in writhing and stretching induced by acetic acid when compared with the standard drug. This reduction in writhing and stretching might be due to blockade of the release of endogenous substance.

Acute anti- inflammatory activity:

Carrageenan induced paw edema method:

The compounds were performed for acute anti-inflammatory activity by carrageenan induced paw edema model in which the carrageenan was injected intra peritoneally and the compounds were administered at the dose of 50 mg/kg through oral route. The edema reduced in the synthesized benzimidazole derivatives from 1hr to 4hr was compared with the standard, which is summarized in Table 6 and its percentage inhibition was shown in the Table 7 .

Table 6 Effect of Anti-inflammatory activity by Carrageenan induced paw edema method

Groups	Paw thickness (mm)				
	15min	**30min**	**1hr**	**2 hr**	**4 hr**
Control 1% carrageenan	6.00***±0.23	6.83***±0.09	7.69***±0.38	8.46***±0.04	8.69***±0.07
Standard-Indomethacin (10 mg/kg)	5.52**±0.17	5.59**±0.14	5.86***±0.11	5.31**±0.17	4.43***±0.14
Compound-1 (50 mg/kg)	4.62**±0.10	4.85**±0.10	5.52***±0.17	4.90***±0.15	4.3***±0.14
Compound-1A (50 mg/kg)	4.7**±0.12	5.16**±0.21	5.50***±0.18	4.67**±0.12	4.50**±0.10
Compound-1B (50 mg/kg)	4.8**±0.06	5.32**±0.17	5.74***±0.02	4.56**±0.08	4.56***±0.14
Compound-1C (50 mg/kg)	5.56**±0.14	5.84**±0.06	6.30***±0.06	5.34**±0.09	5.43**±0.07
Compound-$1A_1$ (50 mg/kg)	4.93±0.10	5.32±0.16	6.9±0.32	6.02±0.21	6.03±0.10
Compound-$1A_2$ (50 mg/kg)	4.92±0.04	4.89±0.04	4.52±0.05	4.50±0.12	4.50±0.19
Compound-$1B_1$ (50 mg/kg)	4.69*±0.09	4.84±0.06	4.81±0.06	5.87±0.07	5.89±0.06
Compound-$1B_2$ (50 mg/kg)	5.43±0.10	5.99±0.10	6.98±0.08	6.89±0.12	6.9±0.03
Compound-$1C_1$ (50 mg/kg)	4.92**±0.09	5.23**±0.10	5.83**±0.36	4.90***±0.15	3.47***±0.14
Compound-$1C_2$ (50 mg/kg)	4.16*±0.21	6.40***±0.09	5.15**±0.06	4.56**±0.15	3.94***±0.12

All the values are mean ±SEM n=3 one way analysis of variance followed Dunnet`s t - test was performed .As a significance level of *p<.05, **p<0.01, ***p<0.001 when compare with the standard.

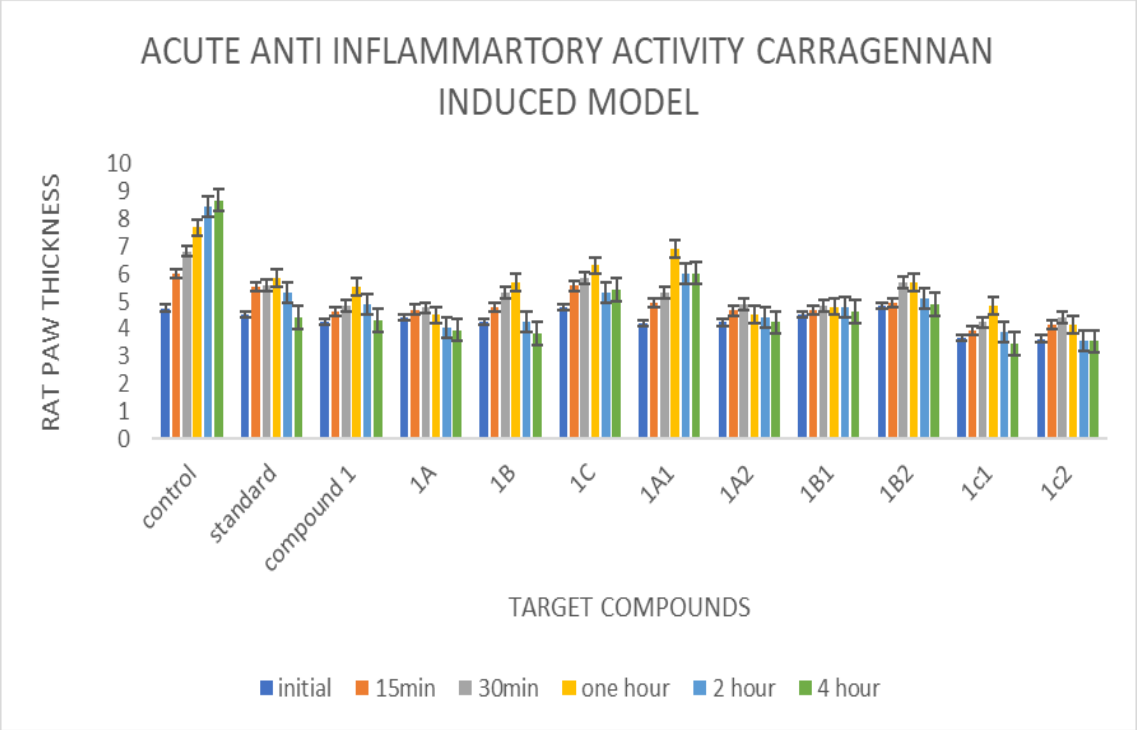


Figure 4 Effect of Anti-inflammatory activity by Carrageenan induced paw edema model

Synthesized compounds have significant anti-inflammatory effect against carrageenan induced paw edema. Carrageenan induced paw edema is one of the fruitful method to detect the orally active anti-inflammatory agents which shows biphasic response. First phase is mediated through the release of histamine, serotonin and kinins, whereas the second phase is through the release of prostaglandins. Synthesized compounds showed maximum inhibition at 4 hr.

Compounds-$1C_1$, $1C_2$ shows better inhibition, Compounds- 1, 1A, 1B and $1A_2$ shows the equal inhibition, Compounds-1C, $1A_1$, $1B_1$ and $1B_2$ shows moderate inhibition when compared with the standard drug. It might be due to the inhibition of the release of prostaglandins.

Table 7 Percentage inhibition of Anti-inflammatory activity by carrageenan induced paw edema method

GROUPS	15min	30min	1hr	2 hr	4 hr
Standard-Indomethacin(10 mg/kg)	8.00%	18.45%	23.79%	37.23%	49.02%
Compound-1 (50 mg/kg)	23.00%	28.98%	28.21%	42.08%	50.51%
Compound-1A (50 mg/kg)	21.66%	24.45%	28.47%	44.79%	48.21%
Compound-1B (50 mg/kg)	20.00%	22.10%	25.35%	46.09%	47.52%
Compound-1C (50 mg/kg)	7.33%	14.49%	18.07%	36.87%	37.51%
Compound-$1A_1$ (50 mg/kg)	17.83%	22.16%	10.27%	28.84%	30.60%
Compound-$1A_2$ (50 mg/kg)	18.00%	28.40%	41.22%	46.80%	48.21%
Compound-$1B_1$ (50 mg/kg)	21.83%	29.13%	37.45%	30.61%	32.22%
Compound-$1B_2$ (50 mg/kg)	9.5%	12.29%	9.23%	18.55%	30.95%
Compound-$1C_1$ (50 mg/kg)	18.00%	23.42%	24.18%	42.08%	60.06%
Compound-$1C_2$ (50 mg/kg)	30.66%	6.29%	33.02%	46.09%	54.66%

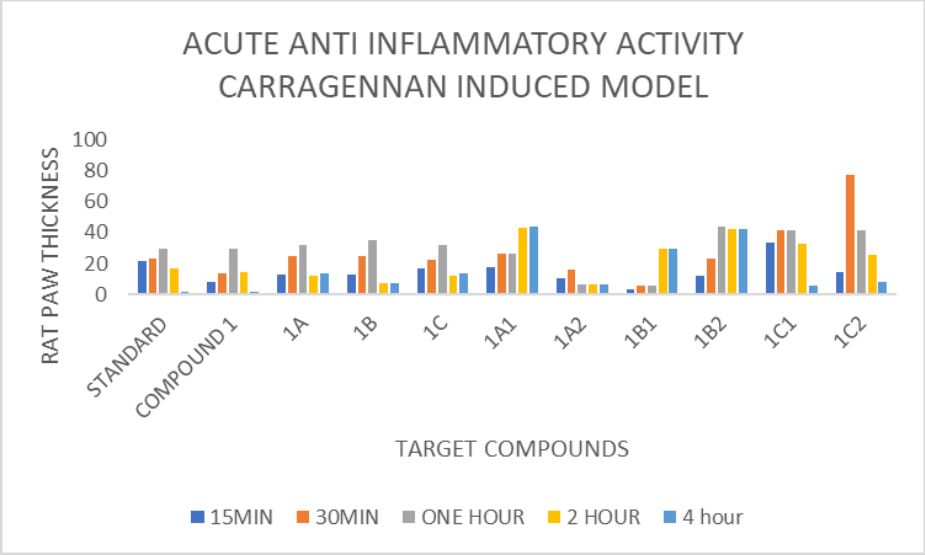


Figure 5 Percentage inhibition of acute anti-inflammatory activity by carrageenan induced paw edema method

Compounds-$1C_1$ (60.06 %), $1C_2$ (54.66 %) shows better the percentage of inhibition, Compounds- 1(49.02 %), 1A (50.51%), 1B (48.21 %) and $1A_2$ (48.21 %) shows the equal percentage of inhibition, Compounds-1C (37.51 %), $1A_1$ (30.60 %), $1B_1$ (32.22%) and $1B_2$ (30.95%) shows moderate significance level when compare with the standard.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Synthesis of benzimidazole was done from the condensation of *O*-phenylenediamine and phthalic acid. Benzohydrazide benzimidazole was synthesized by the substitution of hydrazine compounds at 2nd position benzoic acid of benzimidazole followed by the mannich formation at 1st position of benzimidazole.

Characterization of compounds were studied by its physical, chemical properties and the spectral interpretation by IR, NMR and Mass spectrums.

Substituted Benzimidazoles were subjected to pharmacological screening of analgesic and anti-inflammatory properties.

It was concluded that from the synthesized substituted benzimidazoles, most of the compounds shows better response to targeted studies.

Further this study may be continued for further substitution to make new moieties and will be focused to study the pharmacological screening like anti-microbial, diabetic activities.

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