CODEN: WJAPAC Impact Factor: 5.48 ISSN: 3049-3013

World Journal of Advance Pharmaceutical Sciences

Volume 3, Issue 4, Page: 43-62 Research Article www.wjaps.com

DESIGN AND BIOLOGICAL EVALUATION OF MERCAPTOBENZIMIDAZOLE DERIVATIVES AS EMERGING ANTIMICROBIAL AGENTS

Nasiruddin Ahmad Farooqui*[1], Sourabh Singh[1], Lalita Tyagi[2], Monika[2], Praveen Kumar[1]

[1]Translam Institute of Pharmaceutical Education and Research Meerut, Uttar Pradesh.
[2]Kalka Institute for Research and Advanced Studies, Meerut, Uttar Pradesh.

How to cite this Article: Nasiruddin Ahmad Farooqui*[1], Sourabh Singh[1], Lalita Tyagi[2], Monika[2], Praveen Kumar[1] (2026). DESIGN AND BIOLOGICAL EVALUATION OF MERCAPTOBENZIMIDAZOLE DERIVATIVES AS EMERGING ANTIMICROBIAL AGENTS. World Journal of Advance Pharmaceutical Sciences, 3(4), 43-62.

Article Info

Article Received: 12 February 2026,

Article Revised: 02 March 2026,

Article Accepted: 22 March 2026.

DOI: https://doi.org/10.5281/zenodo.19307077

***Corresponding author:**

Nasiruddin Ahmad Farooqui

Translam Institute of Pharmaceutical Education and Research Meerut, Uttar Pradesh.

ABSTRACT

Benzimidazoles are a group of hererocyclic compounds having excessive structural flexibility, physicochemical stability, as well as promising biological activities. A series of 1, 2-disubstituted mercaptobenzimidazole derivatives has been synthesized by the nucleophilic substitution of various alkyl halides at C-2 of mercaptobenzimidazole, as a new class of compound in this study. Thin Layer Chromatography (TLC), Fourier-Transform Infrared Spectroscopy (FT-IR), and Proton Nuclear Magnetic Resonance (^1H NMR) spectroscopy were used to characterize the synthesized compounds. The produced compounds were examined for antibacterial activity against the bacteria (Staphylococcus aureus, Bacillus subtilis, and Escherich coli) using the agar well diffusion method and ciprofloxacin as a reference drug. From these synthesized derivatives, BK-3 and BK-5 have been found to possess excellent activity and the rest BK-2, BK-4, BK-6 and BK-8 exhibited good activity. The antibacterial activity of these derivatives was not superior to that of ciprofloxacin, but it suggested that 1,2-disubstituted mercaptobenzimidazole could serve as a lead for developing new antibiotics. The present study highlights the significance of the strategic substitution on benzimidazole core to enhance the biological activity and will support further structure-activity relationship (SAR) studies.

KEYWORDS: Benzimidazole, 1,2-disubtituted derivatives, antimicrobial activity, synthesis, characterization, Staphylococcus aureus, Escherichia coli, FT-IR, 1H NMR.

Highlights

1. A new series of 1,2-disubstituted mercaptobenzimidazole derivatives were prepared via nucleophilic substitution at C-2 of mercaptobenzimidazole.

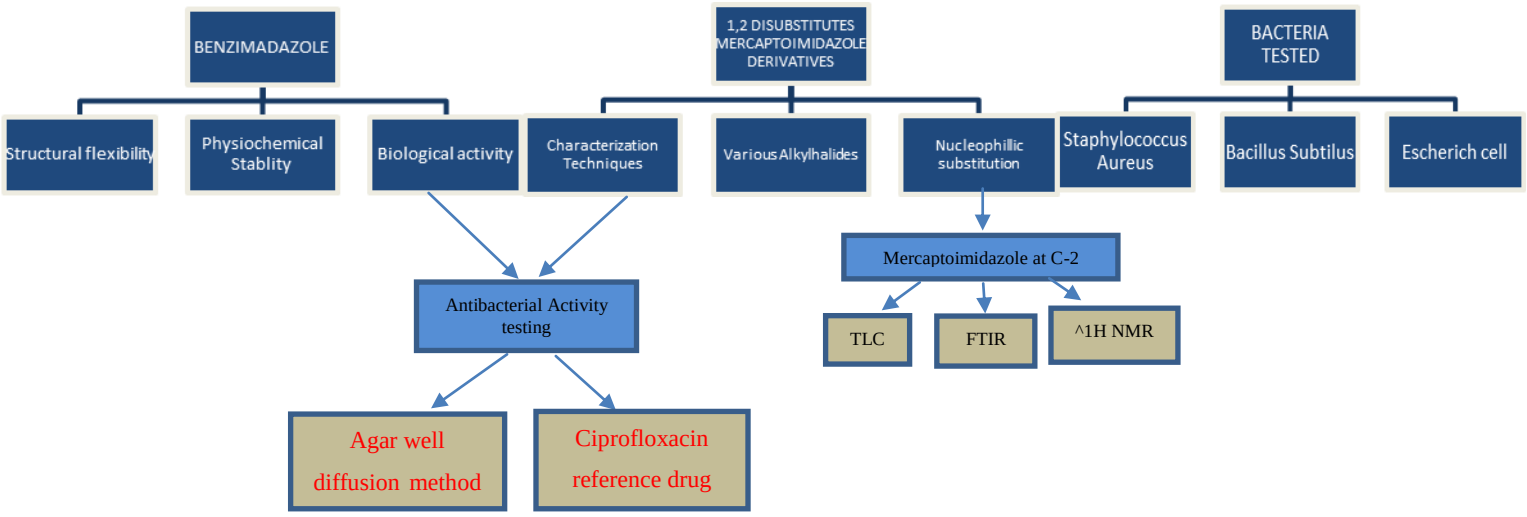
2. The isolates were established structurally through TLC, FT-IR and ¹H NMR analyses.

3. The antibacterial activities were tested towards S. aureus, B. subtilis and E. coli by the agar well diffusion method.

4. Compounds BK-3 and BK-5 showed highly potent anti-bacterial activities whereas BK-2, BK-4, BK-6, and BK-8 displayed good activities.

5. Although the activities were less than that of ciprofloxacin, the compounds could serve as promising lead structures to develop new antibiotics.

Graphical Abstract



1. INTRODUCTION

Benzimidazoles represent a class of structurally robust heterocyclic compounds first identified in 1872. A few years later the same compound was synthesized by Ladenburg by refluxing 3,4-diaminotoluene with acetic acid. The benzimidazoles are also referred to as Benzozimidazoles or Benzoglyoxalines. Benzimidazoles are weak bases; they are weaker than the imidazoles. Benzimidazoles are also acid enough to be generally soluble in aqueous alkali, to provide N-metallic compounds. The acid character of benzimidazoles, like other imidazoles are suggested to result from resonance stabilization of the ion. Characterized by a fused benzene–imidazole system, they exhibit exceptional resistance to acidic, basic, and oxidative degradation, (Narasimhan *et al.*, 2012; Bansal *et al.*, 2019) making them suitable scaffolds in medicinal and industrial chemistry. Their physicochemical attributes, including a dipole moment of approximately 4D and hydrogen bonding via N–H–N interactions, contribute to their stability and solubility profiles, which are modifiable through strategic substitution at the 1- and 2-positions. (Bansal et al., 2021; Choudhary & Joshi, 2019). Substituted benzimidazoles demonstrate intramolecular chelation, further influencing behavior in biological and non-biological matrices. (Kaur et al., 2019; Rollas & Küçükgüzel, 2007).

Mechanistically, benzimidazoles function as selective anthelmintics by targeting tubulin and inhibiting microtubule polymerization, thereby disrupting cellular transport in helminths with minimal toxicity to the host. (Lacey, 1990; Horton, 2000; Ceballos et al., 2009). Pharmacokinetically, their low water solubility limits gastrointestinal absorption—a feature exploited in localized intestinal treatments. (Savjani et al., 2012; Lipinski, 2000). Upon absorption, hepatic metabolism through cytochrome P450 oxidation, hydrolysis, and subsequent conjugation leads to biliary excretion. (Zanger & Schwab, 2013; Rendic & Di Carlo, 1997; Choudhuri et al., 2010).

1.1 Uses of Benzimidazole

benzimidazole derivatives useful in the textile industry as wetting, emulsifying and/or foaming agents or as dispersants for dying.2-Aminobenzimidazoles have found use in the preparation of fluorescent dyes for such preparation as inks to take clothes to the teller. Under such circumstances, 2-mercaptobenzimidazole and the like benzimidazole derivatives have come to be employed as well for various purposes in the photographic field. They decrease photographic "Fog" and increase contrast as well as speed, so they have been utilized tn photographic developing and fixing solutions. 2 mercaptobenzimidazoles has also been found to be useful as a rubber antioxidant. (Pathare, B., & Bansode, T, 2021)

1.2 Properties of Benzimidazole

Benzimidazole with the imide nitrogen (i.e, hydrogen at l-position) is generally more soluble in polar solvents and less soluble in hot water but sparingly soluble in ether and almost insoluble in benzene. Benzimidazoles are weak bases, less basic than the imidazoles by some measure. They are usually soluble in dilute acids. The more acidic benzimidazoles may be dissolved in solutions that are less basic than sodium carbonate solution including potassium carbonate solution. 2(3H)-benzimidazolone was slightly soluble in dilute sodium hydroxide solution. (Alamgir et.al, 2007)

1.3 Mechanism of Action

The main action of the benzimidazole is a result of their binding to the protein tubulin thereby inhibiting polymerization of tubulin to microtubules. By binding to tubulin, it inhibits self-association of subunits and also the “capping” of the microtubule on the associating end of the microtubule. It is reported that benzimidazole may also target mammalian tubulin, however these agents are

lethal only to helmnthes and they have little toxicity to their hosts as anthelmintics. (Lacey, E., & Gill, J. H. 1994)

1.4 Metabolism

It is also not very water soluble and consequentially is poorly absorbed from the GI tract (fat containing meal increases absorption). Poor absorption might be advantageous as the drugs are generally used to treat gastrointestinal helminthes.

So far as absorbed, they undergo nearly immediate deactivation in the liver and pass out into the bile. The aglycone is generally readily and almost completely metabolized with a predominance of oxidative and hydrolytic degradation routes. The oxidative reaction of phase I is usually a cytochrome P-450 catalyzed reaction and can be followed by conjugation in stage II. (Zhao et. al, 2021)

1.5 Natural Products Containing Benzimidazole Nucleus

The Benzimidazole ring does not seem to be particularly common in nature. But the 5,6-dimethylbenzimidzole unit has now been demonstrated to be a component of the vitamin B12 structure very recently. Acid-hydrolysis of Vitamin B 12 gives three closely-related substances called compounds – alpha, beta & gamma. Component γ is 5, 6-dimethylbenzimidazole. (Kabi et.al, 2022).

1.6 Antimicrobial Agents

1.6.1 Definition and Characteristics

Antimicrobials are substances that kill or inhibit bacteria, fungi, and protozoans, functioning as microbiocidal (killing) or microbiostatic (growth-inhibiting) drugs. (Kohanski et al., 2010; Spellberg & Gilbert, 2014). Disinfectants are antimicrobials applied to inanimate surfaces. (McDonnell & Russell, 1999).While antibiotics technically denote microbial metabolites, the term now broadly refers to any antibacterial agent. (Clardy, Fischbach, & Walsh, 2006; Davies & Davies, 2010). Internal infections cannot be cured by sterilization or pasteurization; instead, chemotherapeutic antimicrobials—both natural antibiotics and synthetic compounds are administered. (Davey et. al, 2015).

1.6.2 Antimicrobial Agents' Mode of Activity

Antimicrobial drugs have multiple modes of action: Figure 1

- The inhibition of the synthesis of cell walls
- The inhibition of DNA/RNA synthesis,
- cytoplasmic membrane,
- metabolism,
- protein synthesis

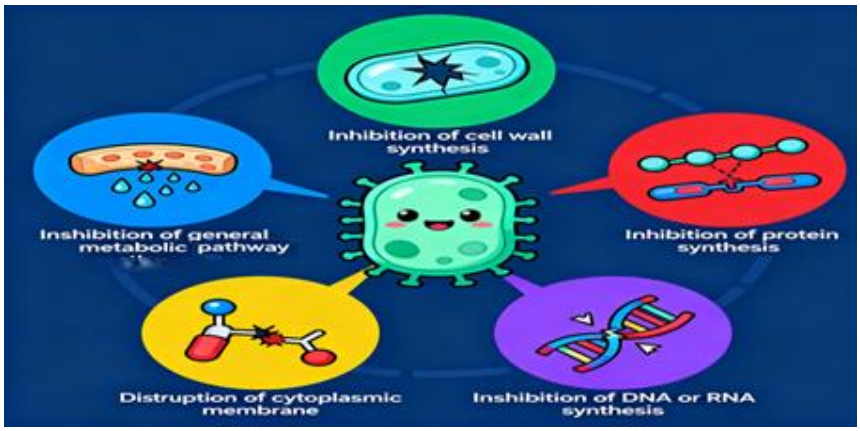


Fig 1: Mechanism of Action of Antimicrobial Agents.

1.6.2.1. Inhibition of Bacterial Cell Wall Synthesis

The peptidoglycan mesh that makes up the bacterial cell wall is inflexible. N-acetylmuramic acid (NAM) and N-acetylglucosamine alternate to form peptididoglycan. (NAG) chains that are joined by peptide bonds. (Silhavy, et.al, 2010). (Fig-2)

- **Penicillin:** Only in developing bacteria that are producing new peptidoglycan does this antibiotic prevent the fusing of the extracellular matrix. It prevents the polypeptide chains of the cell wall peptidoglycan from being cross-linked by the transpeptidation enzymes. Additional antibiotics that block bacterial cell walls include Glycopeptides, Ampicillin, and Cephalosporin. (Vollmer et al. 2008 and Walsh 2003).(Fig-3)
- **Vancomycin:** Many bridges that unite NAM subunits in Gram-positive bacteria are disrupted as this antibiotic. (Bugg et al., 1991).
- **Bacitracin:** This antibiotic blocks the secretion of NAG and NAM from the cytoplasm of Gram-positive bacteria. (Schnell et al., 2019).

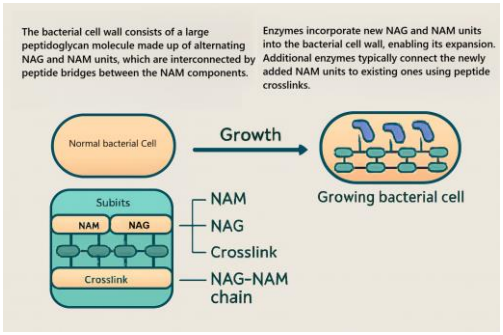


Fig 2: Growth of bacterial cell wall.

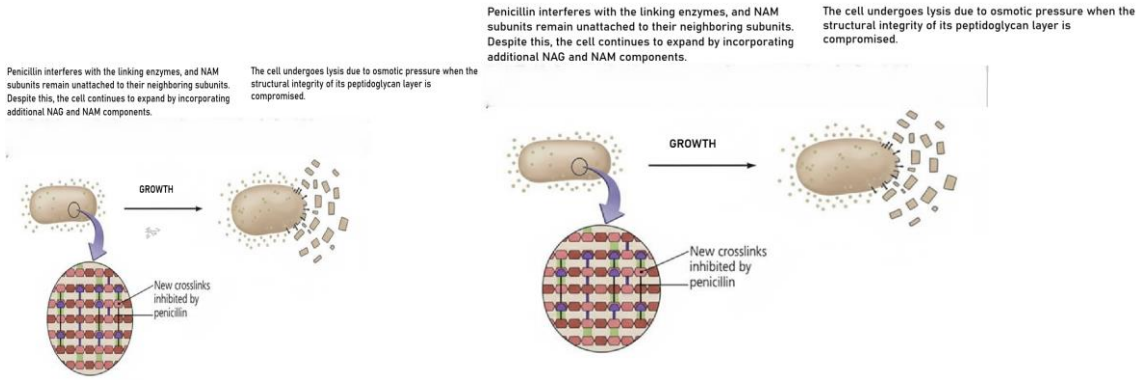


Fig 3: Penicillin inhibit bacterial cell wall.

1.6.2.2. Inhibition of protein synthesis

- Ribosomes are primary structures for production of protein in a cell. (Alberts et al., 2015). They have two major subunits: the subunits 30S and 50S. The 50S subunit also creates peptide bonds between amino acids, and both are essential for reading codons and initiating protein synthesis. (Berlanga, M, 2010; Nelson et.al, 2008). 70S Ribosomes formation shown in fig-4.

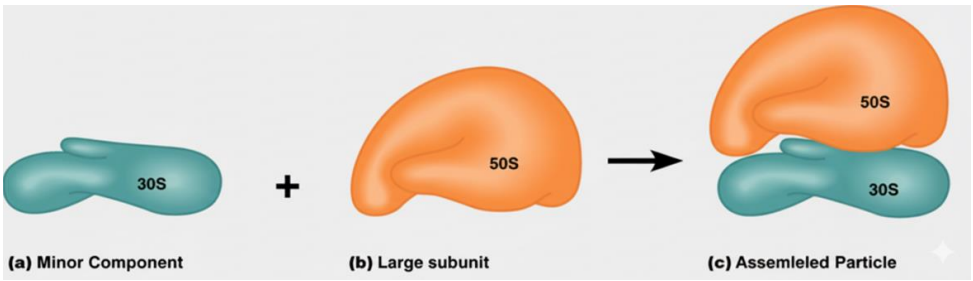


Fig 4: Formation of 70S Ribosome.

- **Aminoglycosides:** These drugs change the shape of the 30S subunit, causing the mRNA to be misread and inhibiting protein synthesis. Examples include Streptomycin and Gentomycin. (Kotra et al., 2000; Krause et al., 2016)
- **Tetracycline:** This antibiotic blocks the docking site of tRNA on the ribosome. (Chopra & Roberts, 2001).
- **Chloramphenicol:** This antibiotic stops peptide chain synthesis and elongation by stopping peptidyl transferase from working on the 50S ribosomal subunit. (Wilson, 2014).
- **Macrolides:** These drugs bind to the 50S ribosome and prevent its movement from one codon to the next, halting translation. An example is Erythromycin. (Kannan et al., 2014).

1.6.2.3. Disruption of cytoplasmic membranes

Plasma membranes are phospholipid bilayers. Fungi have a sterol called ergosterol, whereas human membranes have cholesterol. (Ghannoum & Rice, 1999; Odds et al., 2003). Three antifungal drugs use this difference:

- **Polyenes:** Attach to ergosterol in the membrane. (Gandra et al., 2021).

- **Azoles:** Inhibit ergosterol synthesis. (Bhattacharya et al., 2020).
- **Polymyxin:** It binds to plasma membrane and disrupts its structure and properties of permeability. Effective against Gram-negative bacteria. Ex. *Pseudomonas.* (Falagas & Kasiakou, 2005).

1.6.2.4. Inhibit metabolism

Antibiotics that competitively inhibit important enzymes can prevent metabolic pathways from working. We refer to these useful medications as antimetabolites. Ex. - Trimethoprim, Sulfonamides. (Zhao et al., 2021; Bush & Bradford, 2019).

- **Sulfonamides**

These medications work by competing with p-aminobenzoic acid to block the metabolism of folic acid. Sulfonamides inhibit DNA/RNA synthesis, which in turn inhibits the production of proteins. (Sköld 2000).

- **Trimethoprim**

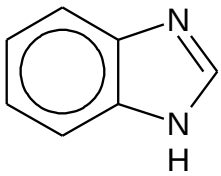
It inhibits the enzyme dihydrofolate reductase, which prevents the formation of tetrahydrofolate. (Gordillo et al., 2021).

1.6.2.5. Inhibit DNA/RNA synthesis

The typical nucleotides found in DNA and RNA are mimicked by an array of substances known as nucleotide analogs. They stop additional replication, transcription, or translation when they get absorbed into DNA and RNA. (Jordheim et al., 2019; Götte & Feld, 2021).

- **Quinolones:** These drugs specifically attack DNA replication by targeting an enzyme associated with DNA uncoiling (DNA gyrase). Examples include Ciprofloxacin (Cipro) and Ofloxacin. (Rodrigues & silva, 2025; Collins & Osheroff, 2024; Leyn et al., 2024).
- **Rifampin:** This drug inhibits DNA-dependent RNA polymerase, an enzyme used in transcription, thus blocking protein synthesis. It is used to fight *Mycobacterium tuberculosis*. (Sudzinová P, et al., 2023; Yang KB, et al. 2023).

NUCLEUS PROFILE

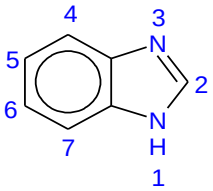


1*H*-benzimidazole

2. Chemical and physical properties of Benzimidazole (NCBI, 2025)

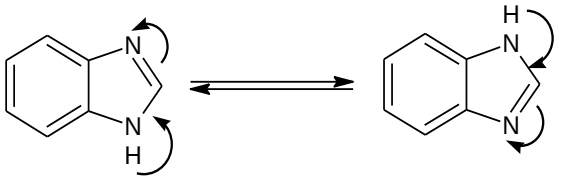
Properties	Information
Synonyms	Benzimidazole, Azindol,1,3-Benzimidazole
IUPAC Name	1H-Benzimidazole
Molecular formula	$C_7H_6N_2$
Molecular mass	118.14
Physical State	White Tabular Crystals
Melting point	170-172^oC
B.P	360^0C
Stability	Stable, Combustible
Density	1.23 g/cm^3
Flash Point	360 °C
Enthalpy of Vaporization	54.61 kJ/mol
Dipole moment	3.93 D
Pk_a	8.52
Solubility	Sparingly soluble in water

2.1 Chemistry of Benzimidazole[17]



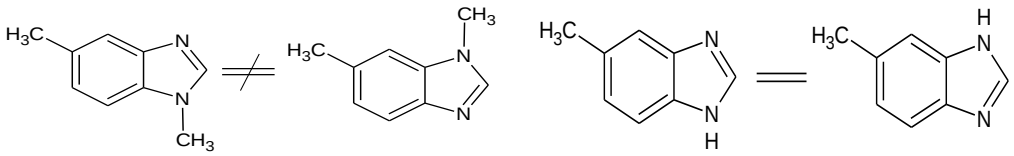
The Benzimidazole has phenyl ring fused with imidazole ring. (Farag et al., 2025; Patel et al., 2023). It also has a hydrogen atom bonded to nitrogen in the 1-position, which is easily tautomerizable.

a) Tautomerism and Isomerism



I II

Benzimidazole exhibits rapid –NH/ =N– exchange, forming tautomers via intermolecular or solvent-mediated processes, making 5- and 6-positions chemically equivalent. (Nieto, 2014). Larger 1-position substituents prevent tautomerism, yielding distinct isomers like 1,5- and 1,6-dimethyl benzimidazole. Dimethylated forms are non-equivalent isomers, while monomethylated forms are tautomeric and equivalent.



I II

3. Method of Preparation: (Alaqeel, 2017)

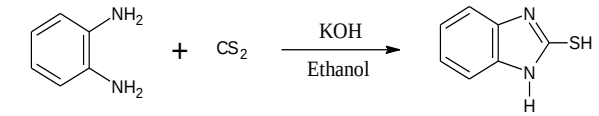
S.No.	Method	Reactants	Conditions	Product
1.	Carboxylic Acid	o-Phenylenediamine + Carboxylic Acid	Reflux	2-Substituted benzimidazole
2.	Esters	Substituted o-Phenylenediamine + Ester	225 °C, 3 hrs	Benzimidazole
3.	Amides	o-Phenylenediamine Dihydrochloride + Amide	240–250 °C	2-Substituted benzimidazole
4.	Nitriles	Nitrile + o-Phenylenediamine HCl	200 °C, Acidic	2-Substituted benzimidazole
5.	Aldehydes	o-Phenylenediamine + Aldehyde	Oxidants (e.g., Cu-acetate, $NaHSO_3$)	2-Substituted benzimidazole
6.	Ketones	o-Phenylenediamine + Ketone	—	2-Substituted benzimidazole
7.	Dibasic Acids	o-Phenylenediamine + Dibasic Acid	Heat	Bis-benzimidazole
8.	Acid Anhydrides	o-Phenylenediamine + Mono/Dibasic Anhydride	Reflux	Substituted benzimidazole
9.	Imino-ethers	o-Phenylenediamine + Benziminomethyl Ether	130 °C	2-Phenyl benzimidazole
10.	Carbon Disulfide	o-Phenylenediamine + CS_2	Alcoholic Solvent ± Alkali	Benzimidazolethiones
11.	Hydrazo Compounds	Hydrazo + CS_2	~150 °C	Benzimidazolethiones
12.	Phosgene	o-Phenylenediamine + Phosgene	Organic Solvent	Benzimidazolone
13.	o-Aminoazo Compounds	o-Aminoazo + Aldehyde	—	N-(Aryl amino) benzimidazole
14.	Urea Pathway	o-Phenylenediamine + Urea → $POCl_3$	DMF, 135–140 °C	2-Chloro benzimidazole
15.	Sulfonate Salt	o-Phenylenediamine + Sulfonate	DMF, 150 °C, 2 hrs	Substituted benzimidazole
16.	Cyanoacetate	o-Phenylenediamine + Cyanoacetate	Fusion	Benzimidazole-2-acetonitrile
17.	Cyanoacetimidate	Ethyl 2-Cyanoacetimidate + o-Phenylenediamine	Fusion	Benzimidazole-2-acetonitrile
18.	Enaminonitriles	o-Phenylenediamine + Enaminonitriles	Cyclocondensation	Benzimidazole-2-acetonitrile
19.	Hydrazides	o-Phenylenediamine + Hydrazides	Fusion	Substituted benzimidazole
20.	Phenoxyacetic Acids	o-Phenylenediamine + Phenoxyacetic Acid	—	Phenoxymethyl-substituted benzimidazole
21.	Cyanogen Bromide	o-Phenylenediamine + Cyanogen Bromide	—	2-Amino benzimidazole

4. MATERIALS AND METHODS

A. Chemicals and Reagents

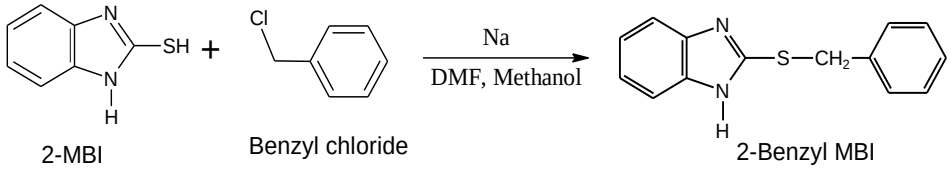
All the chemicals and solvents were of analytical quality and utilized without additional purification. The compounds' melting points were contrasted with those of the reference compounds. (Iqbal et.al, 2023). Thin layer chromatography using silica G and chloroform:methanol as the eluting system was used to verify the compounds' purity. When sprayed with an iodine chamber, the resolved spots appeared as brown spots. The produced compounds were characterized using 1H-NMR and IR spectra.(Bucha, M., et al., 2017).

4.1. 2-Mercaptobenzimidazole Synthesis



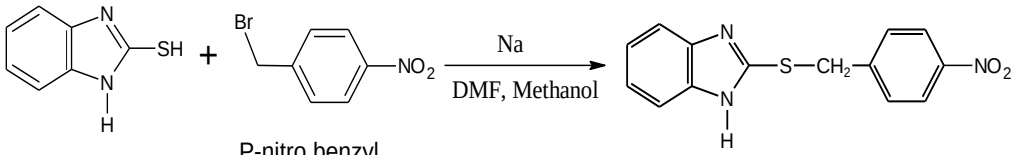
o-phenylenediamine 2-Mercaptobenzimidazole

(1) 2-benzyl mercaptobenzimidazole Synthesis



2-MBI Benzyl chloride 2-Benzyl MBI

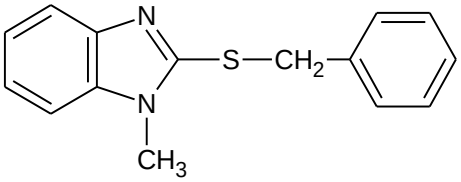
(2) 2-(p-nitro) benzyl mercaptobenzimidazole Synthesis



2-MBI P-nitro benzyl bromide 2-(p-nitro) benzyl MBI

5. ANALYTICAL DATA

1) 2-benzyl-(1-methyl)-mercaptobenzimidazole (BK-1)



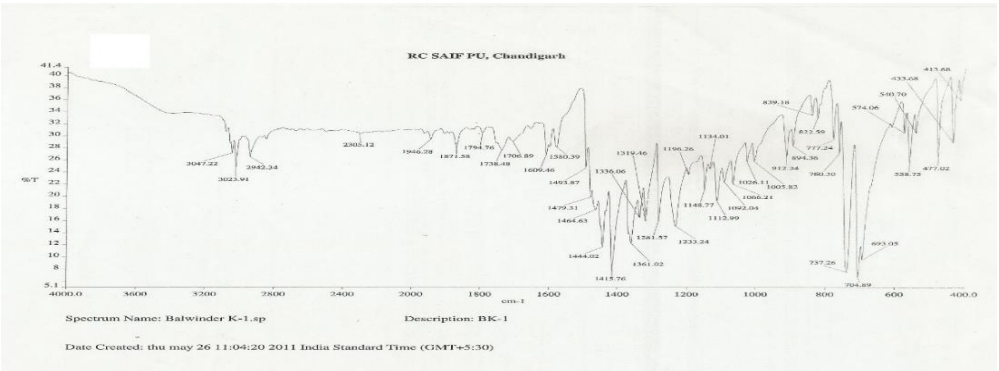


Fig 10 (BK-1): IR data of 2-benzyl-(1-methyl)-mercaptobenzimidazole.

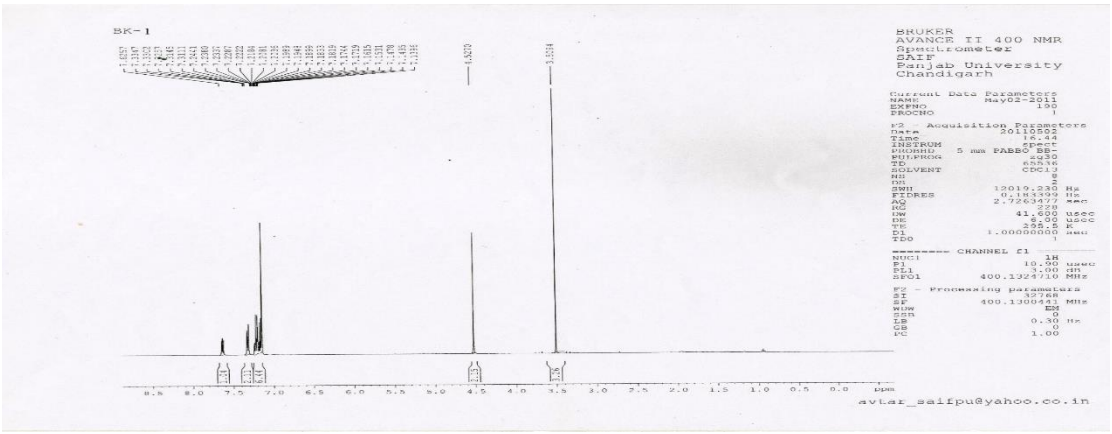
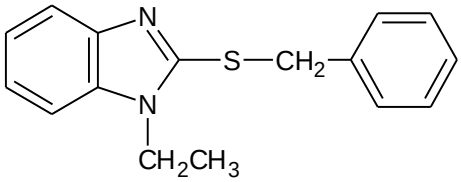


Fig 11 (BK-1): NMR data of 2-benzyl-(1-methyl)-mercaptobenzimidazole.

2) 2-benzyl-(1-ethyl)-mercaptobenzimidazole (BK-2)



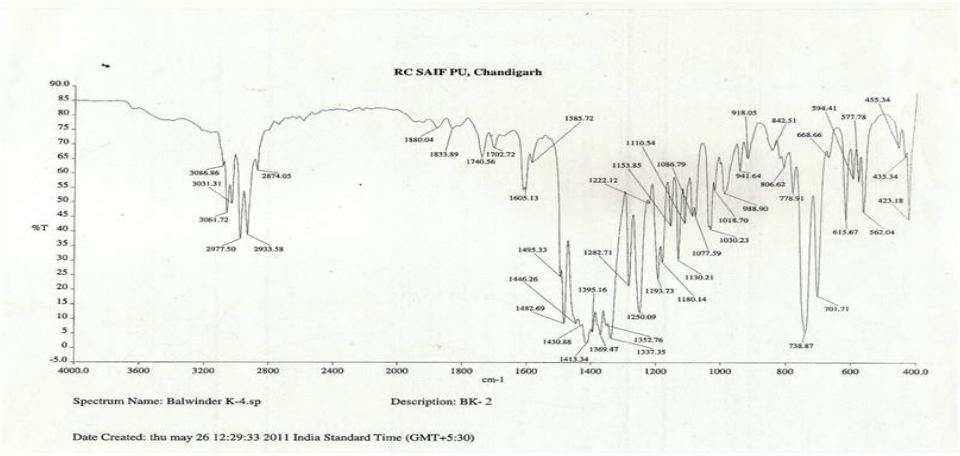


Fig 12 (BK-2): IR data of 2-benzyl-(1-ethyl)-mercaptobenzimidazole.

3) 2-benzyl-(1-propyl)-mercaptobenzimidazole (BK-3)

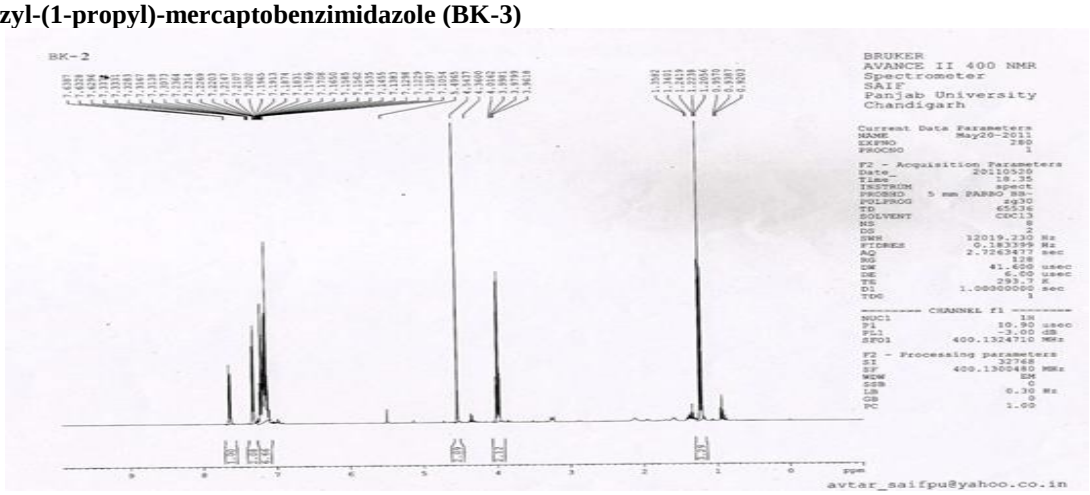
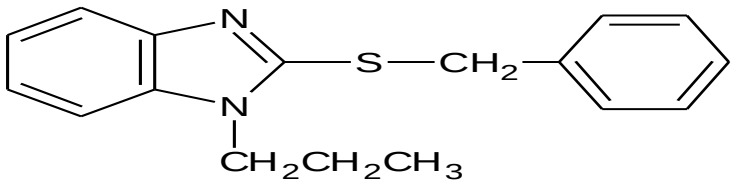


Fig 13: (BK-2): NMR data of 2-benzyl-(1-ethyl)-mercaptobenzimidazole.



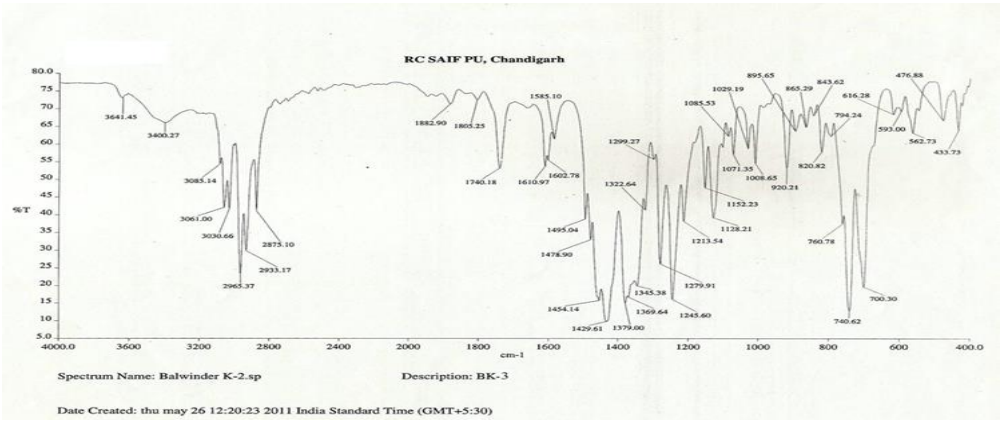


Fig 14 (BK-3): IR data of 2-benzyl-(1-propyl)-mercaptobenzimidazole.

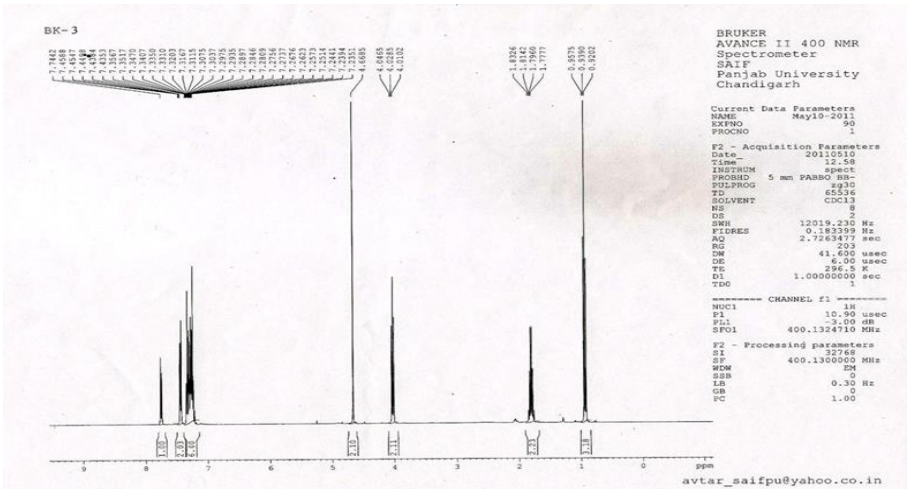
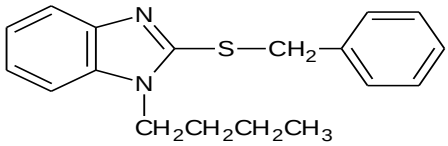


Fig15 (BK-3): NMR data of 2-benzyl-(1-propyl)-mercaptobenzimidazole.

4) 2-benzyl-(1-butyl)-mercaptobenzimidazole (BK-4)



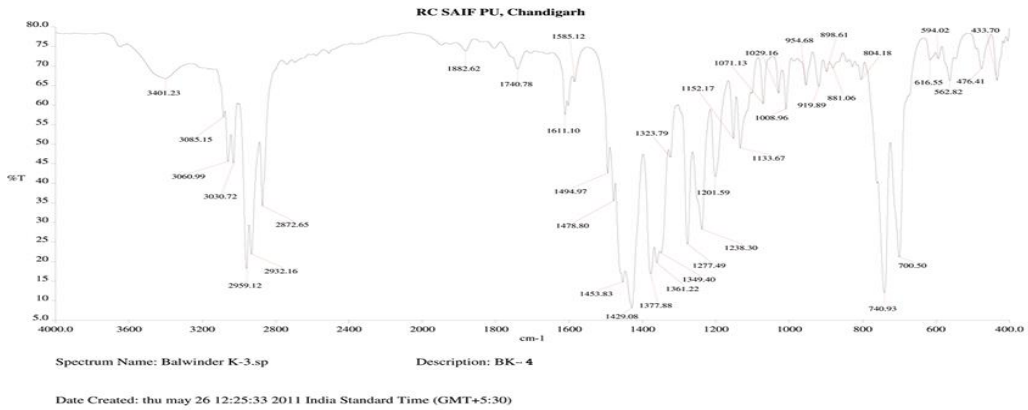


Fig 16 (BK-4): IR data of 2-benzyl-(1-butyl)-mercaptobenzimidazole.

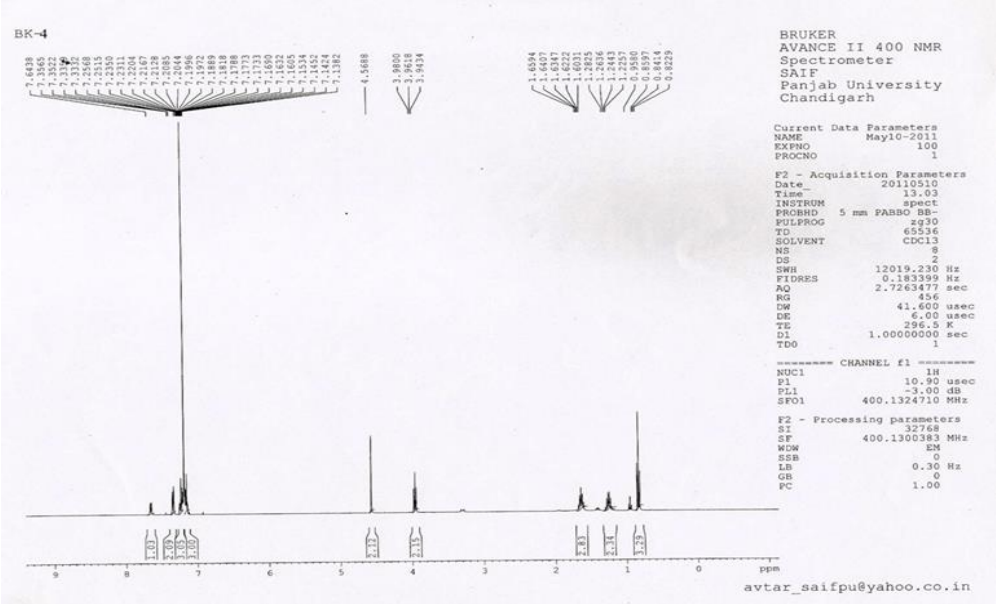
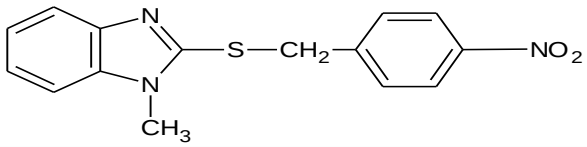


Fig 17 (BK-4): NMR data of 2-benzyl-(1-butyl)-mercaptobenzimidazole.

5) 2-p-nitrobenzyl-(1-methyl)-mercaptobenzimidazole (BK-5)



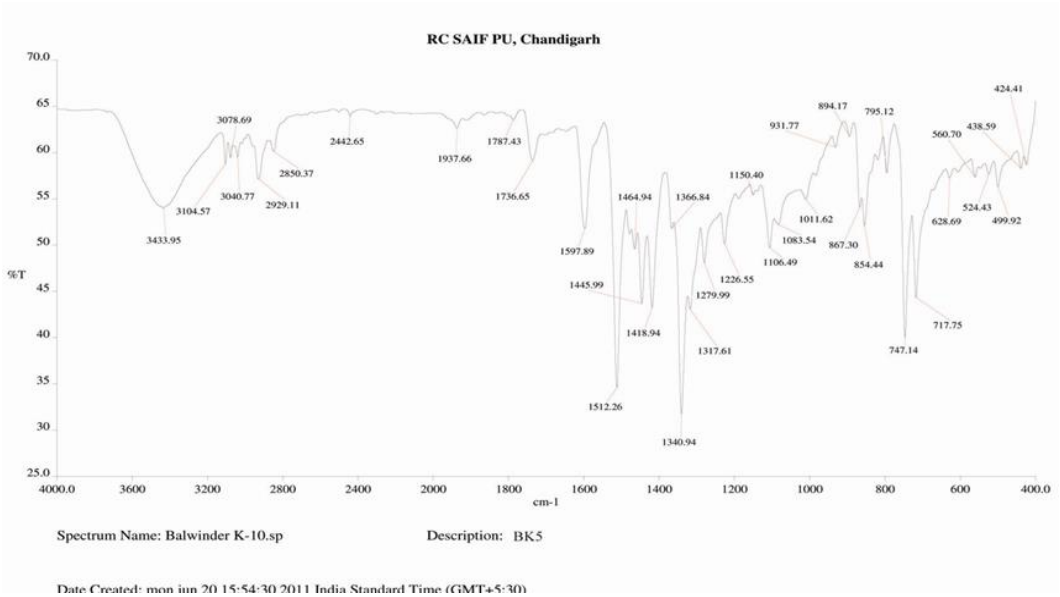


Fig 18 (BK-5): IR data of 2-p-nitrobenzyl-(1-methyl)-mercaptobenzimidazole.

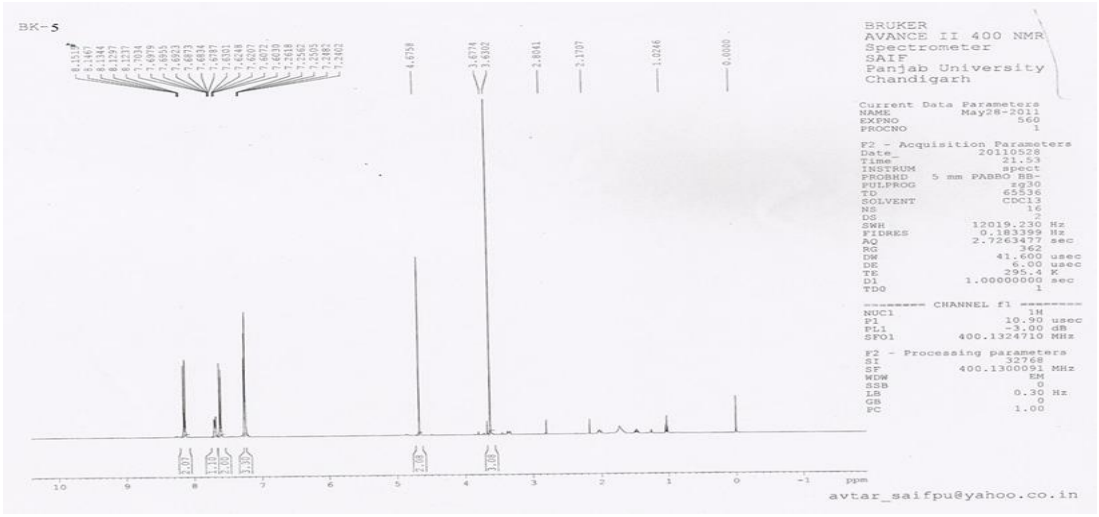
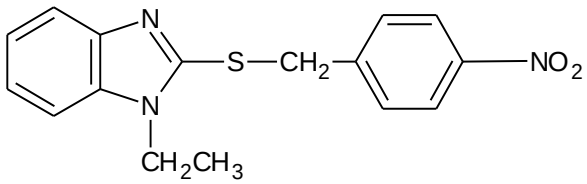


Fig 19 (BK-5): NMR data of 2-p-nitrobenzyl-(1-methyl)-mercaptobenzimidazole.

6) 2-p-nitrobenzyl-(1-ethyl)-mercaptobenzimidazole (BK-6)



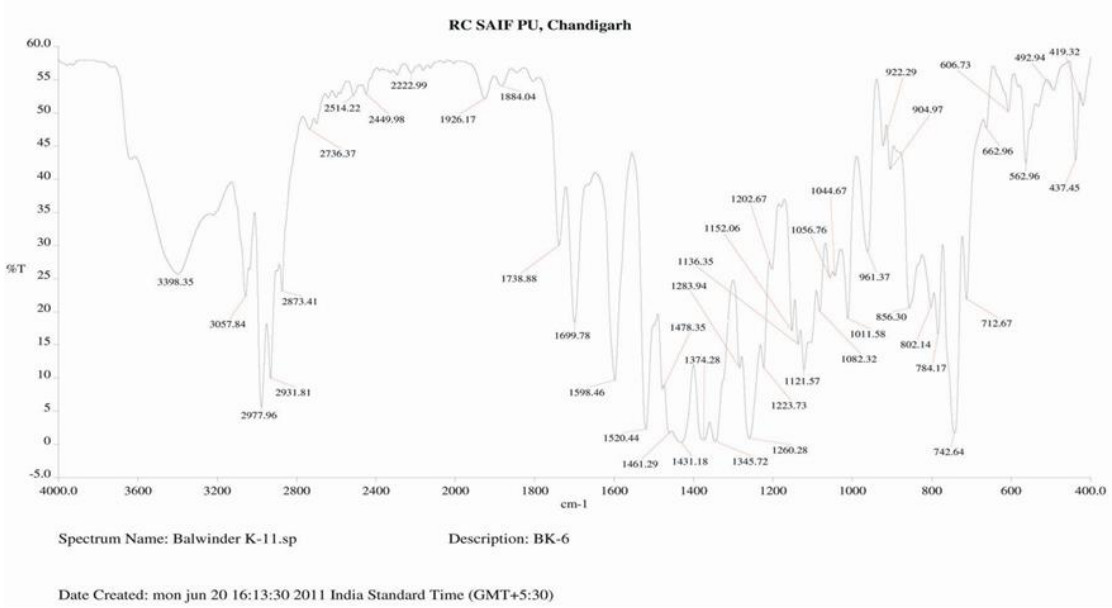


Fig 20 (BK-6): IR data of 2-p-nitrobenzyl-(1-ethyl)-mercaptobenzimidazole.

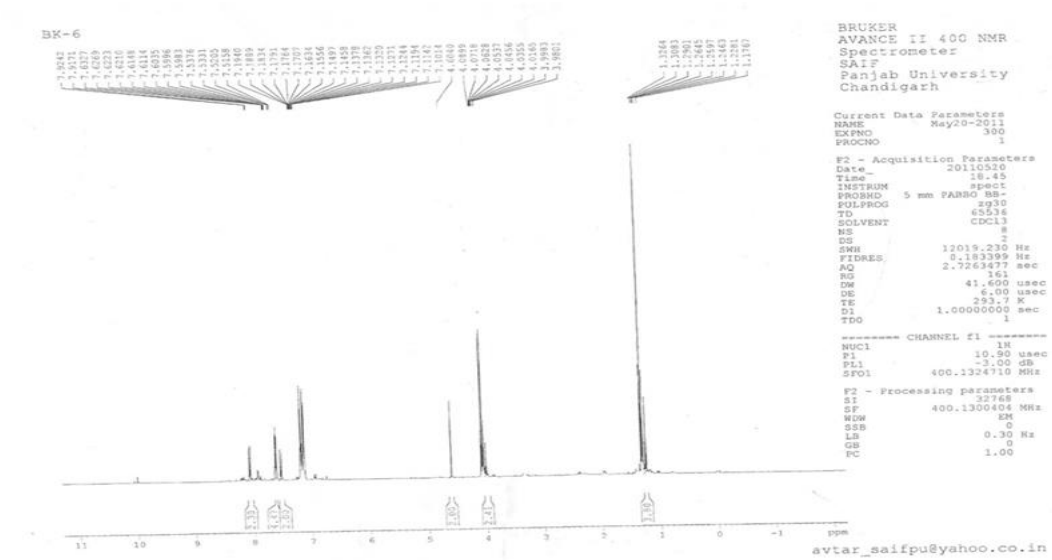
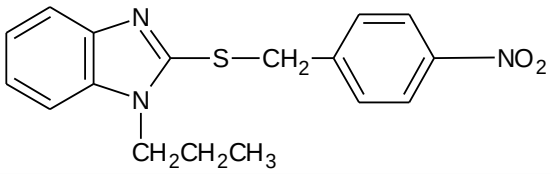


Fig 21 (BK-6): NMR data of 2-p-nitrobenzyl-(1-ethyl)-mercaptobenzimidazole.

7) 2-p-nitrobenzyl-(1-propyl)-mercaptobenzimidazole (BK-7)



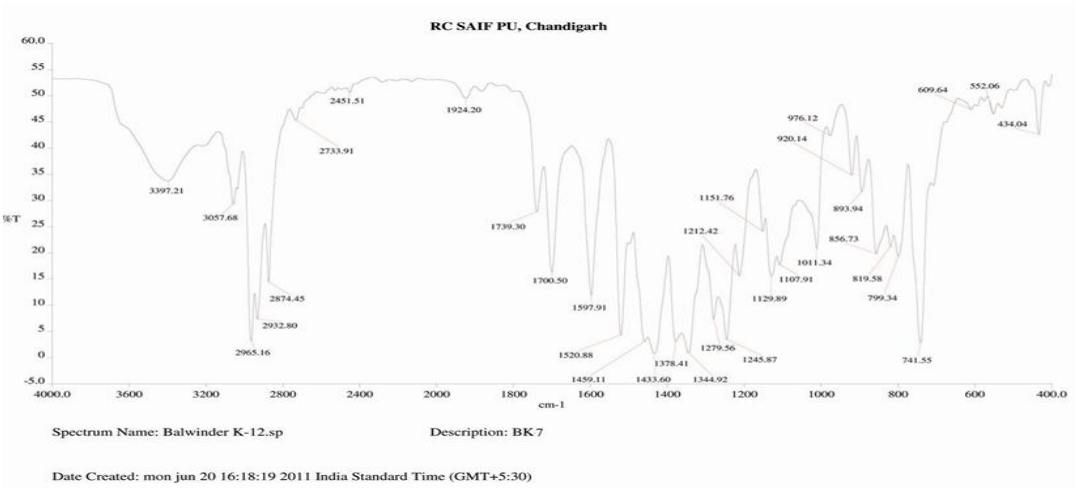


Fig 22 (BK-7): IR data of 2-p-nitrobenzyl-(1-propyl)-mercaptobenzimidazole.

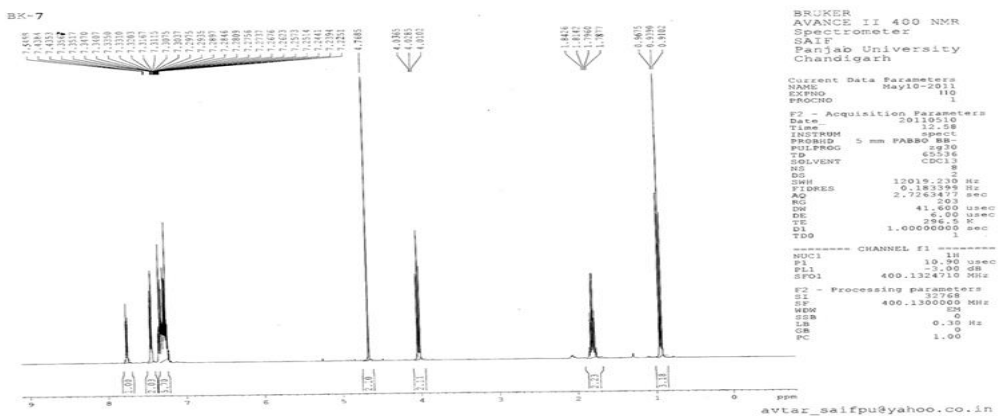
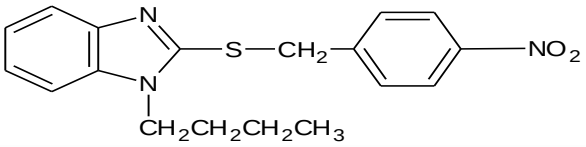


Fig 23 (BK-7): NMR data of 2-p-nitrobenzyl-(1-propyl)-mercaptobenzimidazole.

8) 2-p-nitrobenzyl-(1-butyl)-mercaptobenzimidazole (BK-8)



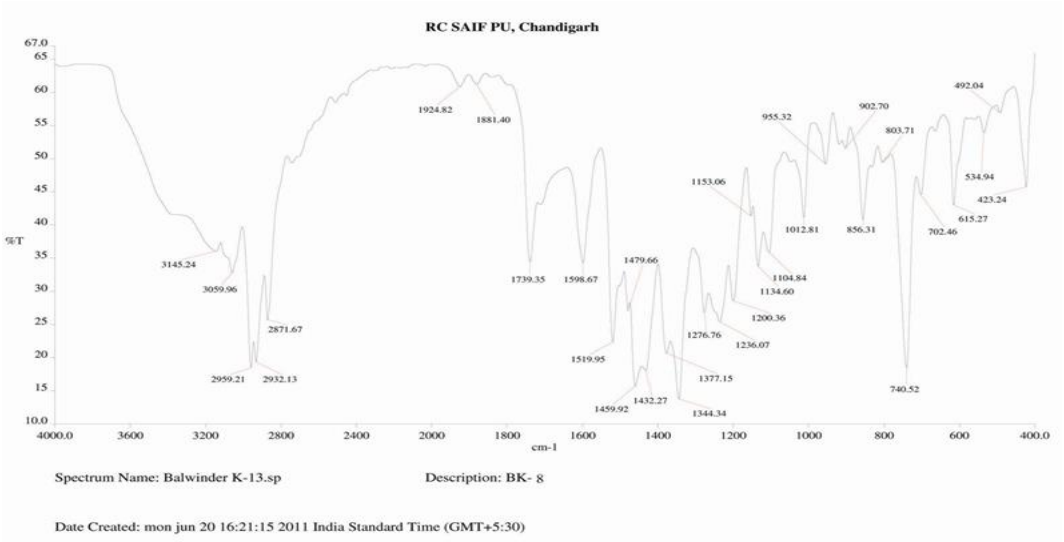


Fig 25(BK-8): IR data of 2-p-nitrobenzyl-(1-butyl)-mercaptobenzimidazole.

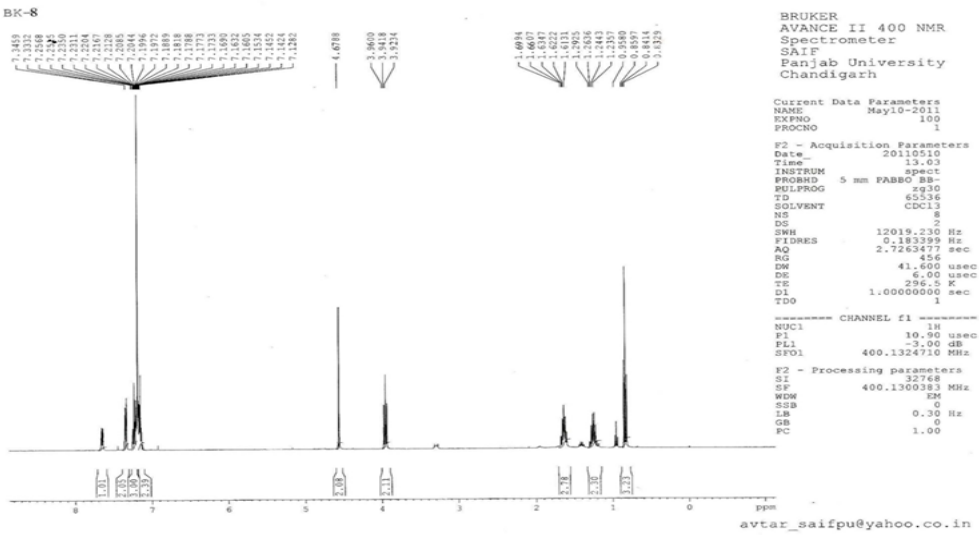


Fig 24 (BK-8): NMR data of 2-p-nitrobenzyl-(1-butyl)-mercaptobenzimidazole.

6. ANTIMICROBIAL ACTIVITY

6.1 Antibacterial Activity

The produced compounds' antibacterial activity against Gram-positive bacteria, such Staphylococcus aureus and Bacillus subtilis, as well as Gram-negative bacteria, like Escherichia coli, was also assessed. The standard drug taken was Ciprofloxacin. (Al-Wahaibi et al., 2021).

After dissolving all of the ingredients in distilled water, the pH was adjusted to 8.0–8.4, a 5M NaOH solution was added, simmered for 10–15 minutes, and the mixture was filtered. Adjust the medium's pH to 7.4±0.2 by adding a dilution of HCl. placed the media in the autoclave for 15 minutes. at 121^{0}C. (Owen, et al, 2005).

6.2 Test Solution Preparation

The solution of 100 µg concentrations of different benzimidazole derivatives was made in DMSO.

6.3 Standard Solution Preparation

10 mg of standard drug was weighed and dissolved in 10 ml to prepare 1000 µg/ml of stock solution. An aliquot of 1ml of this stock solution was further diluted to 10ml to obtain a standard solution of 100µg/ml.

6.4 OBSERVATION

Visually check the zone of inhibition.

7. RESULT AND DISCUSSION

The antibacterial activity of the compounds against S. aureus, B. subtilis, and E. coli was evaluated in vitro using ciprofloxacin as a reference. The Zone of Inhibition for the synthesized drugs against Staphylococcus aureus, Bacillus subtilis, and E. coli is displayed in Table I.

The compounds of 1,2-disubstituted mercaptobenzimidazole exhibit good activity. Compound BK-3 and BK-5 exhibit good activity against Staphylococcus aureus, Bacillus subtilis, and Escherichia coli, whereas compounds BK-2, BK-4, BK-6, and BK-8 exhibit moderate activity against these bacteria.

Table I: Data of Antibacterial Activity of synthesized compounds.

S.No.	Compounds	Zone of Inhibition in mm		
		Staphylococcus aureus	Bacillus subtilis	Escherichia coli
1.	BK-1	05	12	08
2.	BK-2	13	11	0
3.	BK-3	14	19	17
4.	BK-4	0	16	12
5.	BK-5	15	17	13
6.	BK-6	08	13	14
7.	BK-7	11	09	06
8.	BK-8	10	13	16
9.	Ciprofloxacin	25	28	24

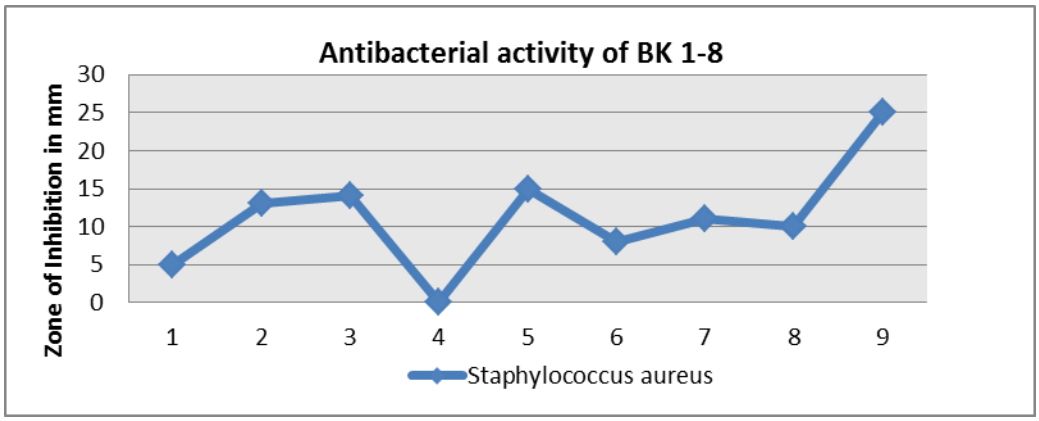


Fig 26: Antibacterial activity of 1, 2-disubstituted mercaptobenzimidazole (S.a)

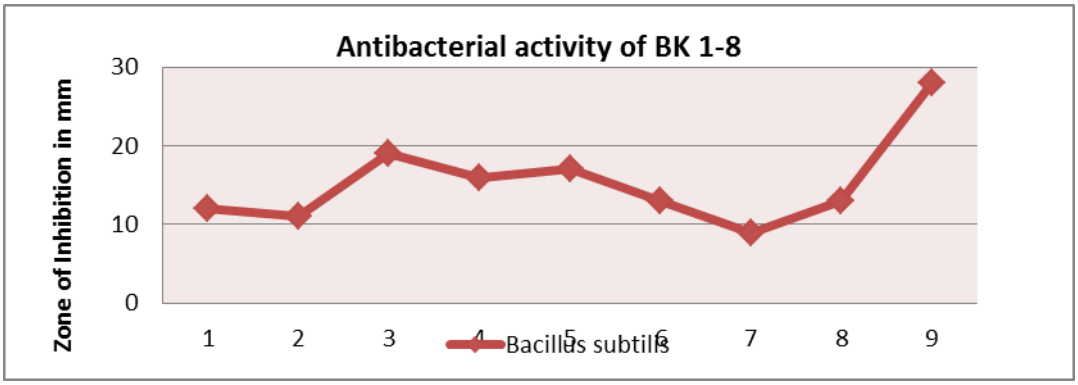


Fig 27: Antibacterial activity of 1, 2-disubstituted mercaptobenzimidazole (B.s)

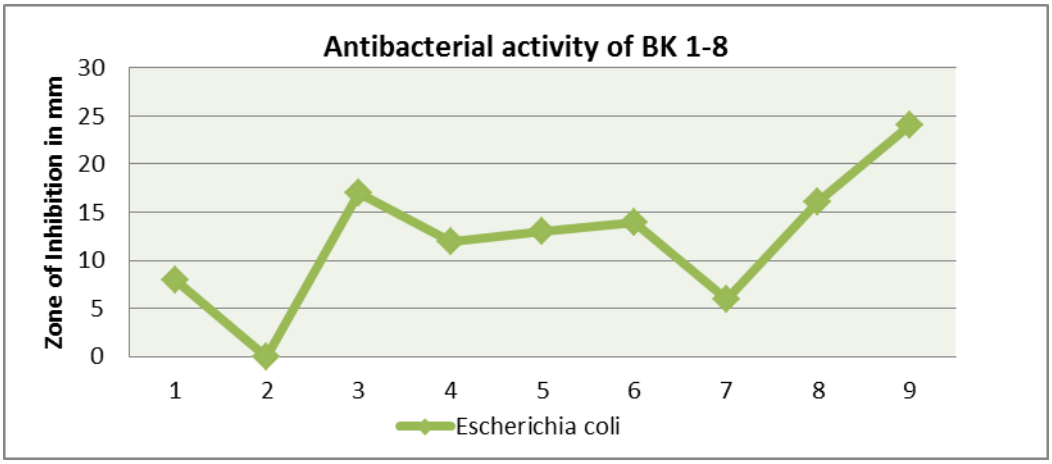


Fig 28: Antibacterial activity of 1, 2-disubstituted mercaptobenzimidazole (E.c)

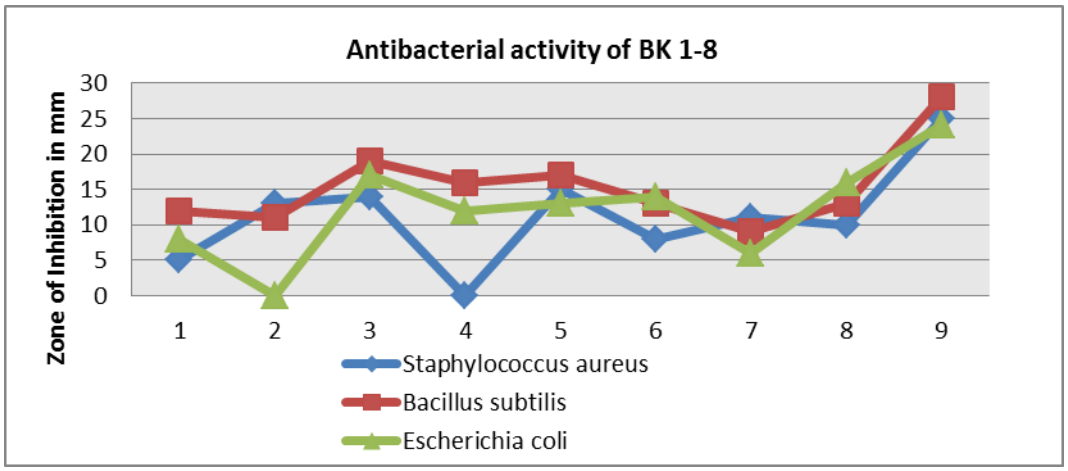


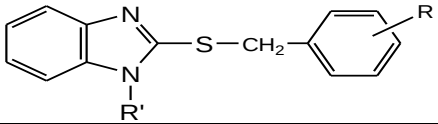
Fig 29- Antibacterial activity of 1, 2-disubstituted mercaptobenzimidazole (S.a, B.s, E.c)

7.1 Chemical Studies

The chemistry approach of the project involve the synthesis of 1, 2-disubstituted mercaptobenzimidazoles compounds. Characterization All newly synthesised compounds were characterized by determination of their melting points, Rf values (TLC), IR and Proton NMR (1H NMR) spectra. IR and 1 H NMR were used for structure confirmation of all the final compounds along with intermediates synthesized.

7.2 Characterization Studies

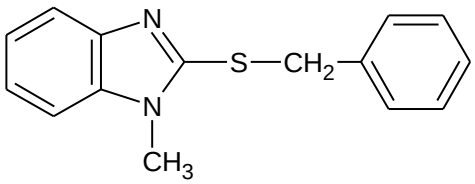
Table II: Physical Data of different synthesized compounds.



S.No.	R	R'	Compound Code	Physical state	% Yield	M.P	R_F Value
1.	H	CH_3	BK-1	Light yellow	74%	70^0C	0.86
2.	H	C_2H_5	BK-2	White	62%	40^0C	0.81
3.	H	C_3H_7	BK-3	Light yellow	64.1%	42^0C	0.79
4.	H	C_4H_9	BK-4	Light yellow	65%	39^0C	0.76
5.	NO_2	CH_3	BK-5	Dark yellow	70%	112^0C	0.80
6.	NO_2	C_2H_5	BK-6	Orange	60.8%	41^0C	0.79
7.	NO_2	C_3H_7	BK-7	Orange	60%	40^0C	0.77
8.	NO_2	C_4H_9	BK-8	Dark brown	63.3%	43^0C	0.75

7.2.1. Characterization Features of Synthesized Compounds

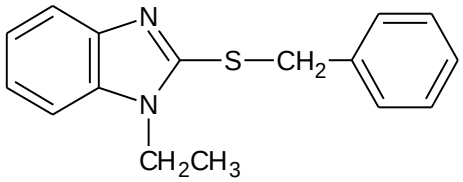
1. Compound (BK-1)



Appearance:	White
Yield:	74%
R_f($CHCl_3$: CH_3OH):	0.86
M.P:	70^0C

- IR (KBr pellets): 3047.22 (Ar C-H, Str), 2942.34 (C-H, Str), 1609.46 (Ar C=C, Str), 1361.02 (C-N, Str), 704.89 (C-S, Str).
- NMR (DMSO): 3.50 (s, 3H, CH_3), 4.52 (s, 2H, CH_2), 7.13-7.62 (m, 9H, Ar H).

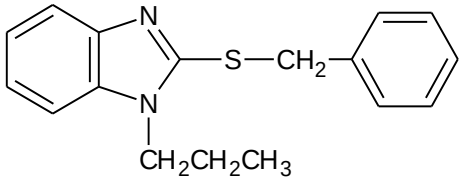
2. Compound (BK-2)



Appearance:	White
Yield:	62%
R_f($CHCl_3$: CH_3OH):	0.81
M.P:	40^0C

- IR (KBr pellets): 3061.72 (Ar C-H, Str), 2977.50 (C-H, Str), 1482.69 (Ar C=C, Str), 1250.09 (C-N, Str), 701.71 (C-S, Str).
- NMR (DMSO): 1.20-1.24 (t, 3H, CH_3), 3.96-4.01 (q, 2H, CH_2), 4.54 (s, 2H, CH_2), 7.10-7.63 (m, 9H, Ar H).

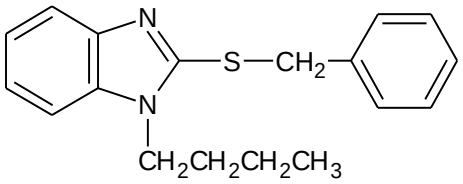
3. Compound (BK-3)



Appearance:	White
Yield:	64.1%
R_f($CHCl_3$: CH_3OH):	0.79
M.P:	42^0C

- IR (KBr pellets): 3061 (Ar C-H, Str), 2965.37 (C-H, Str), 1495.04 (Ar C=C, Str), 1245.60 (C-N, Str), 700.30 (C-S, Str).
- NMR (DMSO): 0.92-0.95 (t, 3H, CH_3), 1.79-1.83 (m, 2H, CH_2), 4.01-4.04 (t, 2H, CH_2), 4.66 (s, 2H, CH_2), 7.23-7.74 (m, 9H, Ar H).

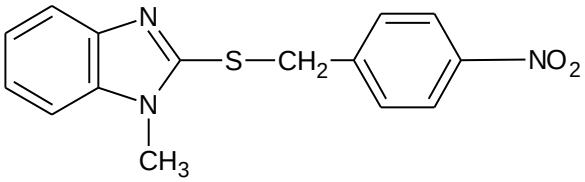
4. Compound (BK-4)



Appearance:	White
Yield:	65%
R_f($CHCl_3$: CH_3OH):	0.76
M.P:	39^0C

- IR (KBr pellets): 3060.99 (Ar C-H, Str), 2959.12 (C-H, Str), 1478.80 (Ar C=C, Str), 1277.49 (C-N, Str), 700.50 (C-S, Str).
- NMR (DMSO): 0.82-0.85 (t, 3H, CH_3), 1.22-1.28 (m, 2H, CH_2), 1.60-1.65 (m, 2H, CH_2), 3.94-3.98(t, 2H, CH_2), 4.56 (s, 2H, CH_2), 7.13-7.64 (m, 9H, Ar H).

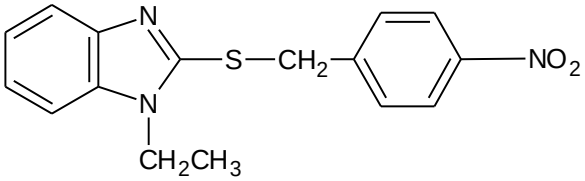
5. Compound (BK-5)



Appearance:	White
Yield:	70%
R_f($CHCl_3$: CH_3OH):	0.80
M.P:	112^0C

- IR (KBr pellets): 3104.57 (Ar C-H, Str), 2929.11 (C-H, Str), 1597.89 (Ar C=C, Str), 1512.26 (N=O, Str), 1340.94 (C-N, Str), 628.69 (C-S, Str).
- NMR (DMSO): 3.63 (s, 3H, CH_3), 4.67 (s, 2H, CH_2), 7.24-8.15 (m, 8H, Ar H).

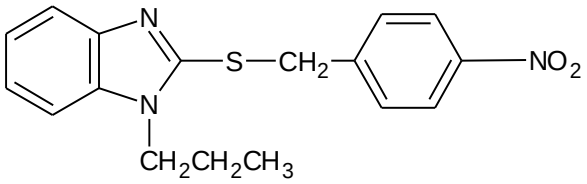
6. Compound (BK-6)



Appearance:	White
Yield:	60.8%
R_f($CHCl_3$: CH_3OH):	0.79
M.P:	41^0C

- IR (KBr pellets): 3057.84 (Ar C-H, Str), 2977.96 (C-H, Str), 1598.46 (Ar C=C, Str), 1520.44 (N=O, Str), 1260.28 (C-N, Str), 606.73 (C-S, Str).
- NMR (DMSO): 1.17-1.40 (t, 3H, CH_3), 3.98.4.08 (q, 2H, CH_2), 4.60 (s, 2H, CH_2), 7.51.7.92 (m, 8H, Ar H).

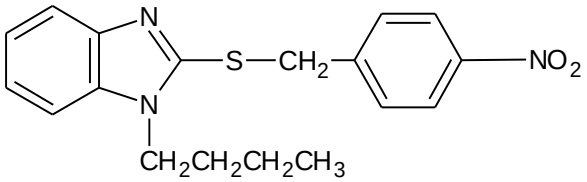
7. Compound (BK-7)



Appearance:	White
Yield:	60%
R_f($CHCl_3$: CH_3OH):	0.77
M.P:	40^0C

- IR (KBr pellets): 3057.68 (Ar C-H, Str), 2965.16 (C-H, Str), 1597.91 (Ar C=C, Str), 1520.88 (N=O, Str), 1344.92 (C-N, Str), 609.64 (C-S, Str).
- The DMSO NMR data are as follows: 0.91-0.96 (t, 3H, CH3), 1.78-1.84 (m, 2H, CH2), 4.01-4.03 (t, 2H, CH2), 4.76 (s, 2H, CH2), and 7.22-7.54 (m, 8H, Ar H).

8. Compound (BK-8)



Appearance:	White
Yield:	63.3%
R_f($CHCl_3$: CH_3OH):	0.75
M.P:	43^0C

• IR (KBr pellets): 1519.95 (N=O, Str), 1344.34 (C-N, Str), 702.46 (C-S, Str), 2959.21 (C-H, Str), 1598.67 (Ar C=C, Str), and 3059.96 (Ar C-H, Str).

- 3.92-3.96 (t, 2H, CH2), 4.67 (s, 2H, CH2), 7.12-7.34 (m, 8H, Ar H), 1.23-1.29 (m, 2H, CH2), 1.61-1.69 (m, 2H, CH2), and 0.83-0.85 (t, 3H, CH3) are the NMR (DMSO) values.

7.3. DISCUSSION

Different benzimidazole derivatives were synthesized. Then obtained product was treated with alkyl halide like methyl iodide, ethyl iodide, propyl bromide and butyl bromide. The synthesized compounds were identified and characterized by the following methods:

- Determination of Melting Point: The Veego melting point apparatus was used to determine the M.P. of the synthesized compound.
- TLC: Methanol and chloroform were the solvents utilized.
- KBr pellets were used for the IR spectroscopy.
- NMR Spectroscopy: DMSO and CDCl3 solvent were used to perform the compound's NMR spectra.
- The cup and plate method was used to screen the produced compounds for antibacterial activity. The compounds were tested against different strains of bacteria Staphylococcus aureus, Bacillus subtilis and Escherichia coli.

While compounds BK-2, BK-4, BK-6, and BK-8 shown moderate action against Bacillus subtilis and Escherichia coli, compounds BK-3 and BK-5 demonstrated excellent action against E. coli, Bacillus subtilis, and Staphylococcus aureus. While considerable antibacterial activity was demonstrated by the synthesized compounds, none of them outperformed the standard reference, Ciprofloxacin.

7.4 SUMMARY AND CONCLUSION

New 1,2-disubstituted mercaptobenzimidazole derivatives were synthesized as per the proposed scheme with good percentage yields. The structure were confirmed and characterized by different physical, analytical and spectral data (^{1}H NMR and FT-IR) and all the data gave positive results.

As 1,2-disubstituted mercaptobenzimidazole derivatives have potent antibacterial activity so it was decided that to determine the antimicrobial activity. While compounds BK-2, BK-4, BK-6, and BK-8 demonstrated moderate activity against Bacillus subtilis and Escherichia coli, compounds BK-3 and BK-5 were shown to have good action against Staphylococcus aureus, Bacillus subtilis, and Escherichia coli.

REFERENCES

1. Narasimhan, B., Sharma, D., & Kumar, P. (2012). Benzimidazole: a medicinally important heterocyclic moiety. Medicinal Chemistry Research, 21(3): 269-283. https://doi.org/10.1007/s00044-010-9533-9.
2. Bansal, Y., Kaur, M., & Bansal, G. (2019). Antimicrobial potential of benzimidazole derived molecules. Mini Reviews in Medicinal Chemistry, 19(8): 624-646. **DOI:** https://doi.org/10.2174/1389557517666171101104024
3. Bansal, R., Kaur, R., & Singla, R. (2021). *Benzimidazole derivatives as potential therapeutic agents: Recent advances and future perspectives*. **Bioorganic Chemistry, 114**: 105084. https://doi.org/10.1016/j.bioorg.2021.105084.
4. Choudhary, R., & Joshi, H. (2019). *Benzimidazole: A biologically active compound and its derivatives—a review*. **International Journal of Pharmaceutical Sciences and Research, 10**(6): 2620–2632. https://doi.org/10.13040/IJPSR.0975-8232.10(6).2620-32.
5. Kaur, R., Chaudhary, S., Kumar, K., & Gupta, G. K. (2019). Recent developments in the synthetic chemistry and biological activities of benzimidazole derivatives. *European Journal of Medicinal Chemistry*, **183**: 111708. https://doi.org/10.1016/j.ejmech.2019.111708.
6. Rollas, S., & Küçükgüzel, Ş. G. (2007). Biological activities of benzimidazole derivatives. *Molecules*, **12**(8): 1910–1939. https://doi.org/10.3390/12081910.

7. Lacey, E. (1990). Mode of action of benzimidazoles. *Parasitology Today*, **6**(4): 112–115. https://doi.org/10.1016/0169-4758(90)90206-U.
8. Horton, J. (2000). Albendazole: A review of anthelmintic efficacy and safety in humans. *Parasitology*, **121**(S1): S113–S132. https://doi.org/10.1017/S0031182099006129.
9. Ceballos, L., Elissondo, M., Sánchez Bruni, S., Denegri, G., & Lanusse, C. (2009). Flubendazole in cystic echinococcosis therapy: Pharmacokinetics and efficacy evaluation in mice. *Parasitology Research*, **105**: 1631–1636. https://doi.org/10.1007/s00436-009-1600-0
10. Savjani, K. T., Gajjar, A. K., & Savjani, J. K. (2012). *Drug solubility: Importance and enhancement techniques*. **ISRN Pharmaceutics**, 2012, Article ID 195727. https://doi.org/10.5402/2012/195727.
11. Lipinski, C. A. (2000). *Drug-like properties and the causes of poor solubility and poor permeability*. **Journal of Pharmacological and Toxicological Methods**, 44(1): 235–249. https://doi.org/10.1016/S1056-8719(00)00107-6.
12. Zanger, U. M., & Schwab, M. (2013). **Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation**. *Pharmacology & Therapeutics, 138*(1): 103–141. https://doi.org/10.1016/j.pharmthera.2012.12.007.
13. Rendic, S., & Di Carlo, F. J. (1997). **Human cytochrome P450 enzymes: A status report summarizing their reactions, substrates, inducers, and inhibitors**. *Drug Metabolism Reviews, 29*(1–2): 413–580. https://doi.org/10.3109/03602539709037591.
14. Choudhuri, S., Cherrington, N. J., Li, N., & Klaassen, C. D. (2010). **Molecular targets of xenobiotic regulation under oxidative stress conditions: Role of nuclear receptors**. *Drug Metabolism Reviews, 42*(1): 109–122. https://doi.org/10.3109/03602530903209230.
15. Pathare, B., & Bansode, T. (2021). Biological active benzimidazole derivatives. Results in Chemistry, 3: 100200. https://doi.org/10.1016/j.rechem.2021.100200.
16. Alamgir, Mahiuddin, David St C. Black, and Naresh Kumar. "Synthesis, reactivity and biological activity of benzimidazoles." Bioactive heterocycles III. Berlin, Heidelberg: Springer Berlin Heidelberg, 2007; 87-118. https://doi.org/10.1007/7081_2007_088
17. Lacey, E., & Gill, J. H. (1994). Biochemistry of benzimidazole resistance. Acta tropica, 56(2-3): 245–262. https://doi.org/10.1016/0001-706x(94)90066-3
18. Zhao, M., Ma, J., Li, M., Zhang, Y., Jiang, B., Zhao, X., Huai, C., Shen, L., Zhang, N., He, L., & Qin, S. (2021). Cytochrome P450 Enzymes and Drug Metabolism in Humans. International journal of molecular sciences, 22(23): 12808. https://doi.org/10.3390/ijms222312808.
19. Kabi, A. K., Sravani, S., Gujjarappa, R., Garg, A., Vodnala, N., Tyagi, U., ... & Malakar, C. C. (2022). An overview on biological activity of benzimidazole derivatives. Nanostructured Biomaterials: Basic Structures and Applications, 351-378. https://doi.org/10.1007/978-981-16-8399-2_9
20. Kohanski, M. A., Dwyer, D. J., & Collins, J. J. (2010). How antibiotics kill bacteria: from targets to networks. *Nature Reviews Microbiology*, 8(6): 423–435. https://doi.org/10.1038/nrmicro2333.
21. Spellberg, B., & Gilbert, D. N. (2014). The future of antibiotics and resistance: A tribute to a career of leadership by John Bartlett. *Clinical Infectious Diseases*, 59(suppl_2): S71–S75. https://doi.org/10.1093/cid/ciu392.
22. McDonnell, G., & Russell, A. D. (1999). **Antiseptics and disinfectants: Activity, action, and resistance**. *Clinical Microbiology Reviews, 12*(1): 147–179. https://doi.org/10.1128/CMR.12.1.147.
23. Clardy, J., Fischbach, M. A., & Walsh, C. T. (2006). *New antibiotics from bacterial natural products*. Nature Biotechnology, **24**(12): 1541–1550. https://doi.org/10.1038/nbt1266.
24. Davies, J., & Davies, D. (2010). *Origins and evolution of antibiotic resistance*. Microbiology and Molecular Biology Reviews, **74**(3): 417–433. https://doi.org/10.1128/MMBR.00016-10.
25. Davey, P. G., Wilcox, M. H., Irving, W., Thwaites, G. (2015). Antimicrobial Chemotherapy. United Kingdom: Oxford University Press.
26. Silhavy, T. J., Kahne, D., & Walker, S. (2010). The bacterial cell envelope. *Cold Spring Harbor Perspectives in Biology*, 2(5): a000414. https://doi.org/10.1101/cshperspect.a000414.
27. Vollmer, W., Blanot, D., & de Pedro, M. A. (2008). Peptidoglycan structure and architecture. *FEMS Microbiology Reviews*, 32(2): 149–167. https://doi.org/10.1111/j.1574-6976.2007.00094.x.
28. Walsh, C. (2003). Antibiotics: Actions, origins, resistance. Washington, DC: American Society for Microbiology. ISBN 1-55581-254-6.
29. Bugg, T. D., Wright, G. D., Dutka-Malen, S., Arthur, M., Courvalin, P., & Walsh, C. T. (1991). *Molecular basis for vancomycin resistance in Enterococcus faecium BM4147: Biosynthesis of a depsipeptide peptidoglycan precursor by the van cluster*. Biochemistry, 30(43): 10408–10415. https://doi.org/10.1021/bi00106a037.
30. Schnell, L., Felix, I., Müller, B., Sadi, M., von Bank, F., Papatheodorou, P., Popoff, M. R., Aktories, K., Waltenberger, E., Benz, R., Weichbrodt, C., Fauler, M., Frick, M., & Barth, H. (2019). Revisiting an old antibiotic: bacitracin neutralizes binary bacterial toxins and protects cells from intoxication. FASEB journal: official publication of the Federation of American Societies for Experimental Biology,

33(4): 5755–5771. https://doi.org/10.1096/fj.201802453R.
31. Alberts, B., Bray, D., Hopkin, K., Johnson, A. D., Lewis, J., Raff, M., ... & Walter, P. (2015). *Essential cell biology*. Garland Science.
32. Berlanga, M. (2010). Review of the book Brock Biology of Microorganisms (11th ed.), by M. T. Madigan & J. M. Martinko (Eds.). International Microbiology, 8(2): 149–150.
33. Nelson, D. L., Lehninger, A. L., & Cox, M. M. (2008). *Lehninger principles of biochemistry*. Macmillan.
34. Kotra, L. P., Haddad, J., & Mobashery, S. (2000). Aminoglycosides: Perspectives on mechanisms of action and resistance and strategies to counter resistance. **Antimicrobial Agents and Chemotherapy, 44**(12): 3249–3256. https://doi.org/10.1128/AAC.44.12.3249-3256.2000.
35. Krause, K. M., Serio, A. W., Kane, T. R., & Connolly, L. E. (2016). Aminoglycosides: An overview. **Cold Spring Harbor Perspectives in Medicine, 6**(6): a027029. https://doi.org/10.1101/cshperspect.a027029.
36. Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews, 65*(2): 232–260. https://doi.org/10.1128/MMBR.65.2.232-260.2001.
37. Wilson, D. N. (2014). Ribosome-targeting antibiotics and mechanisms of bacterial resistance. *Nature Reviews Microbiology, 12*(1): 35–48. https://doi.org/10.1038/nrmicro3155.
38. Kannan, K., Vázquez-Laslop, N., & Mankin, A. S. (2014). Selective protein synthesis by ribosomes with a drug-obstructed exit tunnel. *Cell*, 151(3): 508–520. https://doi.org/10.1016/j.cell.2012.09.018.
39. Ghannoum, M. A., & Rice, L. B. (1999). Antifungal agents: Mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. *Clinical Microbiology Reviews*, 12(4): 501–517. https://doi.org/10.1128/CMR.12.4.501.
40. Odds, F. C., Brown, A. J. P., & Gow, N. A. R. (2003). Antifungal agents: Mechanisms of action. *Trends in Microbiology*, 11(6): 272–279. https://doi.org/10.1016/S0966-842X(03)00117-3.
41. Gandra, S., Tseng, K. K., Arora, A., Bhowmik, B., Robinson, M. L., & Panigrahi, B. (2021). **The rise of Candida auris: Preventing a pandemic of drug-resistant fungi**. *PLOS Pathogens, 17*(8): e1009508. https://doi.org/10.1371/journal.ppat.1009508.
42. Bhattacharya, S., Sae-Tia, S., & Fries, B. C. (2020). **Candidiasis and mechanisms of antifungal resistance**. *Virulence, 11*(1): 574–586. https://doi.org/10.1080/21505594.2020.1772894.
43. Falagas, M. E., & Kasiakou, S. K. (2005). **Colistin: The revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections**. *Clinical Infectious Diseases, 40*(9): 1333–1341. https://doi.org/10.1086/429323.
44. Zhao, X., Liu, M., & Ji, Y. (2021). Mechanisms of action of antimicrobial agents and bacterial resistance. Microbiology Spectrum, 9(1): e01315-20. https://doi.org/10.1128/Spectrum.01315-20.
45. Bush, K., & Bradford, P. A. (2019). Interplay between β-lactamases and new β-lactamase inhibitors. Nature Reviews Microbiology, 17: 295–306. https://doi.org/10.1038/s41579-019-0159-8.
46. Sköld, O. (2000). Sulfonamide resistance: mechanisms and trends. *Drug Resistance Updates*, **3**(3): 155–160. https://doi.org/10.1054/drup.2000.0146.
47. Gordillo, M. A., Romero, M., Hidalgo, A., González-Bello, C., & Alcolea, P. J. (2021). *Trimethoprim analogues as inhibitors of dihydrofolate reductase: Advances in structure-based design and therapeutic applications*. **European Journal of Medicinal Chemistry**, 223: 113641. https://doi.org/10.1016/j.ejmech.2021.113641.
48. Jordheim, L. P., Durantel, D., Zoulim, F., & Dumontet, C. (2019). Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nature Reviews Drug Discovery*, *18*(6): 447–464. https://doi.org/10.1038/s41573-019-0011-3.
49. Götte, M., & Feld, J. J. (2021). Direct-acting antiviral agents for hepatitis C: Structural and mechanistic insights. *Nature Reviews Gastroenterology & Hepatology*, *18*(6): 421–432. https://doi.org/10.1038/s41575-021-00414-1.
50. Rodrigues, C. F., & Silva, F. (2025). The Rise, Fall, and Rethink of (Fluoro)quinolones: A Quick Rundown. Pathogens (Basel, Switzerland), 14(6): 525. https://doi.org/10.3390/pathogens14060525.
51. Collins, J. A., & Osheroff, N. (2024). Gyrase and topoisomerase IV: recycling old targets for new antibacterials to combat fluoroquinolone resistance. ACS infectious diseases, 10(4): 1097-1115. https://doi.org/10.1021/acsinfecdis.4c00128.
52. Leyn, S. A., Kent, J. E., Zlamal, J. E., Elane, M. L., Vercruysse, M., & Osterman, A. L. (2024). Two classes of DNA gyrase inhibitors elicit distinct evolutionary trajectories toward resistance in gram-negative pathogens. npj Antimicrobials and Resistance, 2(1): 5. https://doi.org/10.1038/s44259-024-00021-y.
53. Sudzinová, P., Šanderová, H., Koval', T., Skálová, T., Borah, N., Hnilicová, J., ... & Krásný, L. (2023). What the Hel: recent advances in understanding rifampicin resistance in bacteria. *FEMS Microbiology Reviews*, *47*(6): fuac051. https://doi.org/10.1093/femsre/fuac051.
54. Yang, K. B., Cameranesi, M., Gowder, M., Martinez, C., Shamovsky, Y., Epshtein, V., ... & Nudler, E. (2023). High-resolution landscape of an

antibiotic binding site. *Nature*, *622*(7981): 180-187. https://doi.org/10.1038/s41586-023-06495-6.

55. National Center for Biotechnology Information. (2025). PubChem Compound Summary for CID 5798, Benzimidazole. Retrieved October 23, 2025, from https://pubchem.ncbi.nlm.nih.gov/compound/Benzimidazole.
56. Farag, B., Zaki, M. E., Elsayed, D. A., & Gomha, S. M. (2025). Benzimidazole chemistry in oncology: recent developments in synthesis, activity, and SAR analysis. *RSC advances*, *15*(23): 18593-18647. DOI: 10.1039/D5RA01077B.
57. Patel M, Avashthi G, Gacem A, Alqahtani MS, Park H-K, Jeon B-H. A Review of Approaches to the Metallic and Non-Metallic Synthesis of Benzimidazole (BnZ) and Their Derivatives for Biological Efficacy. *Molecules*, 2023; 28(14): 5490. https://doi.org/10.3390/molecules28145490.
58. Nieto Gómez, C. I. (2014). An experimental and theoretical NMR study of NH-benzimidazoles in solution and in the solid state: proton transfer and tautomerism. https://doi.org/10.3762/bjoc.10.168.
59. Alaqeel, S. I. (2017). Synthetic approaches to benzimidazoles from o-phenylenediamine: A literature review. Journal of Saudi Chemical Society, 21(2): 229-237. https://doi.org/10.1016/j.jscs.2016.08.001.
60. Iqbal, N., Ashraf, M. A., Gul, A. R., Bae, J., Iqbal, N., Park, T. J., & Cho, E. J. (2023). Construction of a Pentacyclic Framework Enabled by Nickel Catalysis. *Organic Letters*, *25*(4): 647-652. https://doi.org/10.1021/acs.orglett.2c04228.
61. Bucha, M., Eppakayala, L., Malothu, R., Kudle, K. R., & Merugu, R. (2017). *Synthesis and molecular docking for antiinflammatory studies of 2-(arylmethyl)-1-ethyl-1H-benzo[d]imidazol-5-amines*. **Asian Journal of Chemistry, 30**(1): 29–33. https://doi.org/10.14233/ajchem.2018.20784.
62. Al-Wahaibi, L. H., Al-Busaidi, A. A., Al-Maqbali, M., Al-Kharousi, Z., & Al-Harthy, H. A. (2021). Design, synthesis, and antibacterial screening of some novel ciprofloxacin-based heterocycle hybrids. *Pharmaceuticals*, *14*(5): 399. https://doi.org/10.3390/ph14050399.
63. Owen, H. R., Wengerd, D., & Miller, A. R. (2005). Culture medium pH is influenced by basal medium, carbohydrate source, gelling agent, and medium storage method. *Plant Cell, Tissue and Organ Culture*, *22*(3): 153–162. https://doi.org/10.1007/s11240-005-0412-4.