International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 32, Issue 4, April 2026 **ijppr.humanjournals.com** ISSN: 2349-7203

Design, Molecular Docking, Synthesis, and *In-Vitro* Evaluation of Some Novel Benzimidazole Derivatives as Anti-Tubercular Agents

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Received: 19 February 2026 Revised: 28 February 2026 Accepted: 20 March 2026

ABSTRACT:

Background The development of multidrug-resistant strains of Mycobacterium tuberculosis and the drawbacks of the available chemotherapeutic drugs have made tuberculosis (TB) a major worldwide health concern. The benzimidazole scaffold is an achievable structure for the development of new drugs since it is acknowledged as a heterocyclic nucleus with a variety of pharmacological features, including strong anti-bacterial and antitubercular activities.**Objectives** Using molecular docking analysis, the current study aimed to develop, synthesize, and evaluate several of new benzimidazole derivatives for their possible antitubercular activity as well as their interactions with particular M. tuberculosis target enzymes.**Methods** They used rational drug design techniques to produce a number of derivatives of benzimidazoles. To predict the affinity of binding and interactions against specific M. tuberculosis target enzymes, molecular docking studies have been carried out. Promising derivatives were created using developed synthetic processes based on in-silico screening results, and they were then characterized using suitable analytical techniques. The in vitro antitubercular activity of the produced compounds was further evaluated.**Result** The suggested way of action was confirmed by docking experiments that showed optimum binding affinities and important molecular interactions with the selected target enzymes. When compared to standard drugs, several of synthesized compounds exhibited significant inhibitory activity against M. tuberculosis, according to an in vitro antitubercular evaluation.**Conclusion** Owing to the results, benzimidazole derivatives show promising as lead compounds for the development of new antitubercular drugs. For greater structural optimization and comprehensive pharmacological research targeted at addressing drug-resistant TB, our study establishes an excellent framework.

Keywords : Tuberculosis (TB), Mycobacterium tuberculosis, Benzimidazole derivatives, Molecular docking, Antitubercular agents, Drug design and synthesis, Multidrug-resistant tuberculosis.

INTRODUCTION:

Mycobacterium tuberculosis, the organism which causes tuberculosis (TB), typically affects the lungs. When persons with TB in their lungs cough, sneeze, or spit, Tuberculosis gets transmitted via the air. Only a tiny percentage of germs must be inhaled by an individual to cause infection. Ten million people suffer the disease tuberculosis (TB) each year. Tuberculosis is the leading infectious killer in the world, killing 1.5 million people annually despite being a preventable and curable illness. In addition to being the primary cause of death for HIV-positive individuals, Tuberculosis plays a significant role in the development of antibiotic resistance. Although Tuberculosis is found worldwide, the majority of people suffering from it reside in low- and middle-income nations. Approximately 50% of all TB patients can be found to being the primary cause of death for HIV-positive individuals, TB plays a significant role in the development of antibiotic resistance. Although TB is found worldwide, the majority of TB patients reside in low- and middle-income nations. Eight countries—Bangladesh, China, India, Indonesia, Nigeria, Pakistan, the Philippines, and South Africa—are home to roughly half of all TB patients[1].

Tuberculosis illness types:

By Status of Infection:

Latent TB: While microbes are present in the human body but do not exhibit any symptoms or spread easily, bacteria can eventually become active, particularly in cases when immunity is reduced.

Active TB: The bacteria are multiplying and causing fever, cough, and additional symptoms, and the infected individual can spread the infection.

By Infection Site:

The most prevalent type of TB, pulmonary TB (lung TB), affects the lungs and causes weariness, chest pain, and coughing, occasionally with blood in it.

Tuberculosis external of the lungs, or extrapulmonary:

Impacts other bodily parts,

Tuberculosis of the lymph nodes:

Expanded lymph nodes in the armpits and neck[2].

Treatment:

The disease tuberculosis is treatable. A typical six-month treatment of four antibiotics is used to treat it. Isoniazid and rifampicin are common medications. The infection caused by germs may not always react to the usual medications. The patient in this instance has Tuberculosis that is resistant to medication. Drug-resistant tuberculosis requires more extensive and intricate treatment[3].

If you have latent tuberculosis, you may only need to take one or two medications. Several medications are necessary for active tuberculosis. The following drugs are frequently prescribed to treat tuberculosis:

- Isoniazid.
- Rifampicin.
- Rifabutin.
- Pyrazinamide.
- Ethambutol.

If you have drug-resistant tuberculosis or any other health complications, you can be prescribed additional medications[4].

Target:

- **ENZYME NAME:** Mycobacterium
- **CLASSIFICATION:** Transferase
- **TYPE:** Protein
- **CHAINS:** A
- **ORGANISM:** Mycobacterium tuberculosis.
- **PDB ID:** 6P9K

- **RESOLUTION:** 1.70Å

Structure:

The 6P9K protein structure has proven KasA as a promising target for the discovery of novel and effective antitubercular drugs. By inhibiting essential cell wall synthesis, it provides the structural data essential to create drugs that are effective against drug-sensitive and drug-resistant strains of Mycobacterium Tuberculosis[5].

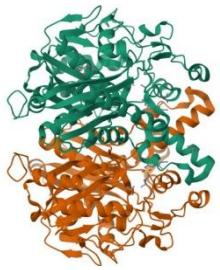


Figure no 1: Structure of 6P9K Protein[6]

Drug design:

Computational approaches to drug discovery and development are quickly becoming more well-liked, widely used, and appreciated. Rational drug design, another name for drug design, is the creative process of developing a novel drug based on understanding about a biological target. A common approach to drug design is computer modeling, which is known as computer-aided drug design (CADD)[7].

Important of Heterocyclic rings:

Heterocyclic systems are acknowledged as being extremely significant because of their demonstrated value in the field of medicinal chemistry. One or more heteroatoms are present in cyclic rings of atoms that are known as heterocyclic compounds. Heterocyclic rings including other heteroatoms, such as phosphorus, iron, magnesium, selenium, etc., are also common, although the most common hetero-atoms are nitrogen, oxygen, and sulfur. Because of its therapeutic uses, heterocycles—the most significant traditional branch of organic chemistry—are gaining more attention in study[8].

Scaffold Moiety:

Benzimidazole is a significant structural motif included in a large number of pharmacologically active and natural compounds. In particular, benzimidazoles may be regarded as auxiliary isosteres of nucleotides with attached heterocyclic cores in their structures. They work well with biopolymers and may be useful in chemotherapeutic applications. Anticancer, antimicrobial, antihistamine, anthelmintic, antioxidant, antihypertensive, antiviral, anticoagulant, and antiulcer activity are just a few of the therapeutic areas in which the benzimidazole moiety serves as a privileged scaffold to synthesize selective drugs of interest[9].

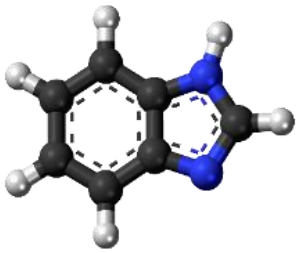


Figure no 2: Ball and Stick model of Benzimidazole.

Methods and materials:

Selection of target

The Protein Data Bank is a crystallographic database that contains three-dimensional structural information about big biological entities such complex collections of proteins, and nucleic acids.

Pharmacophore modelling:

A Pharmacophore is defined as "a set of structural features in a molecule that is recognized at a receptor site and is responsible for that molecule's biological activity". The design of the new transferase inhibitors is based on current inhibitors, their interactions with the protein, and the molecule's common features necessary for biological activity.

Creation of Virtual library:

Using the comprehension of how the ligand reacts to the protein and the shared pharmacophoric characteristics essential for a molecule's biological activity, an inventory of around 35 novel compounds that are strong Transferase inhibitors was developed. The databases were filtered by chemical characteristics including hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), and aromatic ring features.

Creation of a virtual library of ligands and novelty checking:

The ACD Chem Sketch tool is used to sketch the ligands according to the required pharmacophoric characteristics. Utilizing the Pub Chem database, the compounds' uniqueness is confirmed. Since there is no information in the ZINC® database, the developed compounds were regarded as novel[10].

***In-silico* drug likeness screening:**

A qualitative notion, drug similarity is defined by the molecular characteristics of a molecule that influence its absorption, distribution, metabolism, excretion, and toxicity (ADMET). In the early stages of drug development, drug likeness—which is generated from the structures and characteristics of current medications and drug candidates—has been routinely employed to weed out undesired molecules. Hydrophobicity, electronic distribution, hydrogen bonding properties, molecule size, flexibility, and the presence of different pharmacophoric features that affect a molecule's behavior in a leaving organism, such as protein affinity and bioavailability, are the main characteristics. Osiris Property Explorer and Molinspiration, two online software tools, were used to assess the developed ligands for drug similarity[11].

Molinspiration®

Molinspiration® is an online software program which utilizes the Lipinski rule of five to assess ligands in silico pharmacokinetic parameters. In addition to estimating the bioactivity score of ligands, it was used to predict molecular parameters such as molecular weight, log P, total polar surface area, number of atoms, number of rotatable bonds, number of hydrogen bond acceptors and donors, etc[12].

ADMET properties: Osiris Property Explorer

Osiris Property Explorer is an online cheminformatics tool used to assess a chemical compound's potential for toxicity. The results of virtual toxicity are color-coded in either red or green. In this case, green denotes chemicals that are safe and nontoxic, whereas red denotes molecules that are toxic and have unwanted consequences like as mutagenicity, tumorigenicity, irritation, and reproduction. By illustrating the structures using an online tool, the *In-silico* toxicity of the suggested compounds was ascertained[13].

Molecular docking studies:

Molecular docking is typically used to identify the orientation and interaction between proteins and ligands. The present work employed Autodock Tools 4.2(1.5.6) software to determine the ligand binding energy.

Preparation of target:

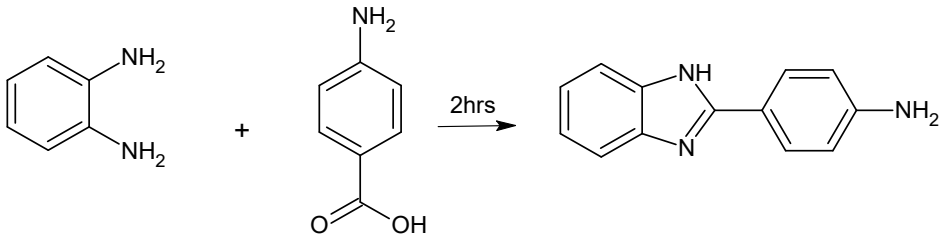
Using Molegro Molecular Viewer, water molecules, cofactors, and protein co-crystallized ligands were eliminated from the crystal structure. The protein chain was saved in a repository work folder called the destination folder after being exported in PDB format from the Molegro Molecular Viewer.

Synthetic Method:

Based on computational approaches, the produced compounds have a high affinity for the target enzymes transferase and are appropriate therapeutic candidates with negligible toxicity. They were selected to be synthesized in the lab.

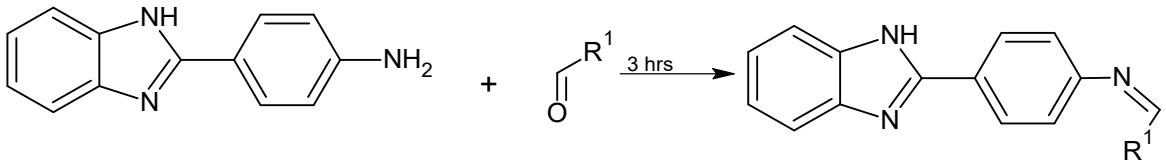
Synthetic scheme:

Step 1:



A mixture of O-phenylenediamine (0.1 mol) and 4-aminobenzoic acid (0.1 mol) had been heated in a water bath for two hours. The reaction mixture was cooled and poured over crushed ice. The bulky white precipitate then stirred in 400 ml of cold water, and a 5 M sodium hydroxide solution was added until the pH reached 7. The remaining was filtered and recrystallized from ethanol.

Step 2:



In a round bottom flask, the substituted aldehydes (0.01mol) were added to the 4- (1H-Benzimidazole-2-yl) aniline (0.01mol) in 30 ml of ethanol containing a few drops of glacial acetic acid, and the mixture was refluxed for 3 hours. It was then cooled to room temperature, poured onto crushed ice, filtered, dried, and recrystallized from ethanol. Another Schiff's base was obtained in the exact same way[14].

UV Spectroscopy:

The substances' absorbance spectra can be measured using UV spectroscopy. The measurements of coloured compounds can be determined and compounds can be identified with the use of these absorption spectra. This molecule absorbs light at a range of wavelengths, yielding an absorption spectrum. Plotting absorbance versus wavelength is all that constitutes an absorption spectrum. The wavelength in the absorption spectrum where the absorbance is highest is known as the λmax. UV spectra were obtained using the UV-1900 Series.

UV-VISIBLE SPECTROMETER.

IR Spectroscopy:

The electromagnetic spectrum's infrared section is the subject of IR spectroscopy. Infrared spectroscopy is the broad term for the study of how molecules interact with infrared light. Determining the functional groups of molecules relevant to both organic and inorganic chemistry is an important use of infrared spectroscopy. IR spectra were collected using an ABB MB3000-PH FT-IR Spectrometer.

Regions of infrared spectrum:

The vast majority of bands displaying functional groups are located between 4000 and 1300 cm-1; an unknown compound's functional group can be identified using its bands. The fingerprint region contains bands that are peculiar to each molecule and range from 1300 cm-1 to 400 cm-1, much like a fingerprint. Only the spectra from various compounds may be compared through these bands[15].

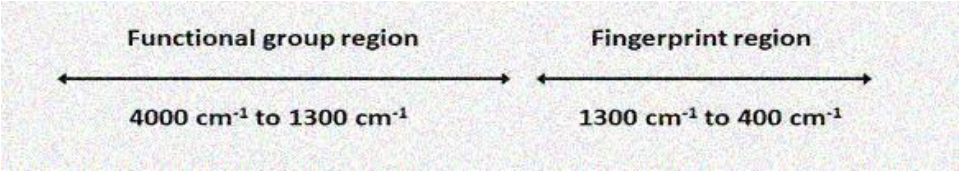


Figure no 3: Regions of infrared spectrum

Nuclear Magnetic Resonance (NMR):

The technique known as nuclear magnetic resonance (NMR) spectroscopy is used to ascertain the molecular structure and composition of a material. The most effective method for learning a compound's structure is nuclear magnetic resonance (NMR). All organic substances are analysed using 1H NMR and 13C NMR spectroscopies. The structure of a molecule can be determined by using proton NMR spectroscopy to investigate the number of equivalent protons and their surroundings. BRUKER Topspin Advance 400 MHZ NMR Spectra were used to record the NMR spectra, with deuterated DMSO serving as the solvent. The basic principle of the NMR phenomenon is the ability of atoms' nuclei to produce chemical information through their magnetic characteristics.

***In-vitro* evaluation of anti-Tuberculosis activity:**

In vitro antituberculosis activity was assessed for all produced molecules A5 and A35 using the Lowenstein-Jensen slope technique, with minor modifications, against the H37RV strain. Comparing the synthesized compounds to the standard medication rifampicin, the in-vitro antitubercular screening revealed that they have good to exceptional antitubercular activity.

$$\% \text{ Inhibition} = (1 - At/A0) \times 100$$

Where,

A= The control's absorbance.

At = The tested compound's absorbance.

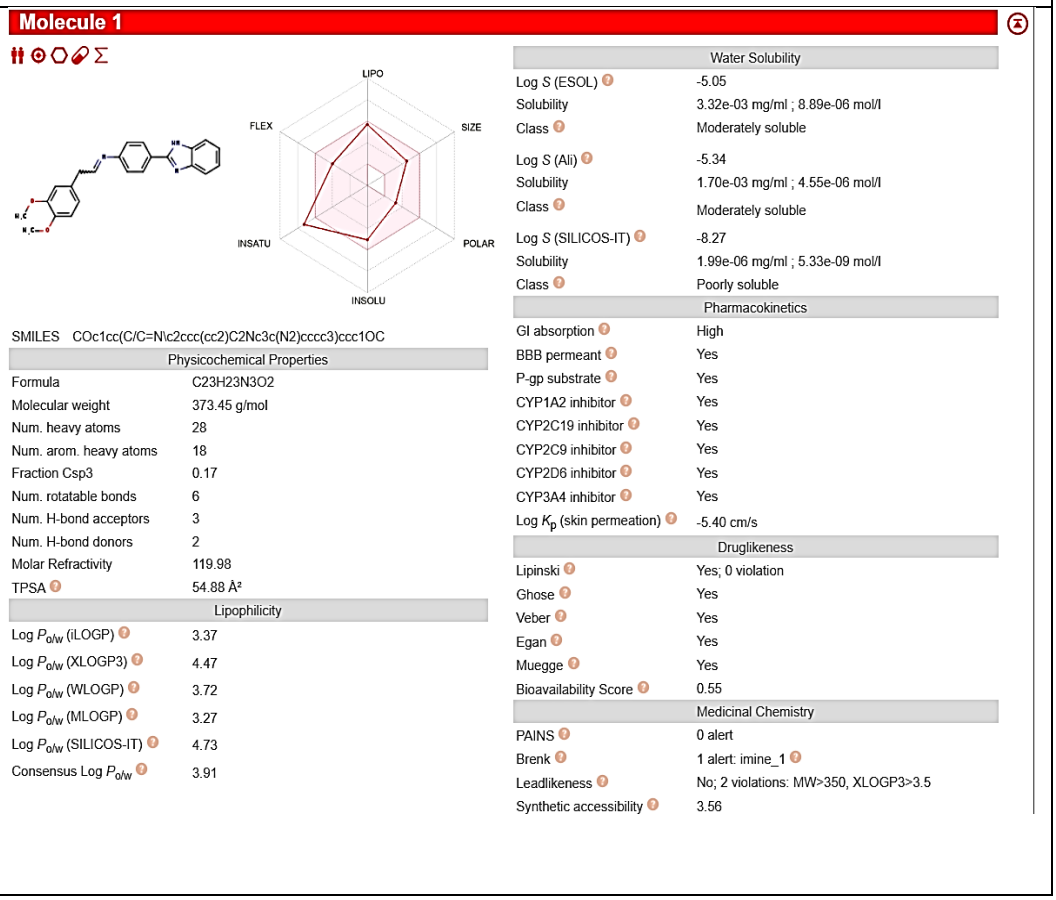
International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 32, Issue 4, April 2026 ijppr.humanjournals.com ISSN: 2349-7203

RESULT AND DISCUSSION:

In-silico drug-likeness properties

The molecular physiochemical properties are predicted using in silico drug-likeness properties software, and the bioactivity score has been calculated to determine the drug's bioavailability when taken orally.

Table no 01: *In-silico* drug-likeness properties

SAMPLE CODE	Swiss ADME Results
A5	

Molecule 1

SMILES COc1cc(C/C=N\c2ccc(cc2)C2Nc3c(N2)cccc3)ccc1OC

Physicochemical Properties	
Formula	C23H23N3O2
Molecular weight	373.45 g/mol
Num. heavy atoms	28
Num. arom. heavy atoms	18
Fraction Csp3	0.17
Num. rotatable bonds	6
Num. H-bond acceptors	3
Num. H-bond donors	2
Molar Refractivity	119.98
TPSA	54.88 Å²

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	3.37
Log $P_{o/w}$ (XLOGP3)	4.47
Log $P_{o/w}$ (WLOGP)	3.72
Log $P_{o/w}$ (MLOGP)	3.27
Log $P_{o/w}$ (SILICOS-IT)	4.73
Consensus Log $P_{o/w}$	3.91

Water Solubility	
Log *S* (ESOL)	-5.05
Solubility	3.32e-03 mg/ml ; 8.89e-06 mol/l
Class	Moderately soluble
Log *S* (Ali)	-5.34
Solubility	1.70e-03 mg/ml ; 4.55e-06 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-8.27
Solubility	1.99e-06 mg/ml ; 5.33e-09 mol/l
Class	Poorly soluble

Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	Yes
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-5.40 cm/s

Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55

Medicinal Chemistry	
PAINS	0 alert
Brenk	1 alert: imine_1
Leadlikeness	No; 2 violations: MW>350, XLOGP3>3.5
Synthetic accessibility	3.56

International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
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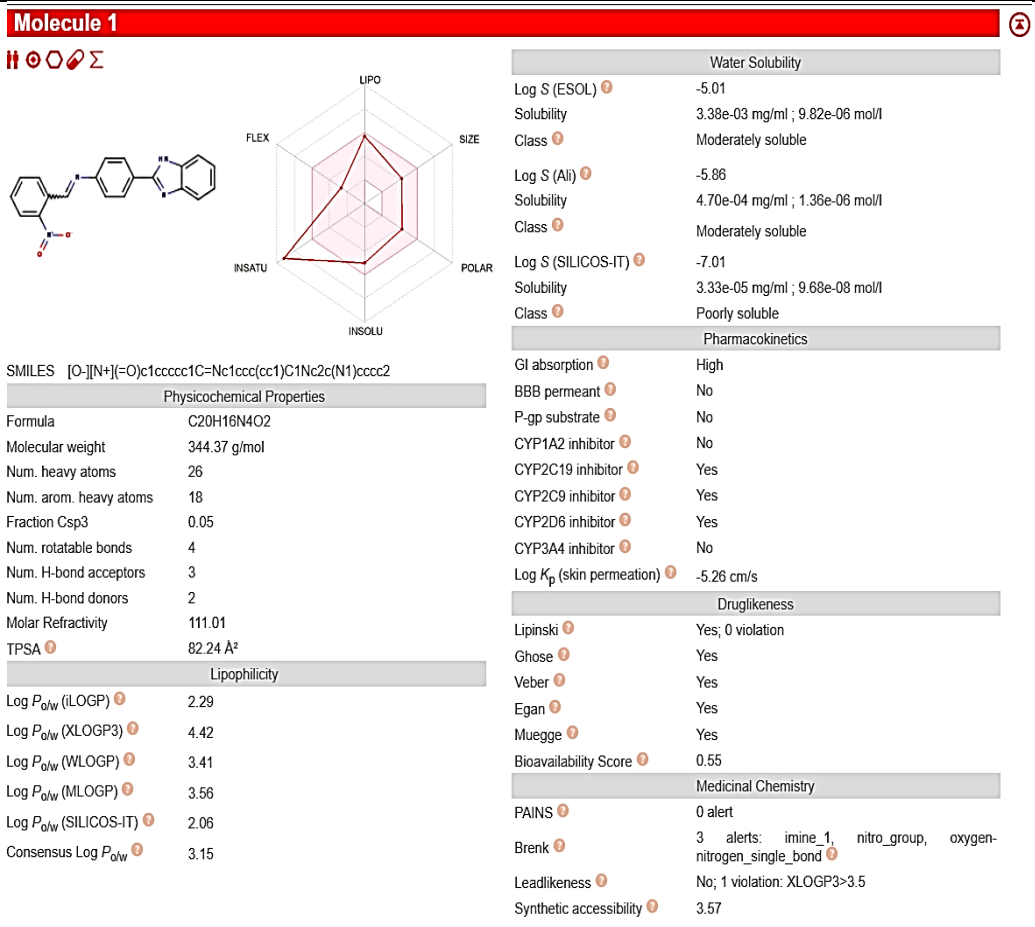
SAMPLE CODE	Swiss ADME Results
A35	

Molecule 1

SMILES [O-][N+](=O)c1ccccc1C=Nc1ccc(cc1)C1Nc2c(N1)cccc2

Physicochemical Properties	
Formula	C20H16N4O2
Molecular weight	344.37 g/mol
Num. heavy atoms	26
Num. arom. heavy atoms	18
Fraction Csp3	0.05
Num. rotatable bonds	4
Num. H-bond acceptors	3
Num. H-bond donors	2
Molar Refractivity	111.01
TPSA	82.24 Å²

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	2.29
Log $P_{o/w}$ (XLOGP3)	4.42
Log $P_{o/w}$ (WLOGP)	3.41
Log $P_{o/w}$ (MLOGP)	3.56
Log $P_{o/w}$ (SILICOS-IT)	2.06
Consensus Log $P_{o/w}$	3.15

Water Solubility	
Log *S* (ESOL)	-5.01
Solubility	3.38e-03 mg/ml ; 9.82e-06 mol/l
Class	Moderately soluble
Log *S* (Ali)	-5.86
Solubility	4.70e-04 mg/ml ; 1.36e-06 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-7.01
Solubility	3.33e-05 mg/ml ; 9.68e-08 mol/l
Class	Poorly soluble

Pharmacokinetics	
GI absorption	High
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-5.26 cm/s

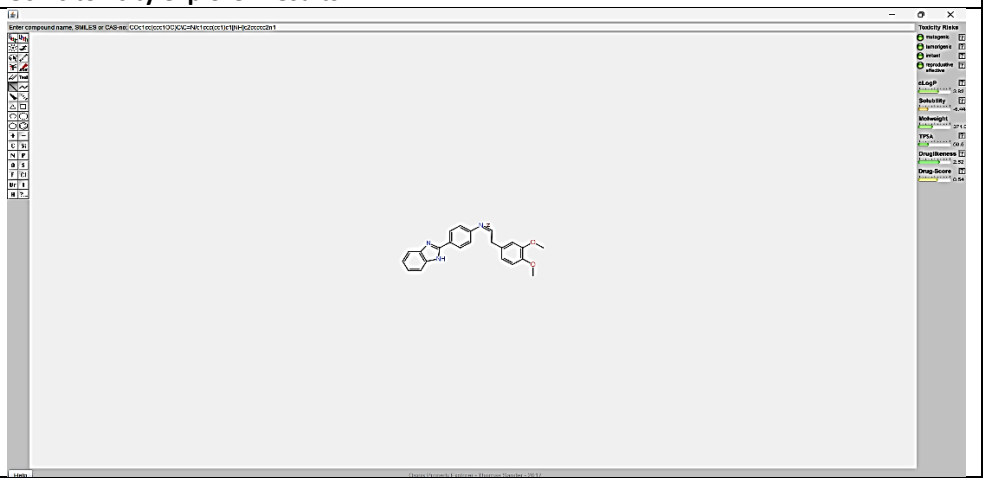
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55

Medicinal Chemistry	
PAINS	0 alert
Brenk	3 alerts: imine_1, nitro_group, oxygen-nitrogen_single_bond
Leadlikeness	No; 1 violation: XLOGP3>3.5
Synthetic accessibility	3.57

Osiris Toxicity Explorer®

The Osiris Toxicity Explorer® software is used to determine whether the newly discovered compounds are toxic or not. When molecules are green, it means they are safe and nontoxic, and when that are red, it means that are potentially harmful.

Table no 03: In silico drug toxicity by Osiris toxicity explorer

SAMPLE CODE	Osiris toxicity explorer Results
A5	

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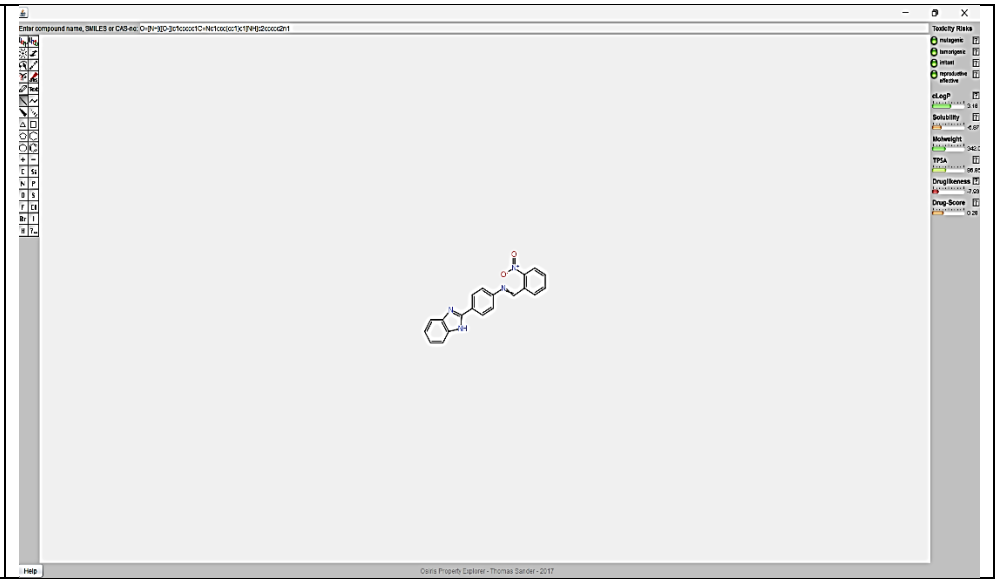
A35	

Table no 04: physiochemical and ADMET properties

Physicochemical and ADMET properties	A5	A35
Mutagenic	No	No
Tumorigenic	No	No
Irritant	No	No
Reproductive effective	No	No
LogP	3.37	2.29
Solubility	-5.05	-5.01
Molecular weight	373.45g/mol	344.37g/mol
Blood-Brain Barrier	No	No
HBA	3	3
HBD	2	2
No rotatable bonds	6	4

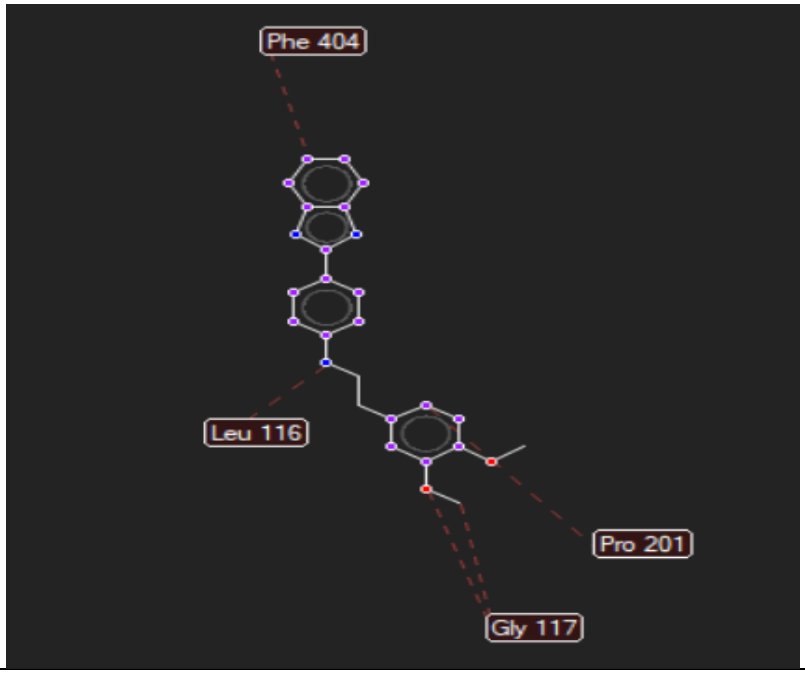
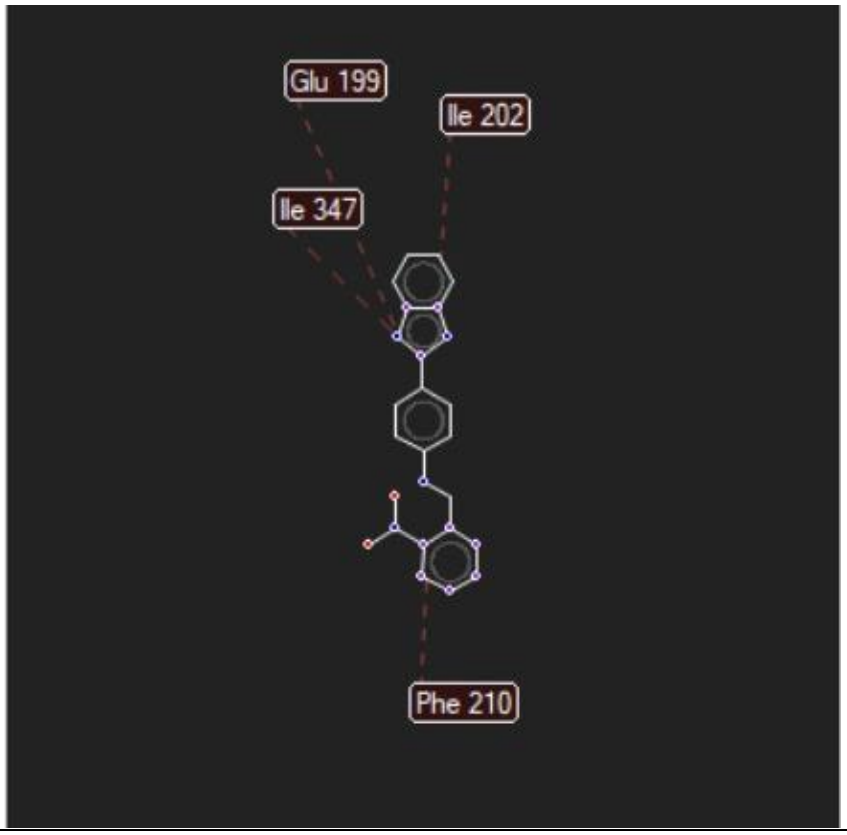
Docking studies:

Using the (PDB) protein databank at a resolution of 1.70Å, the docking study aims to determine the most suitable position for the ligand (benzimidazole derivative) within the receptor-binding site (6P9K). The docked ligands exhibited binding affinities ranging from -6.91 to -9.37 kcal/mol, indicating an effective interaction with the receptor's active regions. They all have strong hydrophobic and hydrogen bonding relationships with the amino acids in the protein. The Discovery Studio 2021 Client program can be used to evaluate the results. A visual analysis is essential for evaluating the program's effectiveness.

Table no 05: Docking scores of the top scored compounds

S.NO	SAMPLE CODE	BINDING ENERGY
1	A5	-7.17
2	A35	-9.31

Table no 06: Binding interaction of the top scored compounds

SAMPLE CODE	Binding interaction Results
A5	
A35	

Characterization:

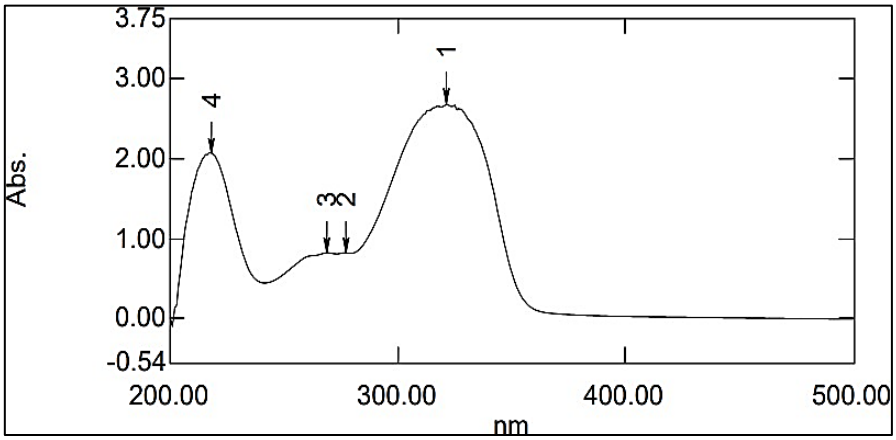


Figure no 4: UV Spectrum of compound A5

UV SPECTRUM OF COMPOUND A5

Table no 07: UV Spectrum of compound A5

S.NO	WAVELENGTH	ABSORBANCE
1.	321.5	2.684
2.	277.0	0.825
3.	269.0	0.826
4.	218.0	2.079

λmax value of the compound A5 was found to be **321.5 nm**.

IR SPECTRUM OF COMPOUND A5

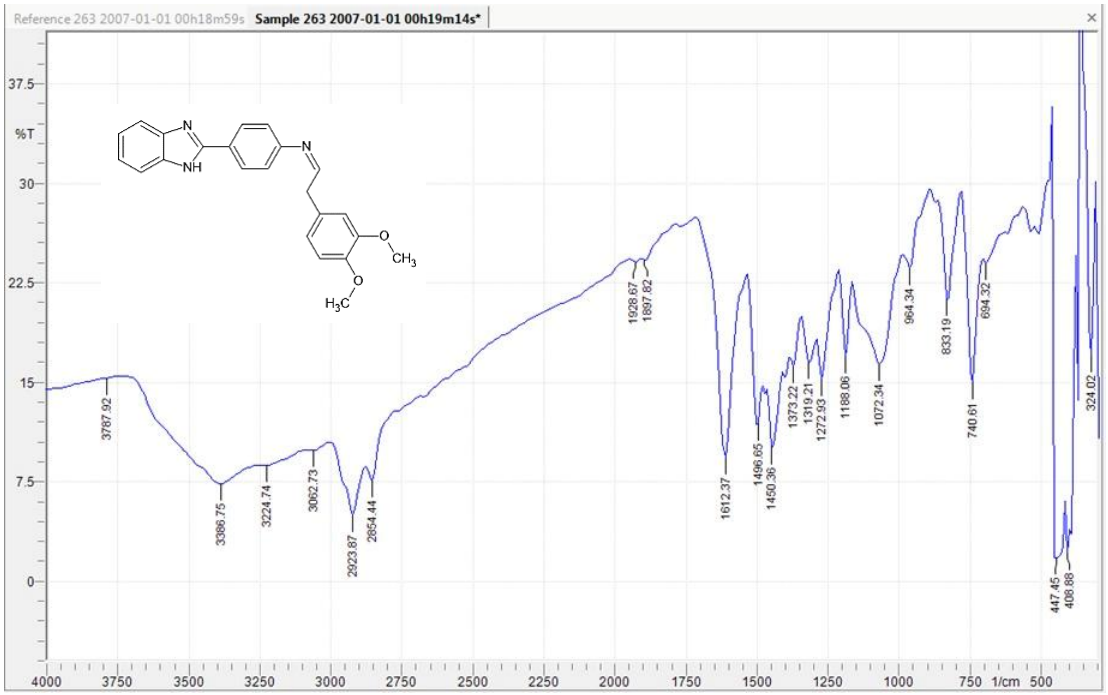


Figure no 5: IR Interpretation of compound A5

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Table no 08: IR Interpretation of compound A5

S.NO	WAVE NUMBER (cm-1)	FUNCTIONAL GROUPS
1.	1496.65	C=C Stretching
2.	1319.21	C-N
3.	1612.37	C=N
4.	3386.75	NH
5.	3062.73	C-H
6.	1072.34	C-O

NMR SPECTRUM OF COMPOUND A5

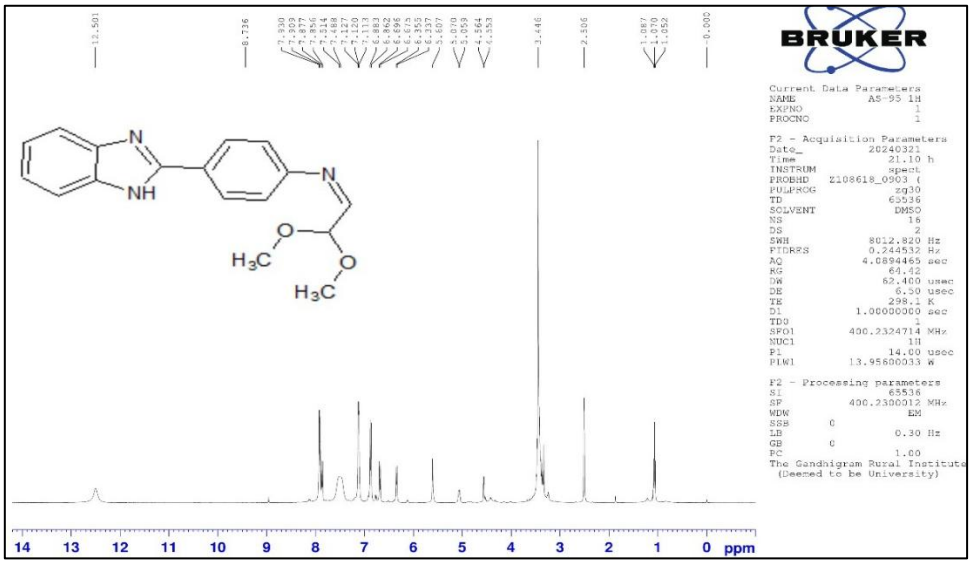

Figure no 6: NMR Interpretation of compound A5

Table no 09: NMR Interpretation of compound A5

S.NO	DELTA VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTON
1.	6.0-7.0	Aromatic CH	Multiple	8
2.	12.50	NH	Singlet	1
3.	8.736	CH=N	Doublet	1
4.	3.446	O-CH3	Singlet	6
5.	5.078	CH (METHINE)	Doublet	1

UV SPECTRUM OF COMPOUND A35

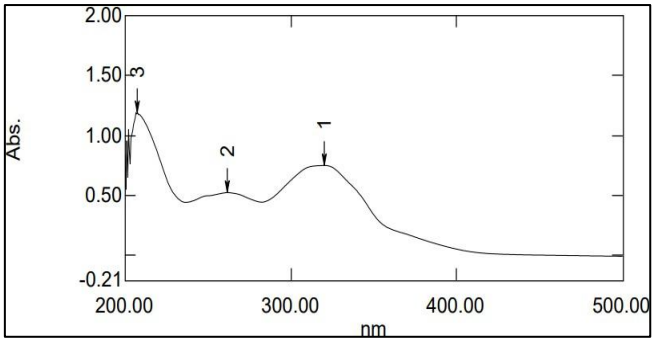

Figure no 7: UV spectrum of compound A35

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Table no 10: UV spectrum of compound A35

S.NO	WAVELENGTH	ABSORBANCE
1.	320.0	0.750
2.	261.5	0.522
3.	207.5	1.189

λmax of the compound A35 was found to be **320.0 nm**.

IR SPECTRUM OF COMPOUND A35

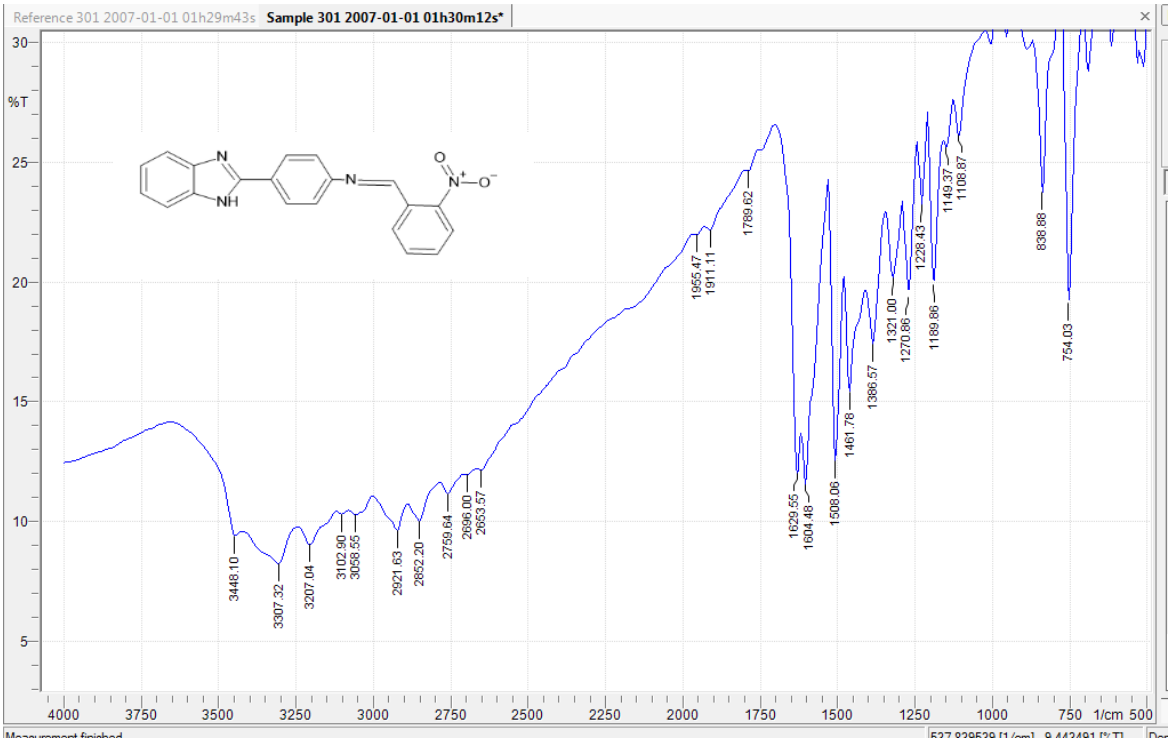

Figure no 8: IR Spectrum of compound A35

Table no 11: IR interpretation of compound A35

S.NO	WAVE NUMBER (cm^{-1})	FUNCTIONAL GROUPS
1.	1461.78	C=C
2.	3058.55	C-H Stretching
3.	838.88	C-H Bending
4.	3307.32	N-H
5.	1604.48	C=N
6.	1321.00	C-N
7.	1508.06	NO_2 Stretching

International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 32, Issue 4, April 2026 ijppr.humanjournals.com ISSN: 2349-7203

NMR SPECTRUM OF COMPOUND A35

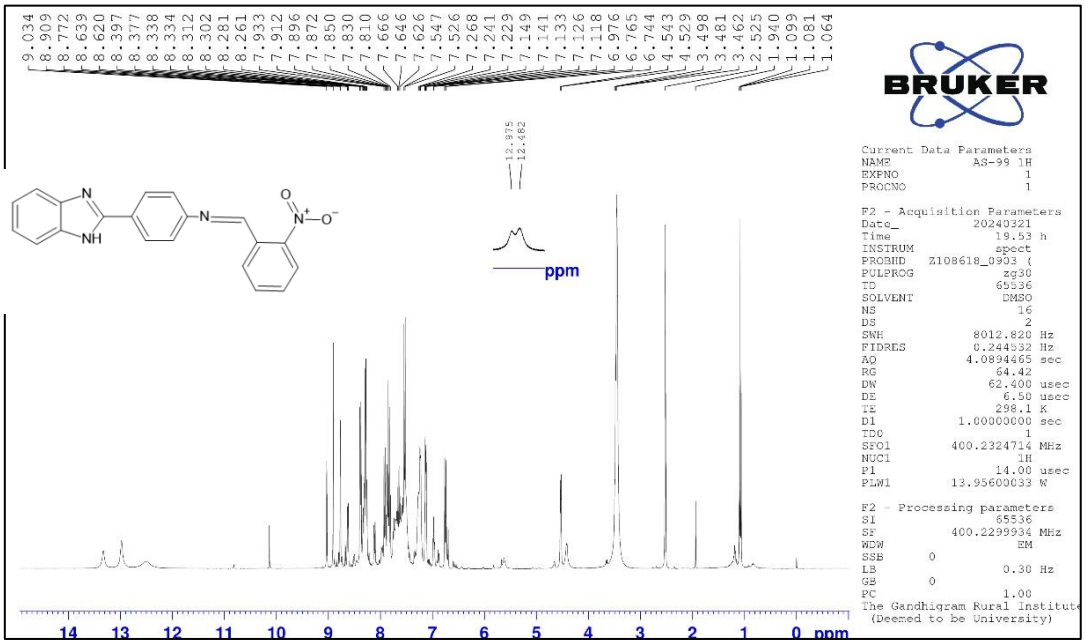

Figure no 9: NMR spectrum of compound A35

Table no 12: NMR interpretation of compound A35

S.NO	DELTA VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTON
1.	7.0-8.0	Aromatic CH	Multiple	12
2.	12.482	NH	Singlet	1
3.	8.772	CH=N	singlet	1

***In-vitro* evaluation of anti-tubercular activity:**

Screening method:

Screening method of compound A35

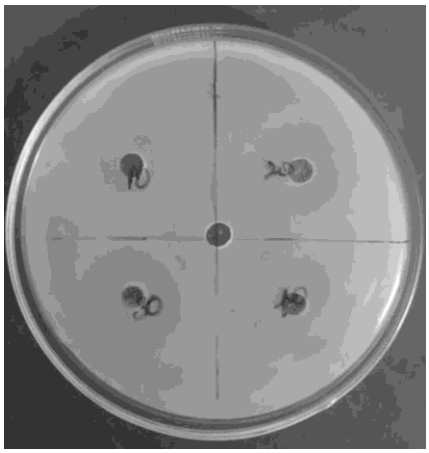

Figure no 10 : Screening method of compound A5

International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 32, Issue 4, April 2026 ijppr.humanjournals.com ISSN: 2349-7203

ANTI-TUBERCULAR ACTIVITY OF A5

Table no 13: Anti-tubercular activity of compound A5

S. No	Strain	Composite				Rifampicin
		10µl	20µl	30µl	40µl	20µl
1	Mycobacterium tuberculosis	14mm	18mm	21mm	25mm	35mm

Screening method of compound A35

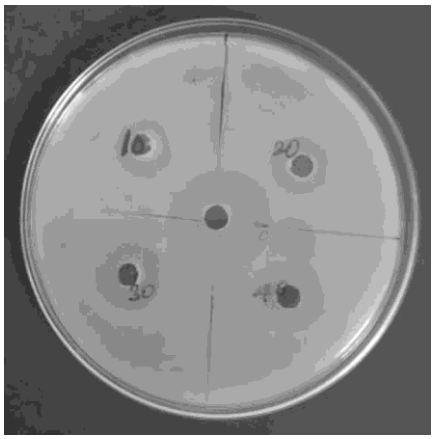

Figure no 11: Screening method of compound A35

ANTI-TUBERCULAR ACTIVITY OF A35

Table no 14: Anti-tubercular activity of compound A35

S. No	Strain	Composite				Rifampicin (C)
		10µl	20µl	30µl	40µl	20µl
1	Mycobacterium tuberculosis	12mm	14mm	18mm	21mm	35mm

Confirmatory test:

MABA- Microplate Alamar Blue Assay

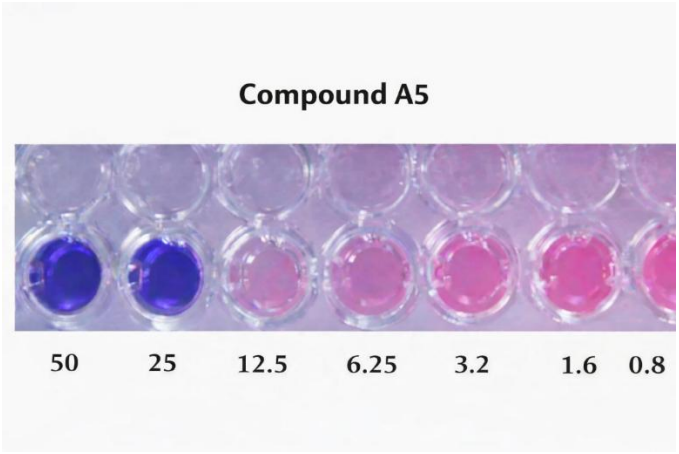

Figure no 12: MABA of compound A5

Table no 15: MABA results and its activity of compound A5

S.NO	µg/ml	COLOUR	ACTIVITY
1	0.8	Pink	-
2	1.6	Pink	-
3	3.2	Pink	-
4	6.25	Pale pink	-
5	12.5	Pale pink	-
6	25	Blue	Moderate
7	50	Blue	Moderate
8	Rifampicin (0.8)	Blue	High

Summary:

The goal of computational drug discovery is to create a novel chemical that may be used to cure or prevent an illness or infection. According to WHO data, tuberculosis sickness can be lethal if left untreated and is typically treated with medications. Thus, designing and synthesizing some innovative anti-tubercular medicines was the goal of the current work.

Mycobacterium TB transferase enzyme was selected as the successful target for anti-tubercular therapy based on the evaluation of the literature. A scaffold library of 35 freshly designed ligands has been created after a review of the literature and an understanding of the inhibitors that are already available and how they interact with enzymes. The program Auto dock 4.2 (1.5.6) was used to perform docking investigations on the ligands. By doing drug-likeness screening using the Lipinski rule of five, predicting favorable ADMET characteristics using online resources like Molinspiration and conducting an *in-silico* toxicity analysis using Osiris Property Explorer, the ligands were further improved. It was discovered that every one of the developed ligands was non-toxic and could be taken orally. Five leads were chosen for synthesis based on their high docking score and synthetic viability. They synthesized both leads. UV, IR, and 1 H NMR spectroscopy was used as analytical techniques to characterize the chemicals. For additional in vitro research, Compounds A5 and A35 with the best docking scores were chosen. The inhibitory concentration was obtained using the in vitro Transferase assay. It was determined that the IC50 value was somewhat powerful. It was discovered to be nearly as effective as the current standard Rifampicin.

Conclusion:

The current study provides information on a number of heterocycles that block the transferase, such as benzimidazole, which are strong anti-tubercular drugs. It prompted us to use molecular docking to forecast the likely interactions between the 35 newly created ligands and the receptor's amino acid constituents. Using Auto dock 4.2 (1.5.6), the 35 compounds were docked against transferase. It was discovered that the two synthetic chemicals, A5 and A35, were non-toxic, exhibited drug-likeness, and complied with Lipinski's rule of 5. A number of 2-substituted benzimidazole derivatives were created, and spectral data helped to clarify their structures. When tested for in vitro anti-tubercular activity, all of the produced compounds showed notable effects. It was discovered to have moderate potency, which is nearly as strong as Rifampicin.

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How to cite this article:
Dr. S. Chandra et al. Ijppr.Human, 2026; Vol. 32 (4): 146-162.

Conflict of Interest Statement: All authors have nothing else to disclose.