Synthesis, Characterization, Molecular Docking, and Biological Evaluation of Novel Schiff Base of Benzimidazole Derivatives as Potential Anti-Tubercular Agents.

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Abstract

This study details the synthesis and biological evaluation of novel Schiff base benzimidazole derivatives. The compounds were synthesized via a three-step reaction sequence involving cyclization of ortho-phenylenediamine with cyanogen bromide, Schiff base formation with substituted aromatic aldehydes, and cyclization with thioglycolic acid catalysed by zinc chloride in presence of 1,4 dioxane as solvent to produce thiazolidine derivatives. Structural confirmation was achieved through FTIR, ^{1}H NMR, ^{13}C NMR, and MS spectra data, then Pharmacological potential was assessed using molecular docking and in vitro assays targeting tuberculosis. Compounds PYK-1, PYK-3 and PYK-5 exhibited significant bioactivities. Particularly, PYK-3 showed enhanced antitubercular efficacy attributed to trimethoxy substitution, which likely increases lipophilicity and target binding. In assays, these compounds demonstrated notable effects on anti-tubercular effects comparable to standard drugs.

Key words: Benzimidazole, Schiff base in-silico study. Anti-tuberculosis, MABA

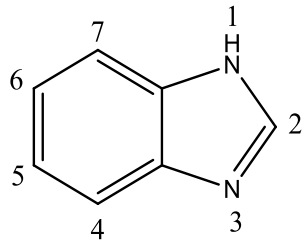
(Multiplate Alamar Blue assay)

1.Introduction:

Benzimidazole is a heterocyclic aromatic compound comprising of a fused benzene ring and an imidazole moiety, resulting in a bicyclic system that forms the core of many biologically active molecules. This scaffold is highly regarded in medicinal chemistry as a privileged structure, owing to its capacity to act as a key pharmacophore and confer a diverse array of therapeutic properties. The classical synthetic route to benzimidazole typically involves

condensing o-phenylenediamine with formic acid, which efficiently generates the parent heterocyclic system.[1]

Structural modification of the benzimidazole ring, particularly by introducing various substituents at the 1-, 2-, and 5-positions, enables the development of compounds exhibiting a wide spectrum of biological activities. [2] Numerous drugs of significant clinical relevance, such as albendazole and mebendazole (both established anthelmintics), as well as omeprazole, an effective proton pump inhibitor used in the management of peptic ulcers and gastroesophageal reflux disease, are based on benzimidazole derivatives. These applications underscore the importance of benzimidazole as a central scaffold in the design and optimization of novel therapeutics within medicinal chemistry research.[3]



Benzimidazole nucleus is a constituent of many bioactive heterocyclic compounds that are of wide interest because of their diverse biological and clinical applications. This interest in benzimidazole chemistry has been increased by the discovery that the 5,6-dimethylbenimidazole moiety is part of chemical structure of vitamin B12. This created interest for researchers who have synthesized variety of benzimidazole derivatives and screened them for their various biological activities.**[4]**

Benzimidazole and its derivatives have been reported to exhibit a wide spectrum of pharmacological activities, including: antioxidant [5], anticancer [6], Anthelmintic [7], antihypertensive [8], antiviral [9], anti-inflammatory [10], antihistaminic [11] analgesic [12] antiprotozoal [13], antiulcer [14] anticoagulant [15], anticonvulsant [16] antifungal [17], antihepatitic B Virus [18] and antibacterial activity [19] anti- tuberculosis [20], anti-diabetic.[21]

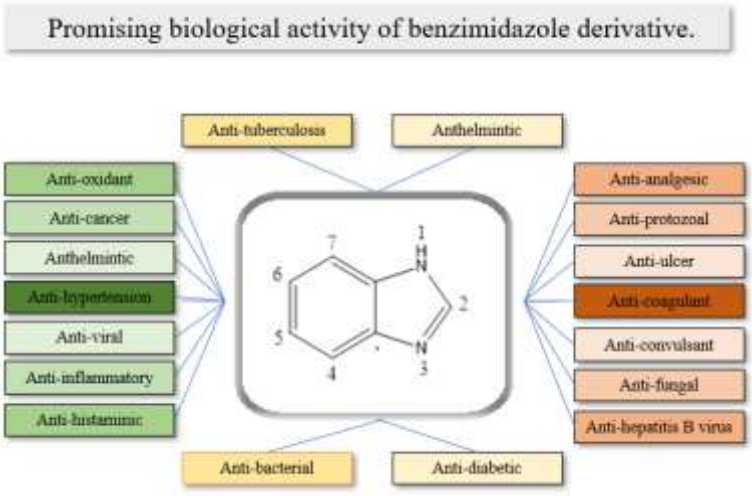
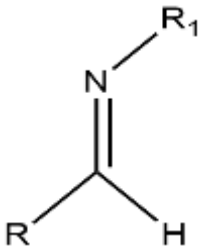


Fig. Schematic representation of biological activity of benzimidazole derivative.

Introduction of Schiff base.



Schiff bases represent a vital category of organic compounds distinguished by the presence of an azomethine (C=N−>*C*=*N*−) functional group, typically generated through the condensation reaction between primary amines and carbonyl-containing compounds such as aldehydes or ketones. This transformation yields a general structure, RHC=N–R_1, in which both R and R_1 groups may comprise alkyl, aryl, cycloalkyl, or variously substituted heterocyclic units, allowing for significant structural diversity.[22] The approach for synthesis of Schiff bases involves combining an aldehyde or ketone with a primary amine under relatively mild conditions, often utilizing an acid catalyst or an appropriate solvent to drive the reaction toward imine formation and enhance product purity.

The resulting C=N double bond not only serves as a reactive site for subsequent functionalization but also enables Schiff bases to coordinate readily with metal ions, expanding their utility in synthetic and coordination chemistry. [23] Due to their ease of synthesis, adaptability, and the remarkable stability of both the free ligands and their metal complexes, Schiff bases have attracted considerable research attention. Their broad spectrum of applications encompasses catalysis, advanced material development, and numerous areas within bioinorganic chemistry, reflecting the significance of their versatile coordination behaviour and their ongoing prominence in contemporary chemical research.[24] Schiff bases are potentially biologically active compounds and have been reported to possess antifungal, anticancer, anticonvulsant and diuretic activities [25].

2.Result and Discussion

1.chemistry

This article's introduction covers the synthesis and biological activities of benzimidazole derivatives. In continuation of this research, we have designed and synthesized a series of novel Schiff bases of benzimidazole derivatives. These derivatives were prepared using three steps synthetic approach to explore their potential therapeutic properties.

Scheme:

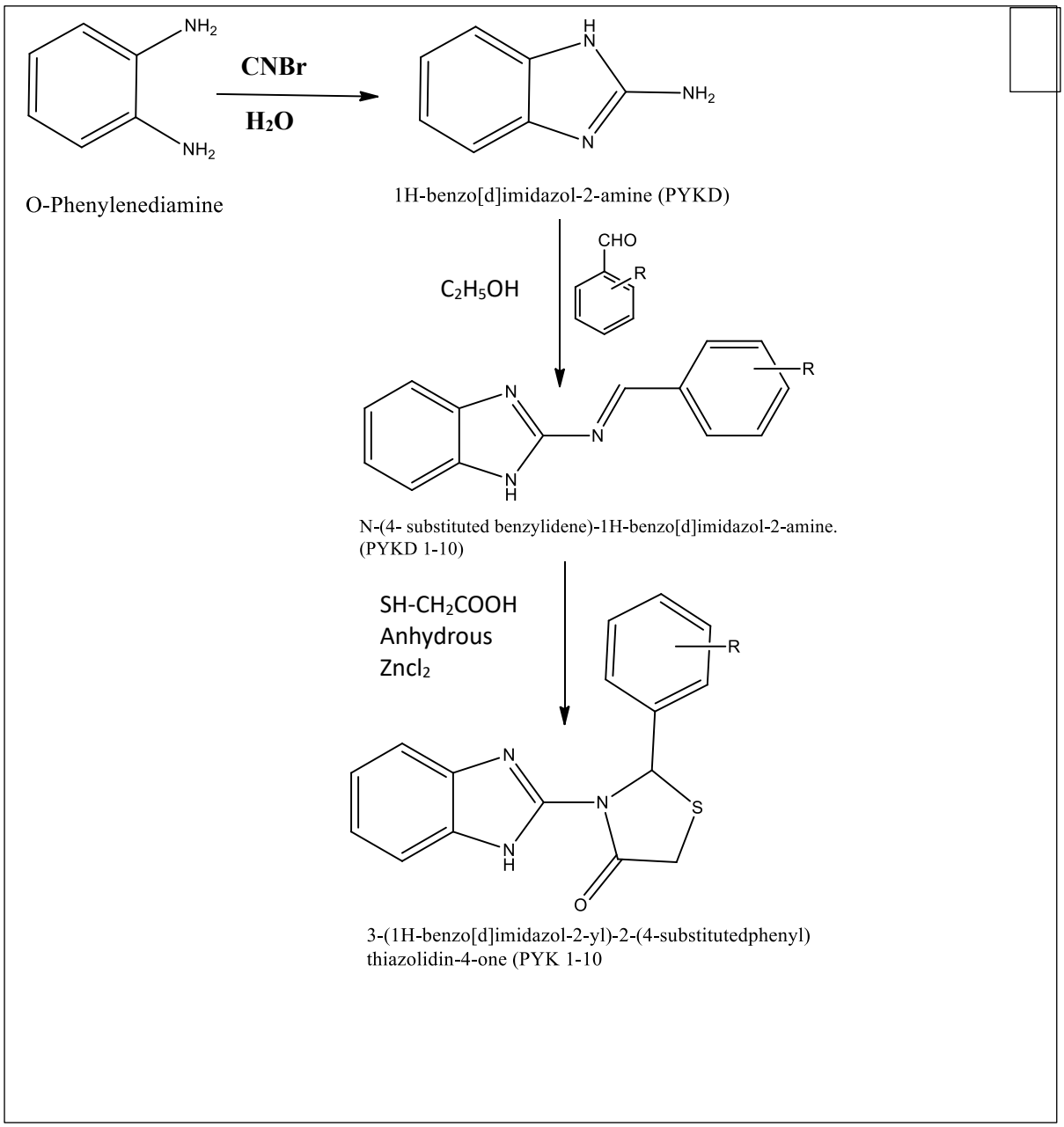
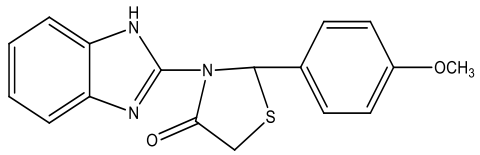
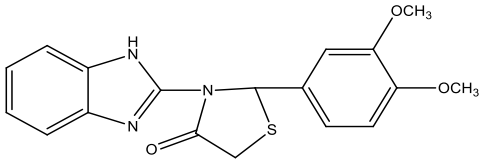
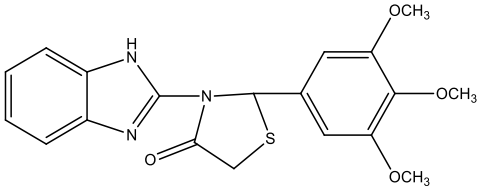
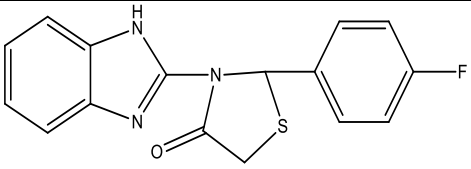
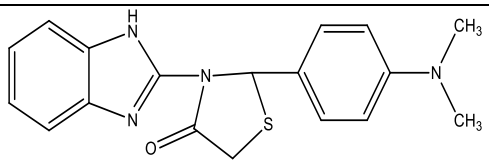
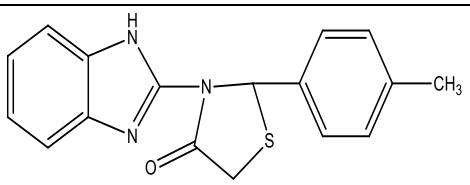
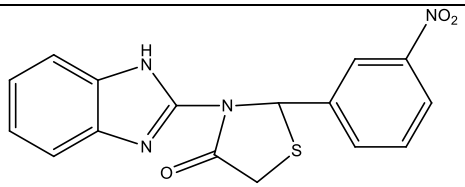
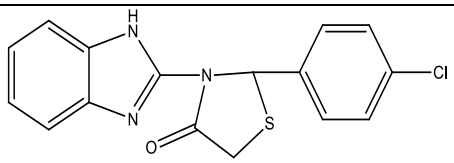
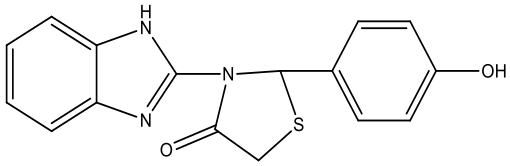
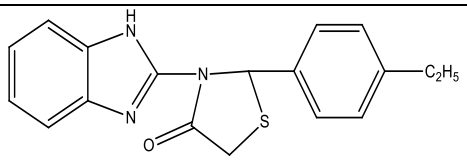

NH2
NH2
CNBr
H2O
H
N
NH2
N
O-Phenylenediamine
1H-benzo[d]imidazol-2-amine (PYKD)
C2H5OH
CHO
R
R
N
N
N
H
N-(4- substituted benzylidene)-1H-benzo[d]imidazol-2-amine.
(PYKD 1-10)
SH-CH2COOH
Anhydrous
Zncl2
R
N
N
H
N
S
O
3-(1H-benzo[d]imidazol-2-yl)-2-(4-substitutedphenyl)
thiazolidin-4-one (PYK 1-10

Table 1: Structure of synthesised Compounds 3-(1H-benzo[d]imidazol-2-yl)-2-(4-substitutedphenyl) thiazolidin-4-one (PYK 1-10)

 **PYK-1**	 **PYK-2**
 **PYK-3**	 **PYK-4**
 **PYK-5**	 **PYK-6**
 **PYK-7**	 **PYK-8**
 **PYK-9**	 **PYK-10**

Step-1: General method of synthesis of 1H-benzo[d]imidazol-2-amine (PYKD).

Cyanogen bromide (3.5 g., 0.034 mole) was added in small portions, with shaking, to a suspension of o-phenylenediamine (4.2 g., 0.34 mole) in water (40 ml), the exothermic reaction mixture was cooled to room temperature and it was stirred for 36hrs. The solution was filtered after standing overnight. Sodium hydroxide (1.4 g., 0.034 mole) in 30 ml of water was then added and the solution was evaporated. The solid thus obtained was filtered and recrystalized from water.

Step-2: General method of synthesis of Schiff base N-(4- substituted benzylidene)-1H-benzo[d]imidazol-2-amine. (PYKD 1-10)

2-aminobenzimidazole (0.01 mol) was refluxed with different substituted aromatic aldehyde (0.01 mol) in ethanol (20 ml) for 6-8 hrs (RT) in presence of glacial acetic acid (few drops). Then the reaction mixture was allowed to cool at RT and the precipitated compound was filtered and dried.

Step-3: General method of synthesis of 3-(1H-benzo[d]imidazol-2-yl)-2-(4-substitutedphenyl) thiazolidin-4-one (PYK 1-10)

An equimolar mixture of compound 2 (0.01), mercaptopuric acid (0.01) and (0.01) in 1,4-dioxane (30ml) containing a small amount of Zinc chloride was refluxed for about 6-8 hours. The resulting product was filtered and cooled in an ice bath to attain room temperature. The solid product was filtered and washed with 10% sodium bicarbonate and recrystallized with alcohol.

<u>Physical properties and spectral characterization of PYK-1</u> 3-(1H-benzo[d]imidazol-2-yl)-2-(4-methoxyphenyl) thiazolidin-4-one. (PYK-1)

Physical Properties: Mol.formula&weight:$C_{17}H_{15}N_3O_2S$,325g/mol, color: Cream, Melting point:171.3 ^{0}C, RF value:0.82, solubility: DMSO, Percentage yield:60%.

IR(KBr)cm^{-1}: 3360 (N-H), 2768 Ar(C-H), 1677 (C=O), 621(C–S-C), 3022(Ar C-H), 1320–1262(C–N).

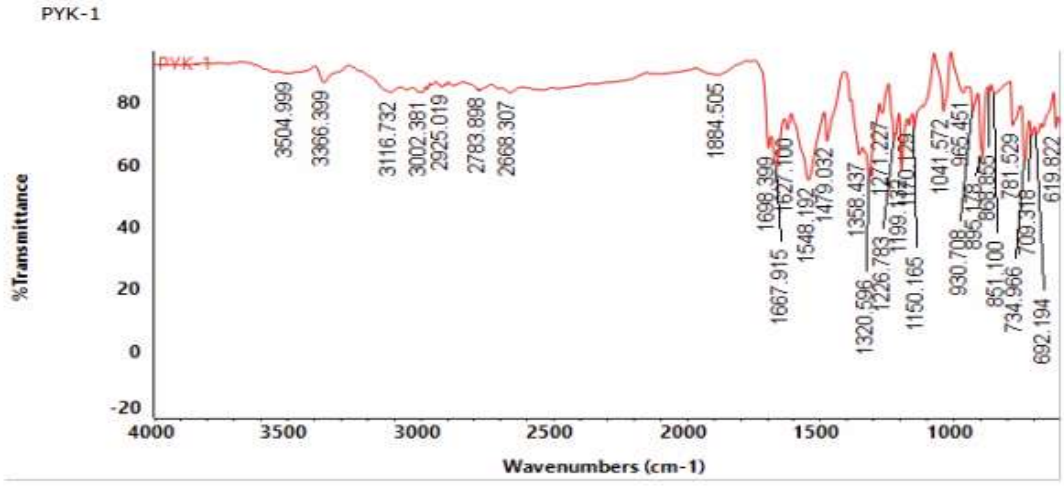


Fig.1.IR of 3-(1H-benzo[d]imidazol-2-yl)-2-(4-methoxyphenyl) thiazolidin-4-onePYK-1).

^{13}C NMR

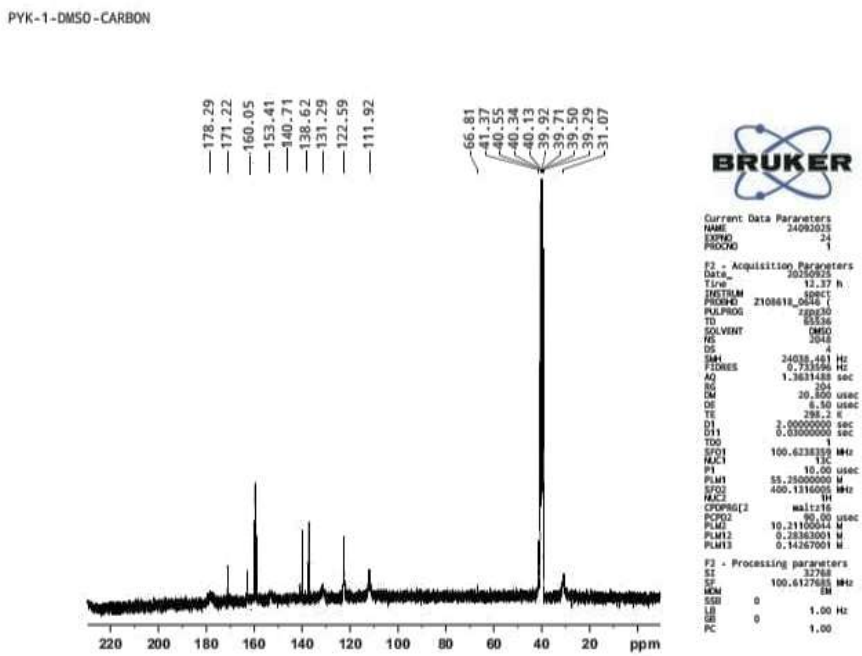
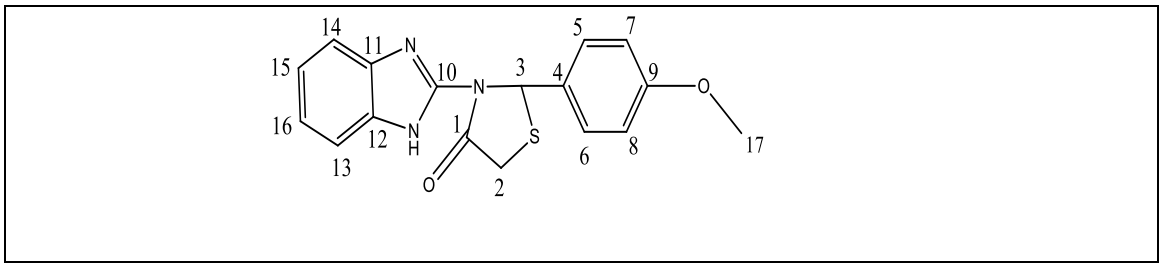
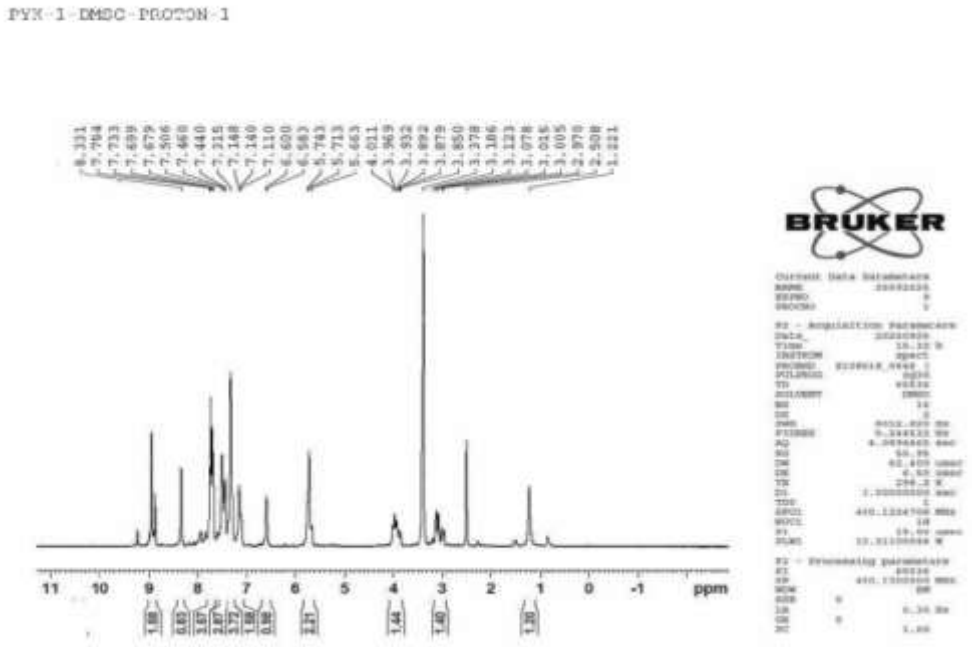


Fig.2.[13]CNMRof 3-(1H-benzo[d]imidazol-2-yl)-2-(4-methoxyphenyl) thiazolidin-4-one (PYK-1).



δ: 171.22(C-1), 31.07(C-2),66.81(C-3),131.29(C-4, C-5, C-6),111.92(C-7, C-8, C-13, C-14),160.05(C-9),153.41(C-10),138.62(C-11, C-12),122.59(C-15, C-16),41.37(C-17) ppm.

[1]H NMR



δ: 3.967,3.892(H-1),6.583(H-2),7.754(H-3, H-4),6.600(H-5, H-6),5.663(H-7),7.110(H-8, H-9),7.315(H-10, H-11) & 3.892(H-12) ppm.

Fig.3.[1] H NMR of 3-(1H-benzo[d]imidazol-2-yl)-2-(4-methoxyphenyl) thiazolidin-4-one (PYK-1).

MS:(m/z):325[M^+]

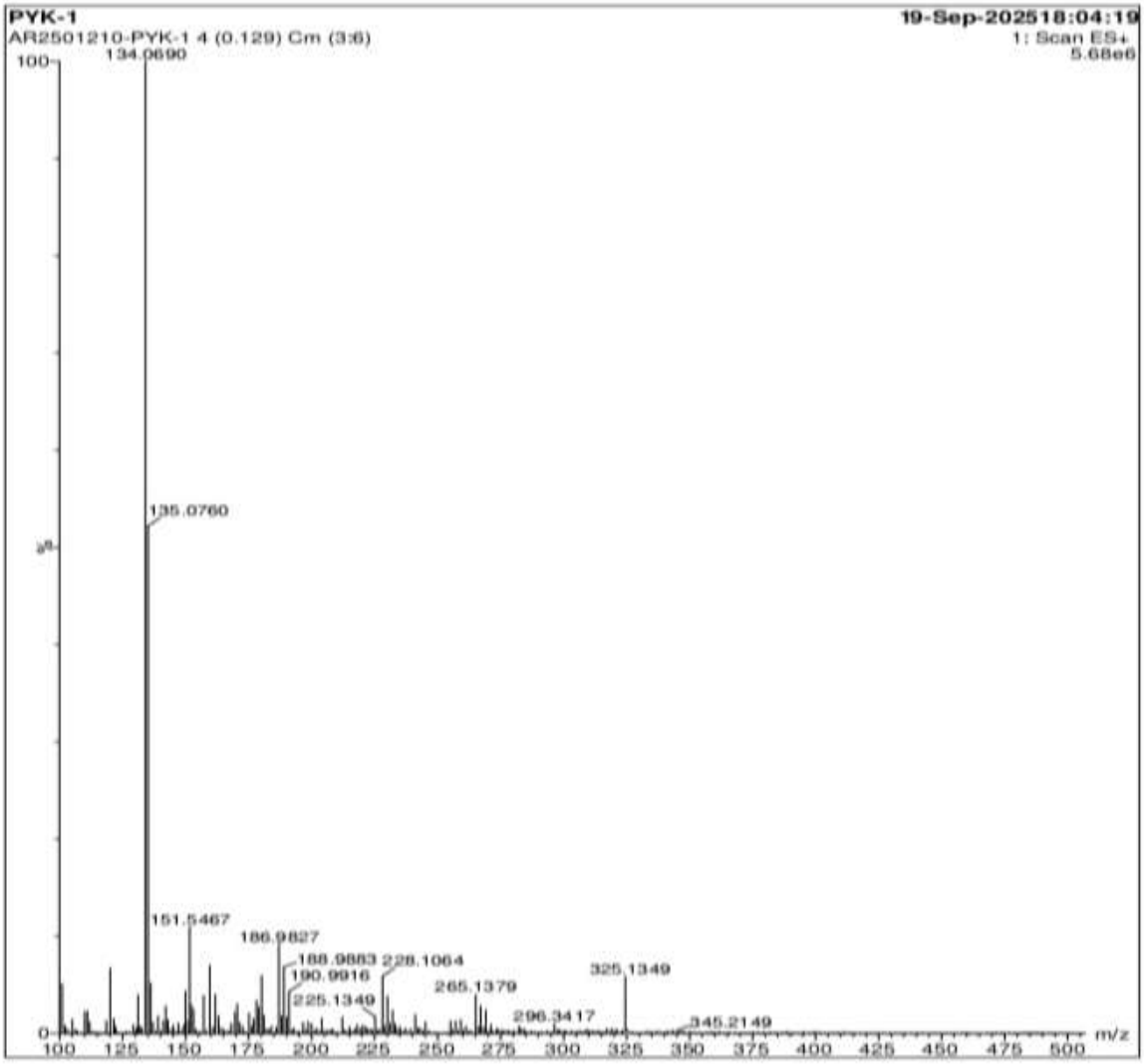


Fig.4.MS of 3-(1H-benzo[d]imidazol-2-yl)-2-(4-methoxyphenyl) thiazolidin-4-one (PYK-1).

Physical properties and spectral characterization of N-(3,4-dimethoxybenzylidene)-1H-benzo[d]imidazol-2-amine (PYK-2).

Physical Properties: Mol formula &weight: $C_{18}H_{17}N_3OS$ 355.10g/mol, color: yellow, Melting point:229C, Rf value: 0.82, Solubility: DMSO, percentage yield:60%.

IR(KBr)cm^{-1}:3371.188 cm^{-1}(N-H),1697.864cm (C=N) cm^{-1}(C=O),1275.086 cm^{-1}(C-O),2783.006 cm^{-1}(O-CH3),1226.270 cm^{-1}(C-N),685.377(C-S-C).

Physical properties and spectral characterization of 3-(1H-benzo[d]imidazol-2-yl)-2-(3,4,5-trimethoxyphenyl) thiazolidin-4-one (PYK-3)

Physical properties: Mol formula & weight:$C_{19}H_{19}N_3O_4S$ 385.41g/mol, color: light beige, Melting point:227 ^{0}C, Rf value:0.82 Solubility: DMSO, Percentage yield:70%

IR(KBr)cm^{-1}:3370(N–H),3119Ar(C–H),2908(C–H),1697(C=N),1667.070(C=O),1199(C–N), 1041 (C–O), 970 (C-H).

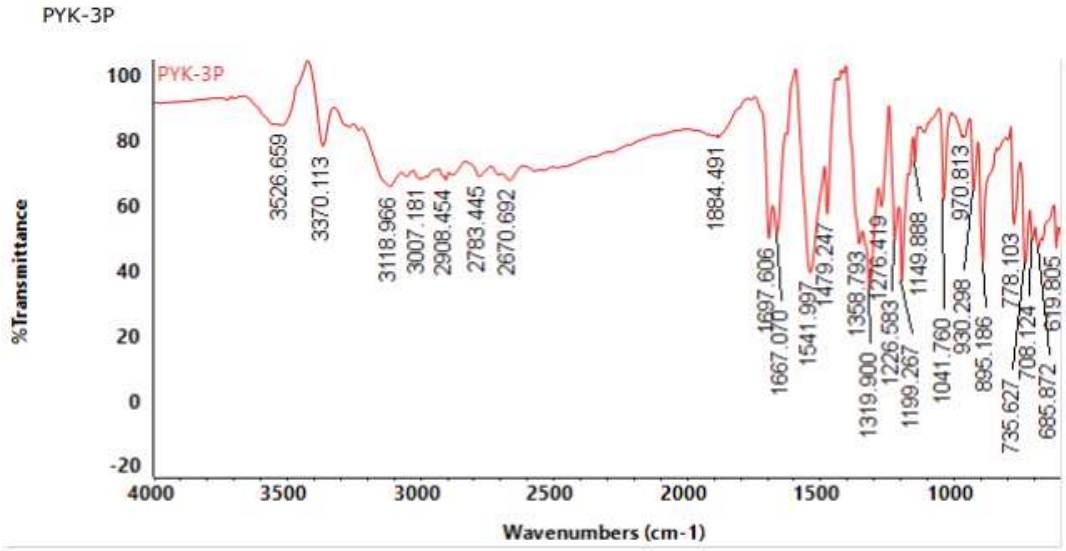


Fig.5. IR 3-(1H-benzo[d]imidazol-2-yl)-2-(3,4,5-trimethoxyphenyl) thiazolidin-4-one (PYK-3).

^{13}C NMR

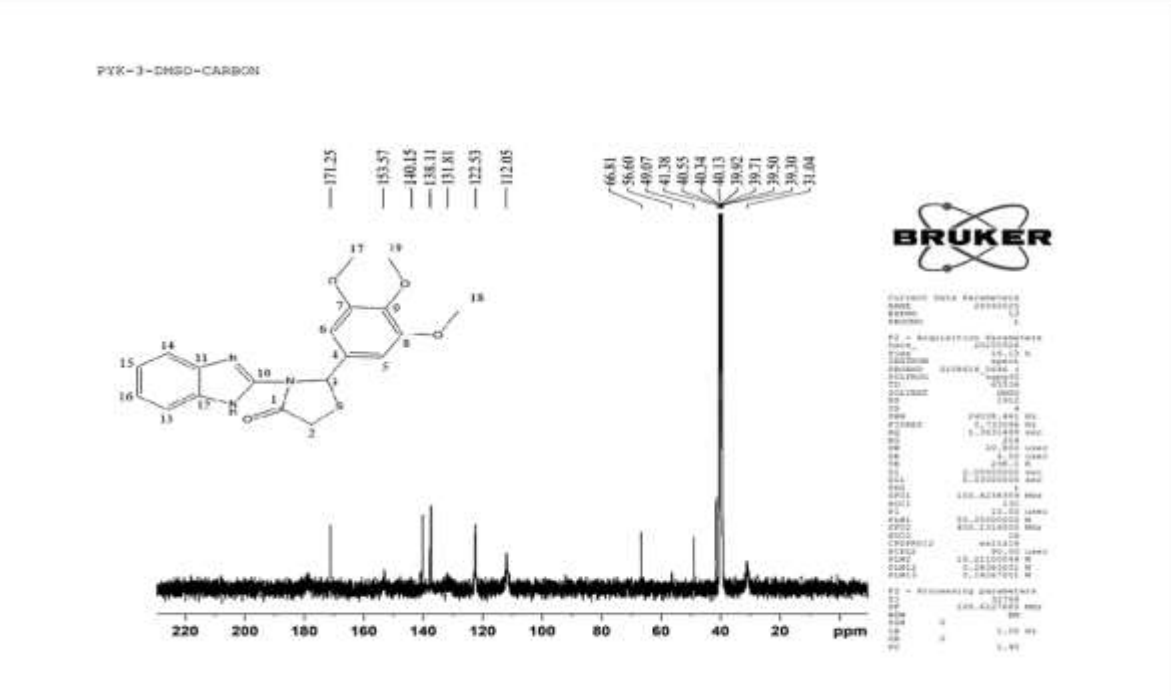


Fig.6. ^{13}C NMR of 3-(1H-benzo[d]imidazol-2-yl)-2-(3,4,5-trimethoxyphenyl) thiazolidin-4-one (PYK-3).

δ:171.25(C-1),39.30(C-2),66.81(C-3),131.81(C-4),,111.10(C-5,C-6),153.57(C-7,C-8),138.81(C-9),140.15(C-10),138.11(C-11,C-12),112.05(C-14,C-15),122.53(C-15,C-16),56.60(C-17,C-18),66.81(C-19).

[1]HNMR

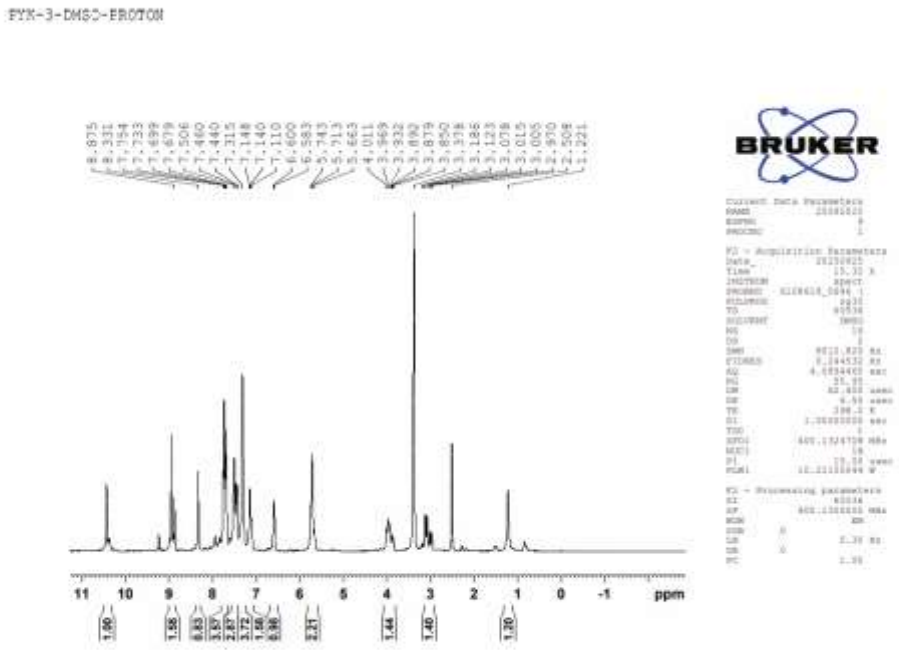
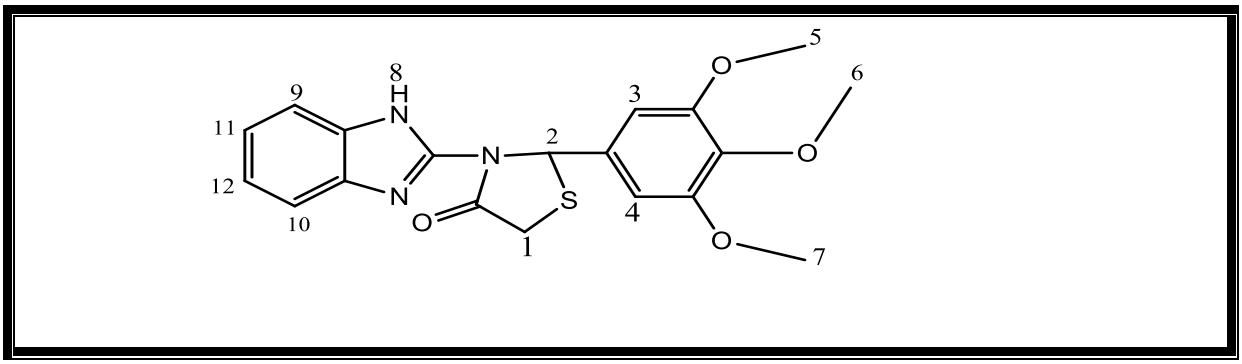


Fig.7. [1]HNMR of 3-(1H-benzo[d]imidazol-2-yl)-2-(3,4,5-trimethoxyphenyl) thiazolidin-4-one (PYK-3).



[1]HNMR:δ:3.969,3.892(H1),6.583(H2),7.110(H3,H4),3.850(H5,H6&H7),5.663(H8), 7.14(H9,H10),7.315(H11,H12).

MS:(m/z):386[M^+]

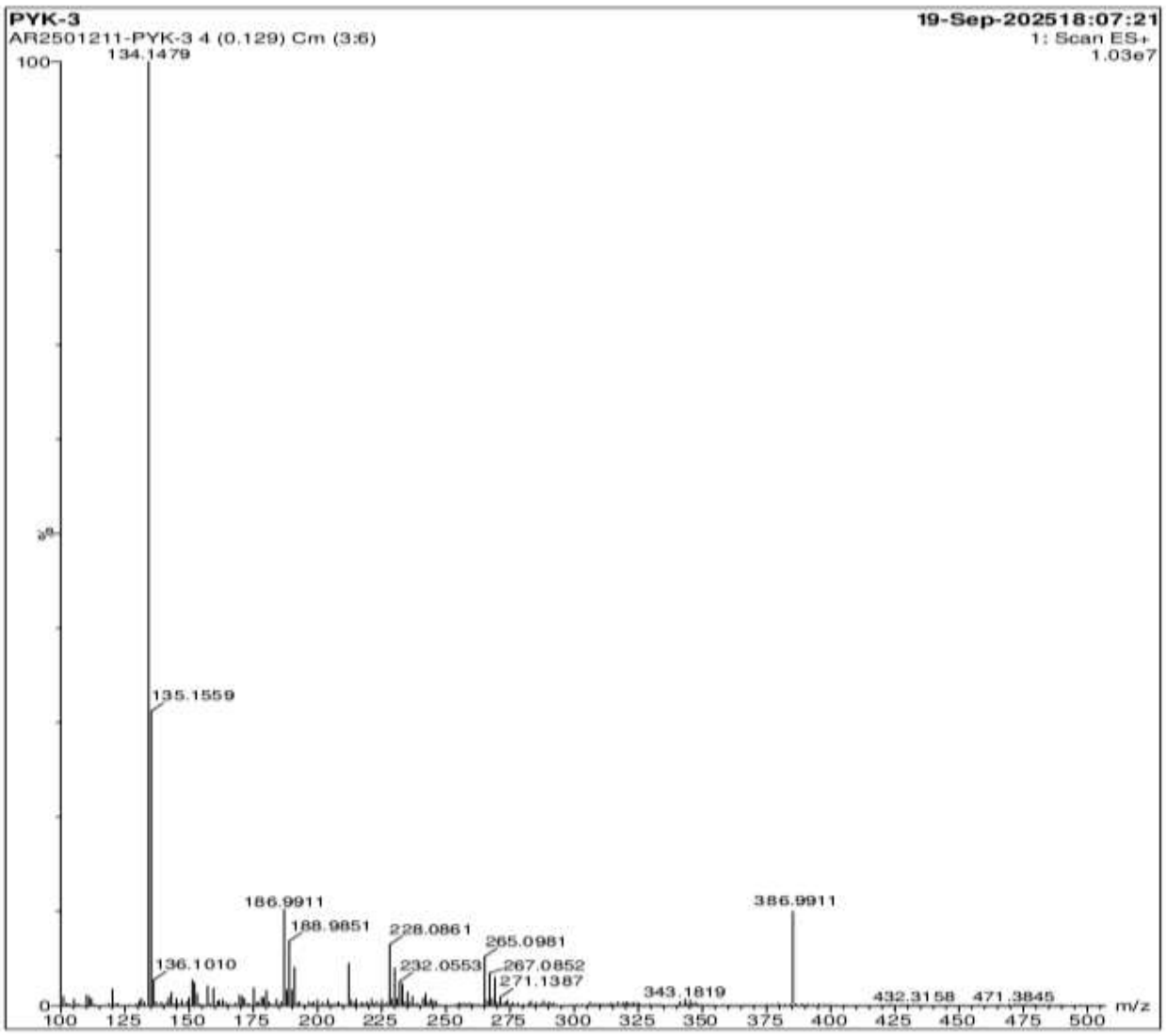


Fig.8 MS of 3-(1H-benzo[d]imidazol-2-yl)-2-(3,4,5-trimethoxyphenyl) thiazolidin-4-one (PYK-3).

Physical properties and spectral characterization of 3-(1H-benzo[d]imidazol-2-yl)-2-(4-fluorophenyl) thiazolidin-4-one (PYK-4)

PhysicalProperties: Molformula&weight:C16H12FN3OS&313.35g/mol,color:beige, Melting point:180 C, Solubility: DMSO, percentage yield:70%.

IR (KBr)cm3379.418cm^{-1} (N-H),1650cm^{-1} (C=O),1044.176cm^{-1}, 621 cm^{-1}(C-S), 1376.978(C-H Methyl)

Physical properties and spectral characterization of 3-(1H-benzo[d]imidazol-2-yl)-2-(4-(dimethyl amino) phenyl) thiazolidin-4-one (PYK-5).

Physical Properties: Mol.formula&weight:C18H18N5OS& 338.12g/mol, color: Pink, Melting point:190 C, Rf value:0.82, solubility DMSO, percentage yield 65%

IR (KBr)cm: 3369.375cm^{-1}(N-H), 3118.978 cm^{-1}Ar(C-H), 1697.907 (C=O), 1357.837 cm^{-1}(C-N), 1149.630 (C-H).

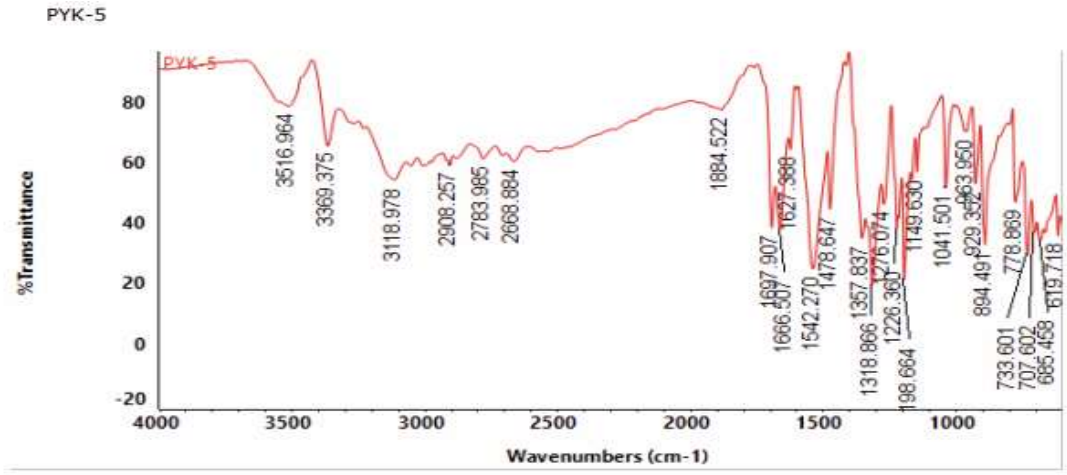


Fig.9.IR 3-(1H-benzo[d]imidazol-2-yl)-2-(4-(dimethyl amino) phenyl) thiazolidin-4-one. (PYK-5)

^{13}CNMR

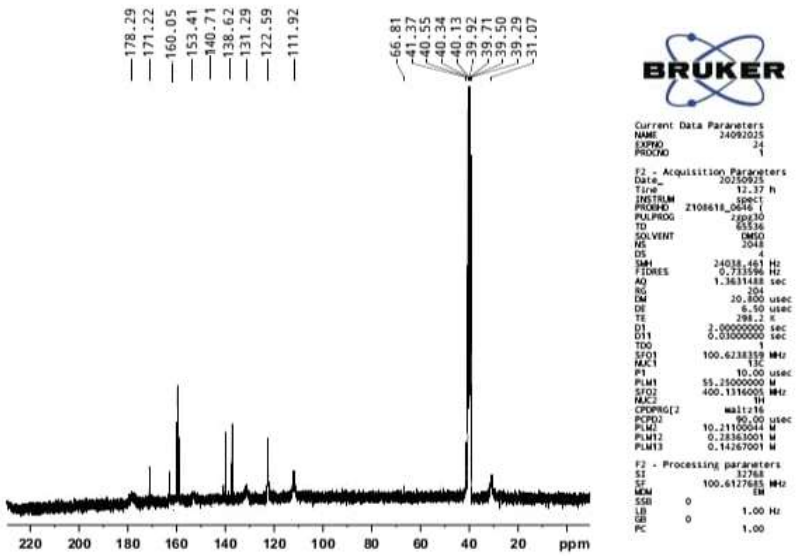
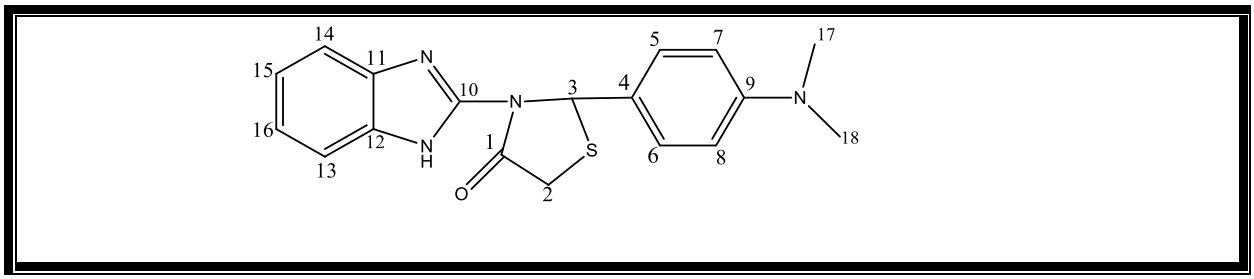


Fig.10. 3-(1H-benzo[d]imidazol-2-yl)-2-(4-(dimethyl amino) phenyl) thiazolidin-4-one. (PYK-5)



[13]CNMRδ:171.22(C-1),31.07(C-2),66.81(C-3),131.29(C-4,C-5.C-6),111.92(C-7,C-8,C-13,C-14),153.41(C-9,C-10),138.62(C-11,C-12),122.59(C-15,C-16),41.37(C-17,C-18).

[1]HNMR

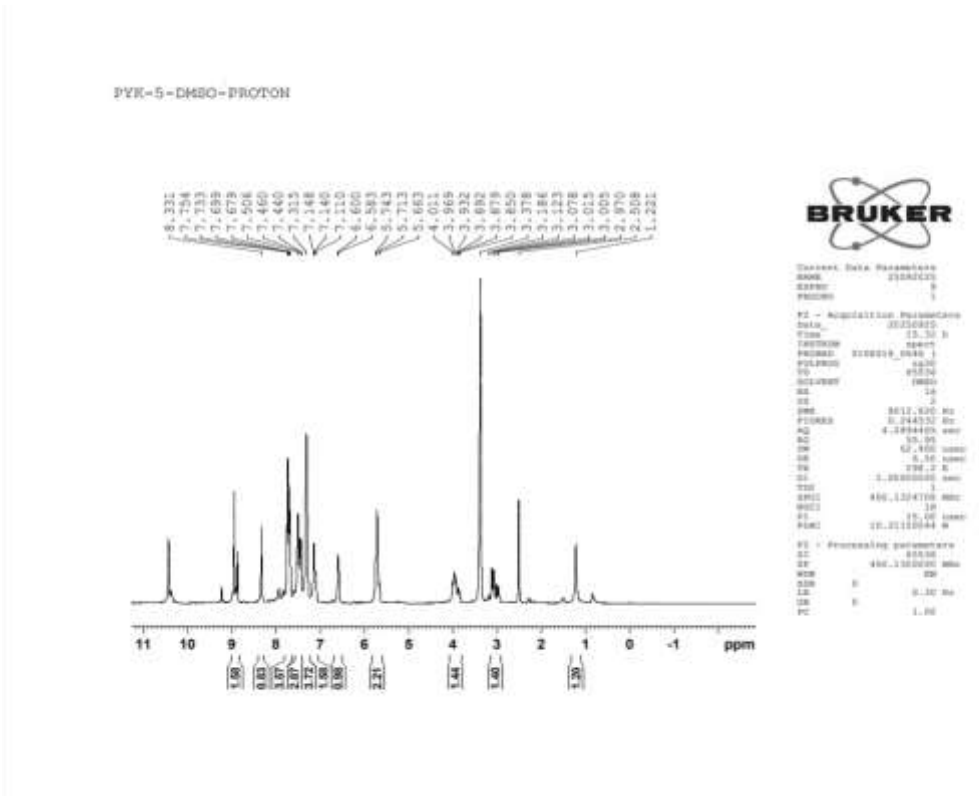
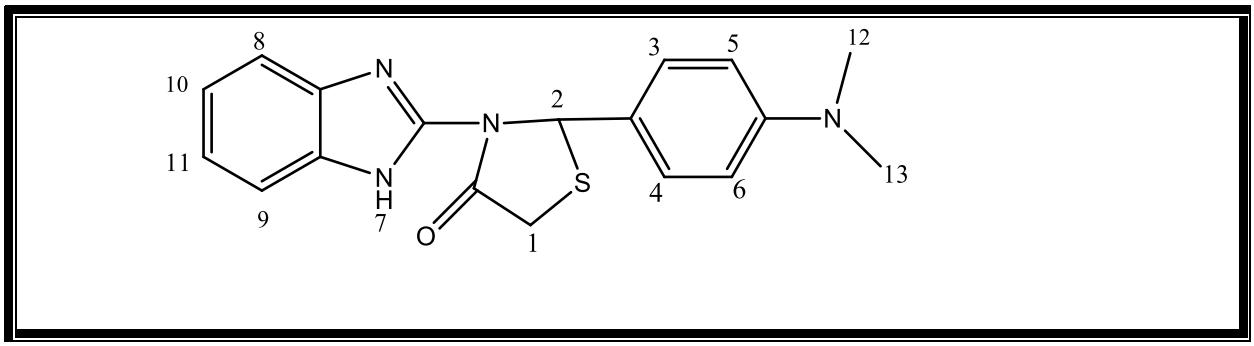
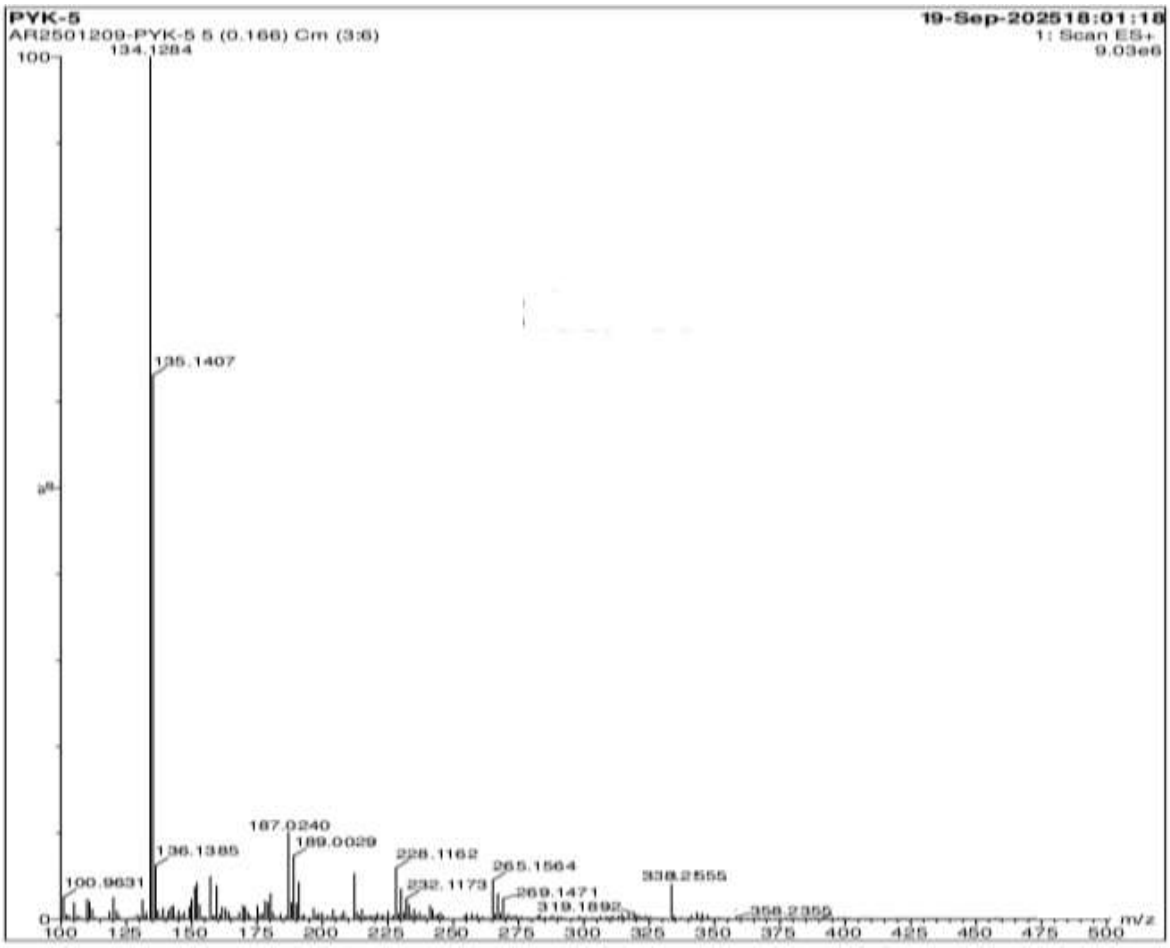


Fig.11. [1]HNMR 3-(1H-benzo[d]imidazol-2-yl)-2-(4-(dimethyl amino) phenyl) thiazolidin-4-one.



[1]HNMR: δ:3.969,3.892(H1),6.583(H2),7.110(C-3, C-4),6.600(C-5, C-6),5.663(C-7),7.140(C-8, C-9),7.315(C-10, C-11),3.123(C-12, C-13)



MS:(m/z):338[M$^-$]

Physical properties and spectral characterization of 3-(1H-benzo[d]imidazol-2-yl)-2-(p-tolyl) thiazolidin-4-one. (PYK-6)

Physical Properties: Mol.formula&weight:$C_{17}H_{15}N_3OS$ 309.09g/mol, color: light beige, Melting point:195 C, Rf value:0.82, Solubility: DMSO, percentage yield:70%.

IR (KBr)cm^{-1}: 3370(N-H),3007 Ar(C-H),1697 (C=N),900-700(C-H), (Ali C-H),1667(C=O).

Physical and spectral characterization of 3-(1H-benzo[d]imidazol-2-yl)-2-(3-nitrophenyl) thiazolidin-4-one. (PYK-7)

Physical properties: Mol formula &weight:$C_{16}H_{12}N_4O_3$ &340.06g/mol, color: Brown, Melting point:167 ^{0}C, Rf value:0.82, Solubility: Methanol, DMSO, percentage yield:70%.

IR (KBr)cm^{-1}: 3370 (N-H),1699(C=O),1614(C=N),1699.348 Ar(C-H) ,675(C-S-C),1531.450(nitro).

Physical properties and spectral characterization of 3-(1H-benzo[d]imidazol-2-yl)-2-(4-chlorophenyl) thiazolidin-4-one. (PYK-8)

Physical properties: Mol.formula&weight:$C_{16}H_{12}ClN_3OS$&329.78g/mol, color: off white, Melting point:210 C, Rf value:0.82, solubility: DMSO, percentage yield:70%

IR (KBr)cm^{-1}: 3235.354(N-H),1677(C=O),1467 Ar(C-H),1373 (C-Cl).

Physical and spectral characterization of 3-(1H-benzo[d]imidazol-2-yl)-2-(4-ethylphenyl) thiazolidin-4-one. (PYK-9)

Physical properties: Mol.formula&weight:$C_{21}H_{15}N_3O_4$&373g/mol, color: off white, Melting point :180 C, Rf value:0.82, solubility: Methanol, DMSO, percentage yield.

IR (KBr)cm^{-1}: 3119.085 (N-H), 2784.278 Ar(C-H),1673.003(C=O).

Physical properties and spectral characterization of 3-(1H-benzo[d]imidazol-2-yl)-2-(4-hydroxyphenyl) thiazolidin-4-one. (PYK-10)

Physical properties: Mol.formula&weight:C16H13N3O2S 311.35g/mol, color: light beige, melting point :190 C, Rf value:0.82, Solubility: DMSO, percentage yield:70%.

IR(KBr)cm^{-1}:3591.785(O-H),3318(N-H) ,1907(C=O),1683(C=N), 975 Ar(C-H).

1. In-silico Physiochemical study of the Novel drugs

A. In-silico ADME STUDY Swiss- ADME and Molilsinsperision

In-silico methods of determination of the physicochemical descriptors and properties is a key role in drug development and target identification. The web tools, such as SwissADME, give the different properties of a drug based on pharmacokinetic properties, druglike nature, and medicinal chemistry friendliness of one or multiple small molecules.

Table 35: *In-silico* ADME properties of the compounds (PYK-1 to PYK-10) from Swiss ADME

Compound code	**PYK-1**	**PYK-2**	**PYK-3**	**PYK-4**	**PYK-5**	**PYK-6**	**PYK-7**	**PYK-8**	**PYK-9**	**PYK-10**
Num. heavy atoms	23	35	27	22	22	22	24	22	22	23
Num. Arom. Heavy atoms	15	24	15	15	15	15	15	15	15	15
Num. Rotatable bonds	3	07	5	2	2	2	3	2	2	3
Num. H-bond acceptors	3	6	5	3	2	2	4	2	3	2
2Num. of H-bond donors	1	1	1	1	1	1	1	1	2	1
Molar refractivity	94.70	140.52	107.68	88.16	102.41	93.17	97.03	93.22	90.23	97.98
Total Polar Surface Area(Å)	83.52	82.84	101.98	74.29	77.53	74.29	120.11	74.29	94.52	74.29
Log Po/w (ilogp)	2.69	3.02	2.66	3.02	2.85	3.06	1.93	3.24	2.29	3.34
Water solubility	Moderately soluble	Moderately solubl e	Moderately solubi	Moderately solubility	Moderately solubl e	Moderately solubl e	Moderately solubl e	Moderately solubl e	Moderately solubl e	Moderately solubl e
GI absorption	High	High	High	High	High	High	High	High	High	High
BBB permeant	No	No	No	yes	yes	Yes	No	yes	No	yes
Drug likeness (violation)	Yes; (0)	Yes; (0)	Yes; (0)	Yes: (1)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)
Lead likeness (violation)	Yes	No; 1 violation:MW>350	No; (1), MW> 350	Yes	Yes	No; 1 violation: XLO	Yes	No; 1 violation:X	Yes	No; 1 violation

						GP3> 3.5		LOG P>3.5		XLO GP3> 3.5

Molinspiration is also a web tool that provides the calculation of important molecular properties (logP, polar surface area, number of hydrogen bond donors and acceptors and others), as well as prediction of bioactivity score for the most important drug targets**.**

Table 33: *In-Silico* Physicochemical studies obtained from Molinspiration

Compound Code	***miLogP***	***TPSA***	***natoms***	***MW (g/mol)***	***nON***	***nOHNH***	***n violation***	***n rotb***	***Volume***
PYK-1	3.19	58.23	23	325.39	5	1	0	3	278.50
PYK-2	2.78	67.46	25	355.42	6	1	0	4	304.05
PYK-3	2.76	76.69	27	385.44	7	1	0	5	329.59
PYK-4	3.29	48.99	22	313.36	4	1	0	2	257.89
PYK-5	3.23	52.23	24	338.44	5	1	0	3	298.86
PYK-6	3.58	48.99	22	309.39	4	1	0	2	269.52
PYK-7	3.06	94.82	24	340.36	7	1	0	3	276.29
PYK-8	3.81	48.99	22	329.81	4	1	0	2	266.49
PYK-9	2.65	69.22	22	311.37	5	2	0	2	260.97
PYK-10	4.04	48.99	23	323.42	4	1	0	3	286.32

B. In-silico toxicity studied (Protox)

The ProTOX-II web server is a prediction scheme that is classified into different levels of toxicity, such as oral toxicity, organ toxicity (hepatotoxicity), toxicological endpoints (such as mutagenicity, carcinogenicity, cytotoxicity, and immunotoxicity), toxicological pathways (AOPs), and toxicity targets, thereby providing insights into the possible molecular mechanism behind such toxic responses.

Procedure:

The structures of synthesized compounds were drawn using Chemsketch and the smiles notation was generated. The generated smiles were pasted in the respective Protox-II web-tools and were runner. The results of toxicity over different organs were tabulated. All the synthesized compounds exhibited level 4 toxicity. All compounds are exhibited level 4 toxicity, with the highest predicted LD50 of 1000mg/kg

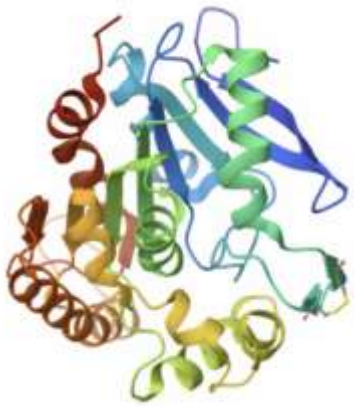
Table 32: *In-silico* Toxicity studies of synthesized compounds.

Compound code	***Predicted LD50 (mg/kg)***	***Predicted Toxicity Class***	***Hepatotoxicity***	***Carcinogenicity***	***Immunotoxicity***	***Mutagenicity***	***Cytotoxicity***
PYK-1	1500	4	Active	In active	In active	In active	In active
PYK-2	1500	4	Active	In active	In active	In active	In active
PYK-3	1500	4	Active	In active	In active	In active	Active
PYK-4	783	4	Active	Inactive	In active	Inactive	Inactive
PYK-5	1127	4	Inactive	In active	In active	In active	In active
PYK-6	1127	4	Active	In active	In active	In active	In active
PYK-7	1127	4	Active	Active	In active	Active	In active
PYK-8	500	4	Active	In active	In active	In active	In active
PYK-9	1500	4	Active	In active	In active	In active	In active
PYK-10	1098	4	Active	In active	In active	In active	In active

C. Molecular Docking

Molecular docking is a bioinformatic modelling method that studies the interactions between two or more molecules to form stable adducts. It predicts the 3D structure of complexes based on ligand and target binding interactions. Molecular Docking generates various possible adduct structures, which are ranked using a scoring function. Docking simulations aim to identify the optimized docked conformer based on the total system energy. However, challenges such as ligand chemistry, receptor flexibility, and effective scoring functions persist.

In silico docking of synthesized for their anti-Tubercular activity.

PDB ID:1SFR (Anti-Tuberculosis Mycobacterium Tuberculosis Antigen 85a Protein).	

Procedure:

- The protein was downloaded from the RCSB website in PDB format and pre-processed using Discovery Studio.
- Next, water molecules and unwanted entities were removed, missing polar hydrogen atoms were added, and energy minimization was performed to optimize the structure.
- The 2D structures of the ligand were created using Chem Draw and saved as mdl.mol files.
- Next, Chem 3D's energy minimization technique was applied to optimize the structures for the lowest possible energy Prepared proteins and ligands were imported into PyRx, assigned as macromolecules and ligands, and saved in. pdbqt format.
- The docking was conducted for each ligand, recording binding affinity and RMSD values. The complex was saved in. pdbqt to analyse interactions.
- The 2D interactions of the ligands with the amino acids of target proteins were visualized using Bio-via Discovery Studio.

***In-Silico* Screening for Anti-tubular Activity:**

The *in-silico* screening was conducted to evaluate the potential anti-tubercular activity of the synthesized compounds. These compounds were evaluated for their interactions with the PDB ID 6SQB receptor, which revealed promising interactions.

Table 31: Binding energy of synthesized compounds with 1SFR (Anti-tubular activity):

SI NO.	Compound codes	Binding Affinity(kcal/mol) (1SFR)
1	PYK-1	-8.2
2	PYK-2	-7.2
3	PYK-3	-8.5
4	PYK-4	--7.9
5	PYK-5	-8.3
6	PYK-6	-7.7
7	PYK-7	-7.9
8	PYK-8	-8.0
9	PYK-9	-8.0
10	PYK-10	-7.8
11	STREPTOMYCIN	-8.1

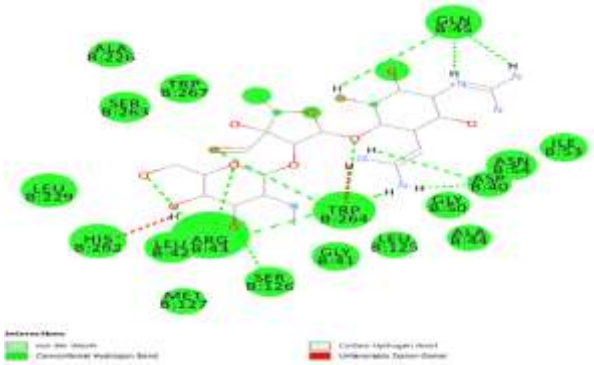


Fig .2d model of affinity of streptomycin with 1SFR Protein.

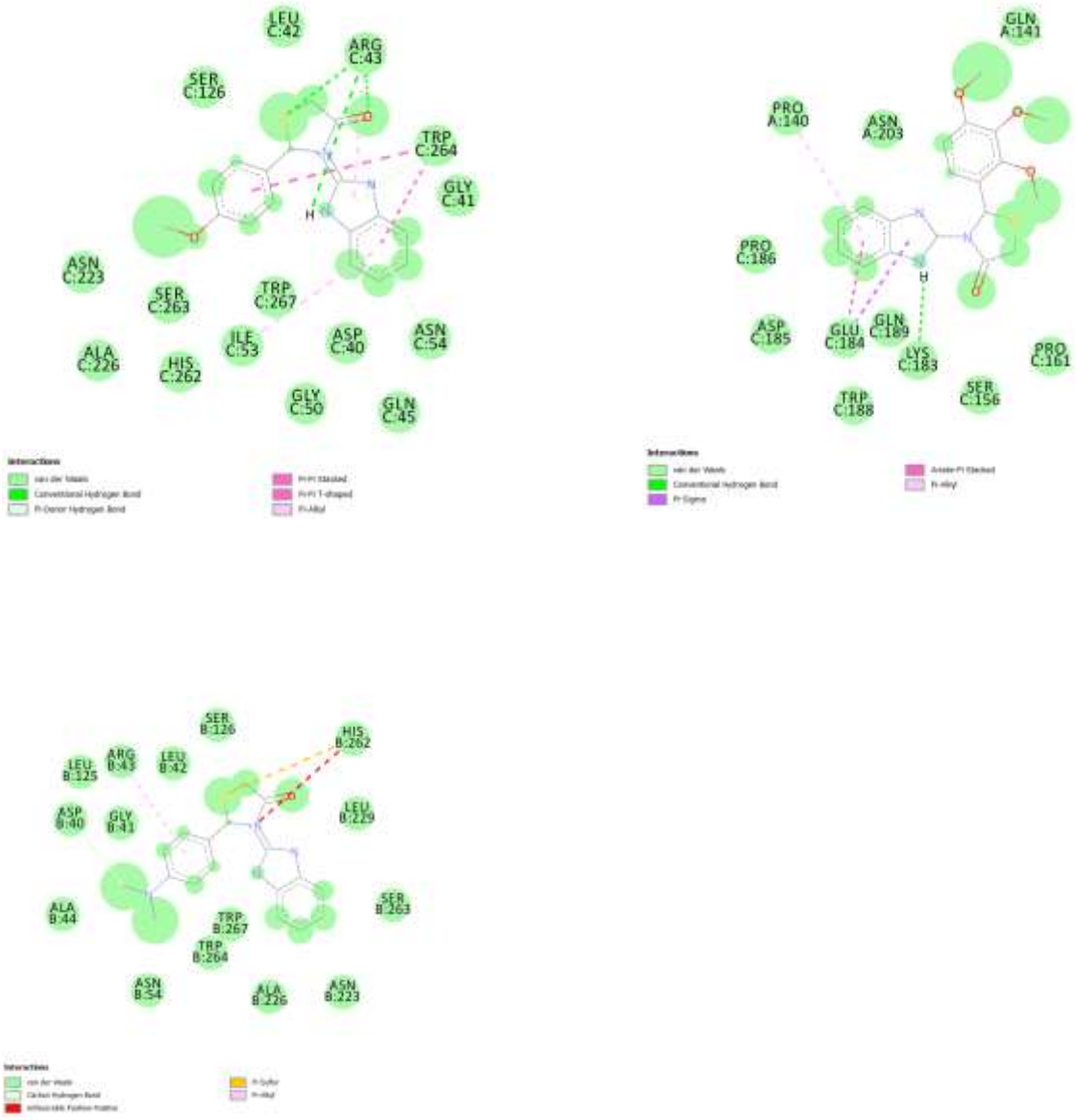


Fig.2d model of affinity of PYK-1, PYK-3 and PYK-5 With 1SFR

D. In-vitro Anti-tubercular activity:

Test and Method Used: Anti-TB test by MABA Assay

Standard Strain used: Mycobacteria tuberculosis (Vaccine strain, H37 RV strain): ATCC No 27294

Procedure:

- The anti-Mycobacterial activity of compounds was performed against M. tuberculosis using the microplate Alamar Blue assay (MABA).
- This methodology is non-toxic, uses a thermally stable reagent, and shows good correlation with the proportional and BACTEC radiometric method.

- Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of the sterile 96-well plate to minimize evaporation of medium in the test wells during incubation.
- The 96-well plate received 100 µl of the Middlebrook 7H9 broth containing Mycobacterium tuberculosis.
- Serial dilutions of compounds were made directly on the plate.
- The final drug concentrations tested were 100 to 0.2 µg/Plates were covered and sealed with parafilm and incubated at 37ºC for five days.
- After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% Tween 80 was added to the plate and incubated for 24 hrs.
- A blue colour in the well was interpreted as no bacterial growth, and a pink colour was scored as growth.
- The MIC was defined as the lowest drug concentration that prevented the colour change from blue to pink.

Anti-tubercular activity

The synthesized novel Schiff base benzimidazole derivatives were evaluated based on their molecular docking scores. Three selected derivatives were tested for their antimicrobial activity against *Mycobacterium tuberculosis* (vaccine strain; ATCC No. 27294). Among them, compounds PYK-1 and PYK-5 demonstrated excellent activity at a concentration of 12.5 µg/ml each, comparable to the standard drug pyrazinamide. In contrast, compound PYK-3 exhibited significant activity at a lower concentration of 6.25 µg/ml, while pyrazinamide showed activity at 3.125 µg/ml.

Standard values for the Anti-Tb test which was performed.

Isoniazid – 1.6µg/ml

Ethambutol – 1.6µg/ml

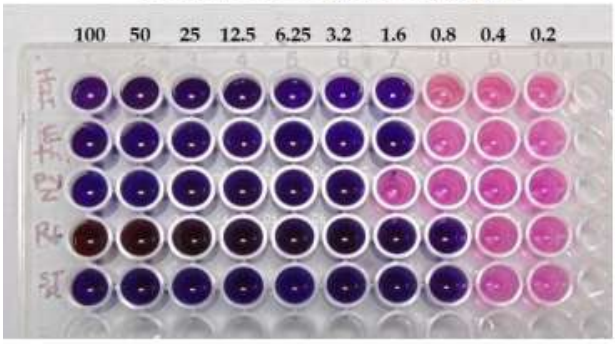
Pyrazinamide- 3.125µg/ml

Rifampicin – 0.8µg/ml

Streptomycin- 0.8µg/ml

Results:

Standard Drug Photograph



Photograph:

SL/No	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	PYK 01	S	S	S	S	R	R	R	R
2	PYK 03	S	S	S	S	S	R	R	R
3	PYK 05	S	S	S	S	R	R	R	R

Note:

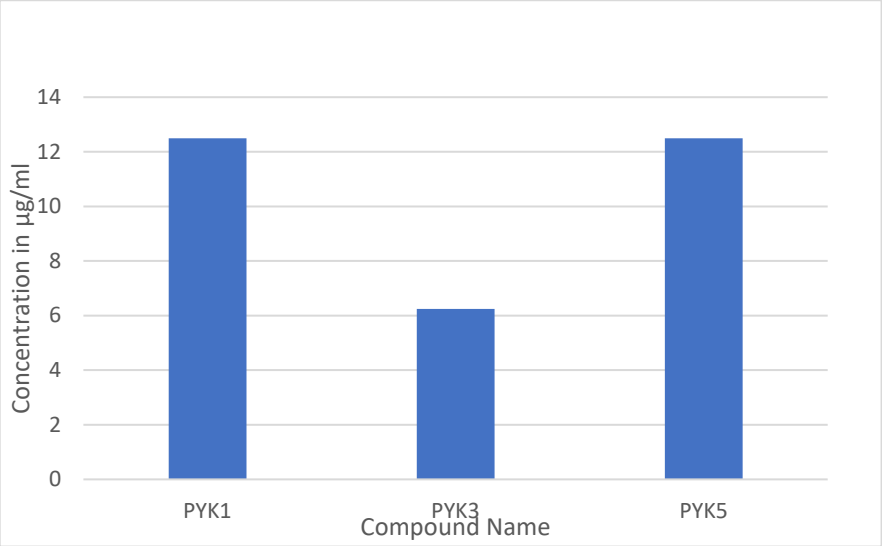
S – Sensitive

R- Resistant

Table 45: MIC Table

Sl. No	Sample	Mic Value

1	**PYK 01**	**12.5 µg/ml**
2	**PYK 03**	**6.25 µg/ml**
3	**PYK 05**	**12.5 µg/ml**



CONCLUSION:

This research study focused on the synthesis and analysis of the pharmacological effects of novel Schiff base Benzimidazole derivative. The compounds were prepared and characterized using FTIR, ^{1}H NMR, ^{13}C NMR, and LC-MS spectroscopy.

The reaction followed three steps. In first step, cyanogen bromide, Ortho phenyl diamine and water allowed for cyclization reaction for 36 hours kept overnight and recrystallised with Sodium hydroxide in water to yield 2-amine benzimidazole, which was confirmed by IR spectroscopy through the observation of various peaks.

The second step involves the formation Schiff base where 2-amine benzimidazole reacts with substituted aromatic aldehyde using ethanol as solvent in presence of glacial acetic acid as catalyst to give Schiff base derivatives. Which was confirmed by IR Spectroscopy through the observation of various peaks. The second step involved the formation of N-(substituted aldehyde)-1H-benzo[d]imidazol-2-amine

Finally, in third step, the intermediate compound undergoes cyclization reaction where it undergoes reaction with thioglycolic acid and 1,4 dioxane in presence of zinc chloride as catalyst to obtain the corresponding benzimidazole derivatives. The reaction was carried out on magnetic stirrer with continuous stirring and refluxing it for 8-12 hours later the reaction was cooled and recrystallized from absolute ethanol to obtain 3-(1H-benzo[d]imidazole-2-yl)-2-(substituted aldehyde) thiazolidine-4-one. The formation of derivative was evaluated through various spectral studies.

The compound 3-(1H-benzo[d]imidazol-2-yl)-2-(4-methoxyphenyl) thiazolidin-4-one (PYK-1), was synthesized through a cyclization reaction involving the Schiff base made from N-(methoxy benzaldehyde)-1H-benzo[d]imidazol-2-amine, using thioglycolic acid and zinc chloride as a catalyst. The identity of this molecule was substantiated by the appearance of the molecular ion (M-) peak 325 in the mass spectrum. Further structural verification was provided by 1H NMR spectroscopic data, which showed characteristic signals for aromatic protons between δ 7.75 and 7.06 ppm, a methoxy group resonating from δ 3.92 to 3.18 ppm, and protons from the thiazolidine ring appearing at δ 5.71–5.60 ppm. This pattern confirms successful cyclization and complete transformation to the intended Schiff base benzimidazole derivative.

The compound 3-(1H-benzo[d]imidazol-2-yl)-2-(3,4,5-trimethoxyphenyl) thiazolidin-4-one (PYK-3) was synthesized via cyclization of the corresponding Schiff base using thioglycolic

acid and zinc chloride as a catalyst. This synthetic method facilitates the reaction at the amide (C=H) site. Confirmation of the molecular structure was achieved through mass spectrometry, which displayed the molecular ion peak (M-) 385. Further structural elucidation was provided by 1H NMR spectroscopy, which revealed characteristic aromatic proton signals at δ 8.75 and 7.94 ppm, thiazolidine ring CH resonances between δ 5.713 and 5.601 ppm, aliphatic proton signals in the region of δ 2.975 to 2.508 ppm, and methoxy group singlets observed from δ 3.970 to 3.186 ppm. These spectroscopic features strongly support the formation of the thiazolidine ring in the benzimidazole scaffold.

The synthesis of 3-(1H-benzo[d]imidazol-2-yl)-2-(4-(dimethylamine) phenyl) thiazolidin-4-one (PYK-5) was achieved via cyclization of the relevant Schiff base, using thioglycolic acid and zinc chloride as catalysts to facilitate the transformation at the amide (C=H) position. The identity and molecular weight of PYK-5 were confirmed by the presence of a molecular ion peak in the mass spectrum M- 338. Structural insights from 1H NMR analysis revealed distinct aromatic proton signals at δ 7.93 and 7.02 ppm, a thiazolidine ring proton appearing between δ 5.71 and 5.60 ppm, a characteristic singlet for the dimethyl amino group resonating at δ 3.01–2.90 ppm, and additional methylene or aliphatic proton signals detected from δ 2.60 to 1.20 ppm. These spectroscopic observations collectively verify the successful formation of the thiazolidine ring within a benzimidazole framework.

Anti- tubular Activity:

In silico anti tubular activity:

The in-silico evaluation of the synthesized compounds was performed using the target protein with PDB ID: 1SFR. Among the tested molecules, PYK-3, PYK-1, and PYK-5 showed the highest binding affinities, with docking scores of −8.5, −8.3, and −8.2 kcal/mol, respectively. PYK-3 and PYK-1 formed significant conventional hydrogen bonds with key residues ARG C:43 and LYS C:183, respectively. In contrast, the standard drug streptomycin and the co-crystallized ligand exhibited strong hydrogen bonding interactions involving GLN B:45, ASP B:40, TRP B:264, and SER B:126. These interactions underscore the critical amino acid residues contributing to ligand binding in the active site.

In-vitro anti-tubercular activity:

The synthesized novel Schiff base benzimidazole derivatives were evaluated based on their molecular docking scores. Three selected derivatives were tested for their antimicrobial activity against *Mycobacterium tuberculosis* (vaccine strain; ATCC No. 27294). Among them, compounds PYK-1 and PYK-5 demonstrated excellent activity at a concentration of 12.5 µg/ml each, comparable to the standard drug pyrazinamide. In contrast, compound PYK-3 exhibited significant activity at a lower concentration of 6.25 µg/ml. The variation in activity among these derivatives can be attributed to the different substituted phenyl groups on the benzimidazole-thiazolidines scaffold, which likely influence their interaction with the bacterial targets and membrane permeability. The trimethoxy substitution in PYK-3 possibly enhances its lipophilicity and binding efficiency, resulting in better antimicrobial activity at a lower concentration compared to PYK-1 and PYK-5.

The synthesized benzimidazole derivatives demonstrated considerable effectiveness, warranting additional optimization and detailed pharmacological studies. The presence of the methoxy groups on the phenyl ring significantly enhances lipophilicity and facilitates better membrane permeability and binding affinity to biological targets. Methoxy substituents are electron-donating groups that can engage in hydrophobic interactions and potentially hydrogen bonding through oxygen atoms. This enhances the compound's ability to interact with microbial enzymes or receptors, resulting in improved antimicrobial activity.

In conclusion, this research successfully integrated synthetic chemistry with comprehensive biological evaluation to identify novel benzimidazole-based Schiff base derivatives with multifunctional pharmacological properties. These findings provide a valuable foundation for the future design and development of benzimidazole derivatives as therapeutic agents targeting diverse disease pathways

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