

Mapping Therapeutic Networks and Molecular Interactions: Isatin Mannich Bases as Antitubercular leads with Integrated *In vitro* Screening

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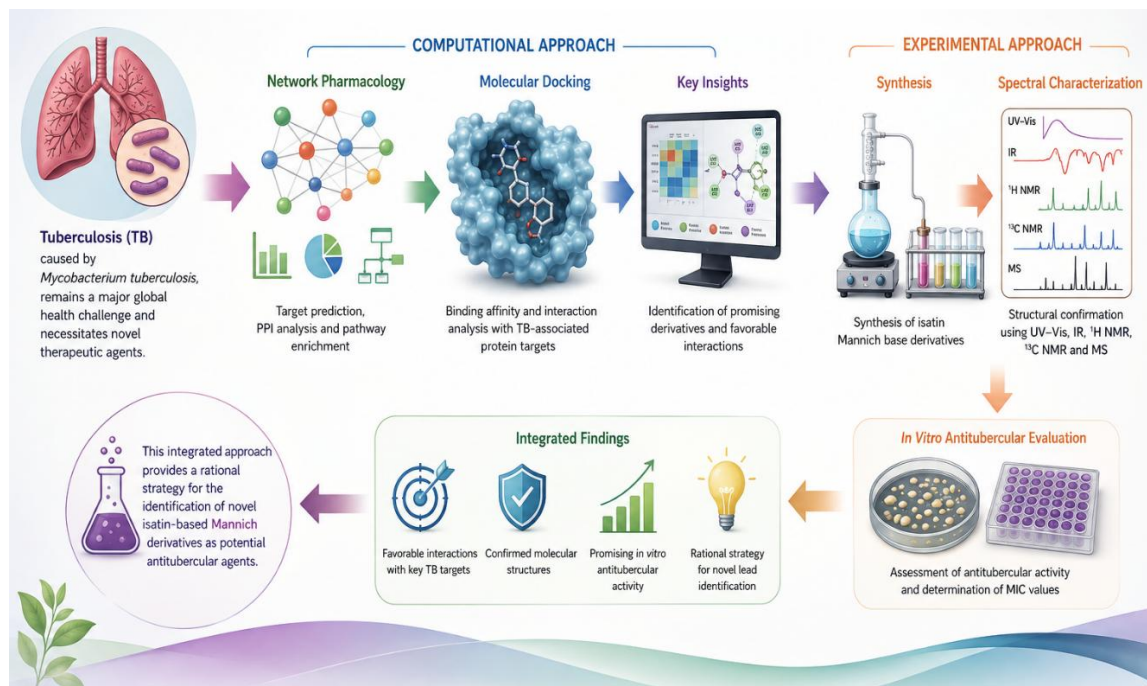
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Abstract

Tuberculosis (TB), caused primarily by *Mycobacterium tuberculosis*, remains a major global health concern and continues to necessitate the discovery of novel therapeutic agents with improved efficacy. In the present study, isatin was selected as a promising heterocyclic scaffold for the design and development of novel Mannich base derivatives targeting tuberculosis. An integrated computational and experimental approach was employed to investigate the therapeutic potential of the designed compounds. Network pharmacology analysis was initially performed to identify potential molecular targets, protein–protein interactions, and disease-associated biological pathways of the isatin derivatives. The predicted targets and enriched pathways provided insights into the possible molecular mechanisms underlying their antitubercular activity. Subsequently, molecular docking studies were carried out against selected tuberculosis-associated protein targets to evaluate the binding affinity and interaction patterns of the designed derivatives within the active sites. Based on the computational findings, the selected isatin mannich bases were synthesized and structurally characterized using UV-Visible, IR, ¹H NMR, ¹³C NMR, and mass spectroscopic techniques. The synthesized compounds were further subjected to *in vitro* antitubercular evaluation to determine their biological activity. The computational investigations indicated favourable interactions of the designed derivatives with the selected therapeutic targets, while spectral characterization confirmed the proposed molecular structures. The *in vitro* findings further demonstrated the biological potential of the synthesized compounds. Overall, the integration of network pharmacology, molecular docking, synthetic chemistry, spectral characterization, and *in vitro* evaluation provides a rational strategy for the identification of novel isatin-based mannich derivatives as potential antitubercular agents.

Keywords: Isatin, mannich bases, Tuberculosis, Network pharmacology, Spectral characterization.

Graphical abstract



I. Introduction: ^[1-4]

Tuberculosis (TB), primarily caused by *Mycobacterium tuberculosis*, remains a major global infectious disease and public health challenge. Despite effective chemotherapy, prolonged multidrug treatment, limited healthcare access, adverse effects such as anti-tuberculosis drug-induced hepatotoxicity, and poor treatment adherence contribute to treatment failure, relapse, and the emergence of drug-resistant strains. First-line therapy includes isoniazid, rifampicin, pyrazinamide, and ethambutol, with second-line agents used for drug-resistant TB. Therefore, the discovery of structurally novel and effective antitubercular agents remains an important objective in medicinal chemistry.

Isatin (1H-indole-2,3-dione) is a versatile heterocyclic pharmacophore with considerable medicinal potential. Its reactive carbonyl groups and readily modifiable framework enable extensive structural diversification and diverse pharmacological activities. Clinically relevant isatin-containing drugs such as sunitinib and nintedanib further demonstrate its therapeutic significance. Isatin derivatives have also shown promising activity against drug-susceptible and drug-resistant *M. tuberculosis*. Mannich modification enables the introduction of aminomethyl functionalities, potentially enhancing molecular interactions and biological properties, providing a rationale for developing isatin-based mannich derivatives as prospective antitubercular agents.

Modern drug discovery increasingly combines computational approaches with experimental validation to accelerate lead identification. In the present study, an integrated in silico strategy involving network pharmacology, molecular docking, and ADMET prediction was employed to investigate the therapeutic potential of designed isatin-based mannich derivatives. SwissTargetPrediction was used for target prediction, while Venny and Cytoscape supported target intersection and network visualization. STRING was employed for protein–protein interaction analysis, and ShinyGO and DAVID database were used for Gene Ontology and pathway enrichment analyses. These approaches provide systems-level insights into potential targets, pathways, ligand–target interactions, and pharmacokinetic properties, supporting subsequent molecular docking and experimental evaluation of the synthesized derivatives.

II. Materials and methods:**1. In silico studies****a) Target Profiling**

A biologically relevant protein target associated with *Mycobacterium tuberculosis* was selected based on its reported therapeutic significance and availability of a suitable three-dimensional structure in the Protein Data Bank (PDB). The selected protein structure was prepared using the Schrödinger Suite, involving structural preprocessing, removal of irrelevant molecules, addition of hydrogen atoms, assignment of appropriate bond orders and protonation states, and energy minimization to obtain a suitable receptor structure for subsequent computational studies. ^[4-7]

b) Virtual screening for lead identification

Virtual screening was employed as an initial lead-identification strategy to prioritize the designed isatin-based mannich derivatives according to their predicted interaction with the selected tuberculosis-associated target using iGEMDOCK v2.1. A curated library comprising

50 drug-like molecules was retrieved from the ZINC database and subjected to virtual screening, with candidate prioritization based on their predicted binding affinities and key ligand–target interaction profiles.

c) In Silico ADMET profiling and lead optimization

The shortlisted compounds were subjected to ADMET prediction using pkCSM to assess their important pharmacokinetic and toxicity-related characteristics. Parameters associated with absorption, distribution, metabolism, excretion, and toxicity were evaluated to identify compounds with comparatively favourable drug-like profiles. The resulting information was considered for prioritizing promising candidates for subsequent computational and experimental investigations.

d) Target Mapping

Network pharmacology was employed to investigate the potential target spectrum and biological mechanisms associated with the selected compounds. SwissTargetPrediction was used for potential target prediction, while Venny was utilized to identify common targets. STRING was employed for protein–protein interaction analysis, and Cytoscape was used to construct and visualize compound–target and protein interaction networks. Functional enrichment and pathway analyses were performed using ShinyGO and DAVID to identify relevant Gene Ontology categories and biological pathways associated with the predicted targets. [8-12]

e) Docking-Guided Validation [13-15]

Molecular docking was performed using the Schrödinger Suite to investigate the binding orientation and interaction profile of the prioritized isatin mannich derivatives within the selected protein target. The prepared ligands and receptor were subjected to docking, and the resulting poses were analyzed based on docking scores and key non-covalent interactions with functionally important amino acid residues. The docking results were subsequently used to support the prioritization of compounds for experimental evaluation.

2. Synthetic Development studies

Step 1: Preparation of N-Methyl-2-acetylbenzimidazole

2-(α -Hydroxyethyl) benzimidazole was synthesized by refluxing o-phenylenediamine (27 g, 0.25 mol) with lactic acid (25.5 mL, 30.6 g, 0.34 mol) for 2.5 h. After cooling, the reaction mixture was alkalized with 10% sodium hydroxide, and the resulting residue was dissolved in boiling water, treated with activated charcoal (2 g) for 15 min, and filtered. The filtrate was cooled to approximately 10 °C, and the separated solid was collected, washed with cold water, dried at 100 °C, and purified. The resulting 2-(α -hydroxyethyl) benzimidazole (8.1 g, 50 mmol) was subsequently oxidized by dropwise addition of potassium dichromate (19.8 g, 150 mmol) to 25% aqueous sulfuric acid (80 mL) with continuous stirring for 20 min, followed by stirring for 2 h. The separated solid was washed with water, suspended in water (50 mL), and treated with 1:1 aqueous ammonia to adjust the pH to 6–6.5. The product was collected, washed, dried, and recrystallized from boiling water to afford 2-acetylbenzimidazole. Finally, the obtained 2-acetylbenzimidazole was treated with sodium borohydride in dimethylformamide (DMF) and stirred at room temperature for 30 min, followed by addition of methyl iodide and further stirring for 30 min. The reaction mixture was

poured into ice-water, and the resulting precipitate was filtered, dried, and collected to obtain the corresponding N-methylated benzimidazole derivative.

Step 2: Synthesis of isatin Schiff bases

Equimolar quantities of isatin (0.005 mol) and the appropriate substituted aromatic amine (0.005 mol) were refluxed in absolute ethanol (30 mL) containing 2–3 drops of glacial acetic acid for 30 min. The solvent was removed, and the resulting Schiff base was isolated and recrystallized from chloroform.

Step 3: Synthesis of N-mannich bases of isatin

The synthesized isatin Schiff bases (0.01 mol) were reacted with equimolar quantities of the respective secondary amines (0.01 mol) and 40% formaldehyde in ethanol (40 mL). The reaction mixture was stirred at room temperature and subsequently refrigerated for 2 days. The precipitated N-Mannich bases were isolated by suction filtration and recrystallized from ethanol.

Scheme

Step 1:

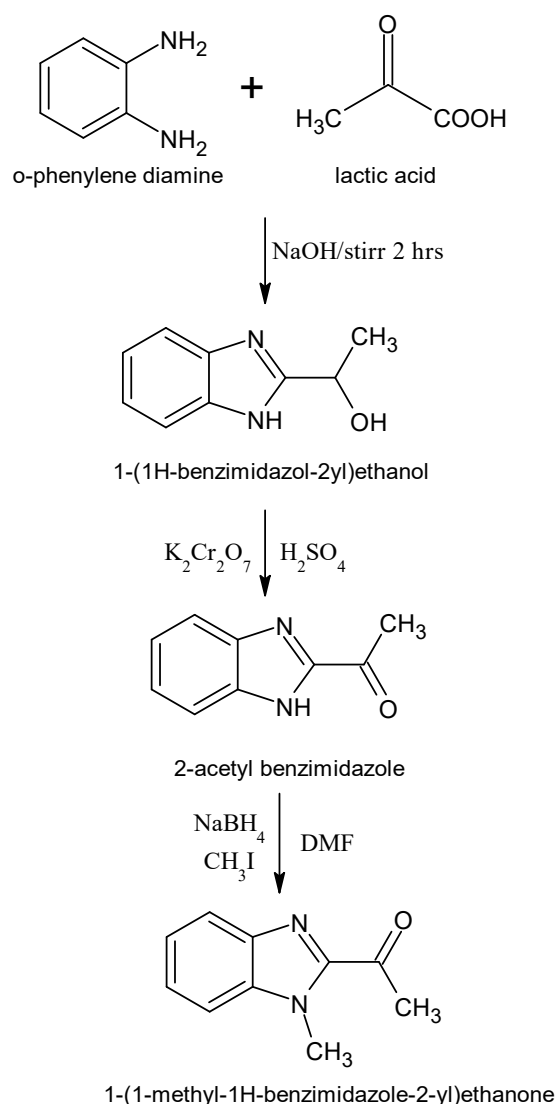


Figure 1 Scheme for preparation of N-Methyl-2-acetylbenzimidazole

Step 2:

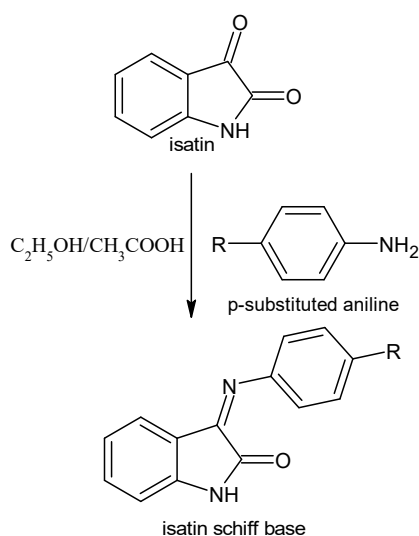


Figure 2 Scheme for synthesis of isatin Schiff bases

Step 3:

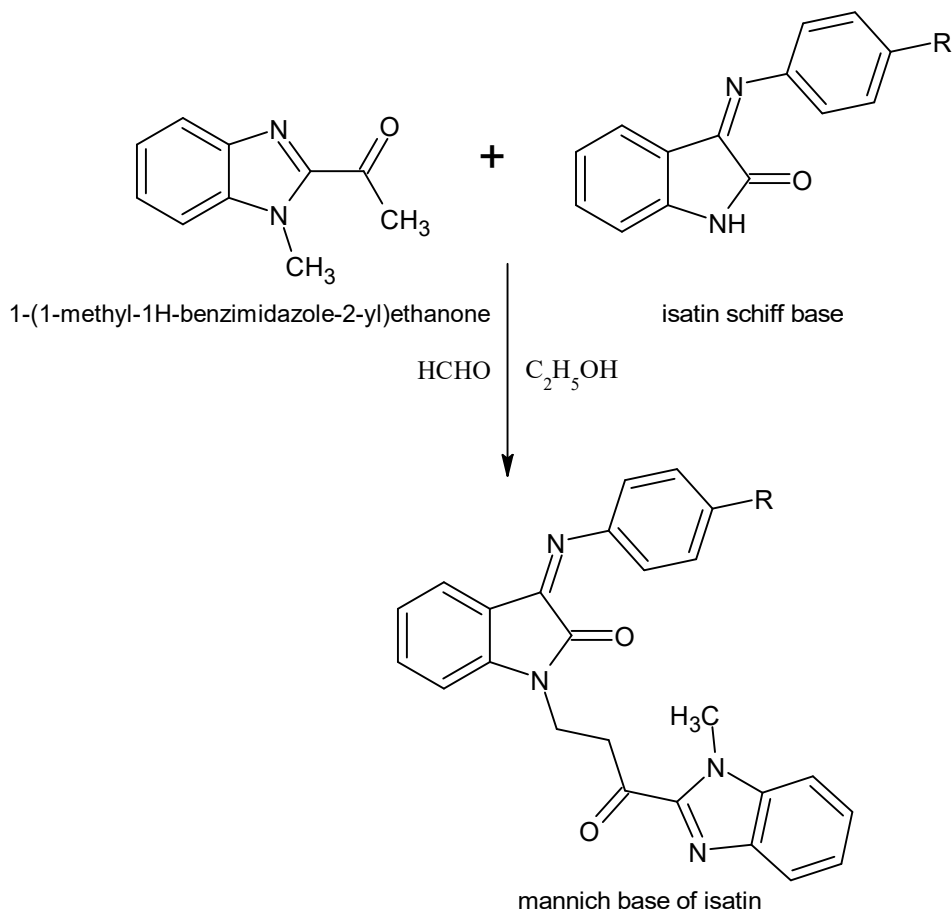


Figure 3 Scheme for Synthesis of N-mannich bases of isatin

Table 1: Chemical substituents of I₁₋₄ derivatives

S.NO	COMPOUND CODE	R
1	I ₁	p-nitro
2	I ₂	p-methoxy
3	I ₃	p-chloro
4	I ₄	p-methyl

3. Analytical characterization

Physicochemical properties, including solubility, melting point, and thin-layer chromatography (TLC), were evaluated for the synthesized compounds. Melting points were determined using a Visible Range Melting Point Apparatus (LABINDIA). UV–Visible spectra was recorded using a JASCO V-530 spectrophotometer and IR spectra was recorded using JASCO FR/IR 410 at the Department of Pharmaceutical Analysis, SRIPMS, Coimbatore. ¹H NMR and ¹³C NMR spectra were recorded on a BRUKER UltraShield NMR spectrometer operating at 400 MHz at VIT University, Chennai. Mass spectral analysis was performed using GC–MS at VIT University, Chennai.

4. Anti-tubercular screening

Anti-tubercular activity of the synthesized isatin Mannich bases was evaluated against *Mycobacterium tuberculosis* using the Pantothenate Synthetase (PanC) MABA assay. The study was performed using recombinant Pantothenate Synthetase as the target enzyme, and the synthesized isatin Mannich bases were evaluated. A suitable standard anti-tubercular drug was used as the reference for comparison of the results.

The MABA method is based on the ability of metabolically active *Mycobacterium tuberculosis* to reduce the blue resazurin dye to pink resorufin. The change in color indicates bacterial metabolic activity. In the presence of an effective anti-tubercular compound, bacterial growth and metabolic activity are inhibited, resulting in no or minimal color change. The MIC is determined as the lowest concentration of the test compound that prevents the characteristic color change from blue to pink.

III. Results and discussion:

1. *In silico* studies

a) Target Profiling

The three-dimensional structure of pantothenate synthetase (PanC) from *Mycobacterium tuberculosis* was retrieved from the RCSB Protein Data Bank with PDB ID: 3IMG. The structure was determined by X-ray diffraction at a resolution of 1.80 Å and contains pantothenate synthetase as a homodimer. PanC is an essential enzyme involved in pantothenate (vitamin B5) biosynthesis, making it an important target for the identification of potential anti-tubercular agents.

b) Lead identification by virtual screening

The docking results of pantothenate synthetase (PDB ID: 3IMG) with the 50 structurally similar ligands are presented in Table 2. The ligands were prioritized based on their predicted binding energies and key protein–ligand interactions. Lead compounds were identified through

virtual screening by selecting ligands exhibiting the most favorable binding energies, along with stable interactions within the active site of pantothenate synthetase.

Table 2: Virtual screening for chosen HITS using iGEMDOCK

S.NO	Compound code	Kcal/mol	S.NO	Compound code	Kcal/mol
1	Zinc_752006	-62.44	26	Zinc_39764271	-85.60
2	Zinc_19995122	-102.53	27	Zinc_39764272	-89.16
3	Zinc_19995125	-107.18	28	Zinc_39764275	-76.08
4	Zinc_19995128	-100.49	29	Zinc_39764276	-76.04
5	Zinc_19995137	-106.43	30	Zinc_71569279	-91.95
6	Zinc_46235100	-104.76	31	Zinc_71569280	-108.26
7	Zinc_44017734	-86.01	32	Zinc_71569282	-100.04
8	Zinc_44240525	-81.43	33	Zinc_71569283	-106.19
9	Zinc_44243009	-109.73	34	Zinc_71569285	-96.07
10	Zinc_62032044	-94.98	35	Zinc_71569288	-108.37
11	Zinc_71566522	-98.69	36	Zinc_71569289	-103.17
12	Zinc_71566957	-117.87	37	Zinc_71569291	-125.28
13	Zinc_71566958	-104.72	38	Zinc_71569354	-106.31
14	Zinc_71567927	-105.28	39	Zinc_71569356	-107.80
15	Zinc_71567928	-98.47	40	Zinc_71569359	-84.23
16	Zinc_71568986	-97.11	41	Zinc_71569361	-101.46
17	Zinc_71568987	-100.02	42	Zinc_71569509	-88.48
18	Zinc_71569178	-105.04	43	Zinc_71569511	-90.26
19	Zinc_71569179	-93.79	44	Zinc_71569512	-93.03
20	Zinc_71569180	-102.03	45	Zinc_71569516	-95.37
21	Zinc_71569181	-95.85	46	Zinc_75169519	-99.08
22	Zinc_71569191	-116.03	47	Zinc_71569521	-100.25
23	Zinc_71569192	-114.98	48	Zinc_71569523	-103.34
24	Zinc_71569193	-109.74	49	Zinc_71569685	-91.81
25	Zinc_71569194	-104.81	50	Zinc_71569699	-101.57

c) In Silico ADMET profiling and lead optimization

In silico ADMET profiling was performed using molinspiration and pkCSM to evaluate the drug-likeness, physicochemical properties, pharmacokinetic behavior, and toxicity profiles of the selected lead compounds. molinspiration was used to determine molecular descriptors and drug-likeness parameters, including molecular weight, miLogP (logP), topological polar surface area (TPSA), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), and rotatable bonds, along with assessment of Lipinski's Rule of Five. The Rule of Five was evaluated based on molecular weight ≤ 500 Da, logP ≤ 5 , HBD ≤ 5 , and HBA ≤ 10 .

Table 3: Drug likeness screening I₁₋₄ by molinspiration

S.NO	Compound code	log p	Molecular weight	No. of H acceptors	No. of H donors	No. of violations
1	I ₁	4.078	455.74	9	1	0
2	I ₂	4.176	440.50	7	1	0
3	I ₃	4.797	444.92	6	1	0
4	I ₄	4.838	422.48	6	0	0

pkCSM was employed to predict important ADMET parameters, including intestinal absorption, water solubility, blood–brain barrier permeability, CYP450 enzyme interactions, renal clearance, total clearance, and toxicity-related endpoints. These computational assessments facilitated the identification and prioritization of compounds with favorable drug-like and pharmacokinetic properties for further development as potential anti-tubercular leads.

Table 4: Drug likeness screening of I₁₋₄ by pkCSM

Compound code	CaCO ₂ permeability	Intestinal absorption	Skin permeability	P-glycoprotein substrate	P-glycoprotein inhibitor	BBB permeability	Volume of distribution (VDss)	CYP3A4 substrate	CYP3A4 inhibitor	CYP2D6 inhibitor	CYP2C9 inhibitor	CYP2C19 inhibitor	Total clearance	Renal OCT2 substrate	AMES toxicity	hERG inhibition	Hepatotoxicity	LD ₅₀	Skin sensitization
I ₁	0.67	100	-2.7	Yes	Yes	-0.8	0.18	Yes	Yes	No	Yes	Yes	0.53	Yes	No	Yes	No	2.57	No
I ₂	1.44	92.6	-2.5	Yes	Yes	-0.5	-0.0	Yes	Yes	No	Yes	Yes	0.80	Yes	No	Yes	Yes	2.67	No
I ₃	1.41	90.0	-2.7	Yes	Yes	-0.5	0.22	Yes	Yes	No	Yes	Yes	0.76	Yes	No	Yes	No	2.69	No
I ₄	1.42	91.4	-2.7	Yes	Yes	-0.3	0.24	Yes	Yes	No	Yes	Yes	0.78	Yes	No	Yes	Yes	2.68	No

d) Target Mapping

i. Target-class distribution of the top 25 targets

The target-class distribution of the top 25 predicted targets demonstrates the functional diversity of the isatin Mannich derivatives. Kinases (20%) formed the major class, including JAKs, EGFR, SRC and AKT1, followed by oxidoreductases and eraser proteins (12% each), and proteases and phosphodiesterases (8% each).

The remaining targets belonged to diverse classes such as phosphatases, transcription factors, nuclear receptors, GPCRs, hydrolases and cytochrome P450. This diversity suggests that the isatin scaffold can interact with multiple protein classes, with its planar aromatic system supporting hydrophobic/ π -interactions and its carbonyl and heteroatom groups enabling

hydrogen bonding. Mannich substitution further modulates polarity, basicity and target recognition, potentially contributing to the observed anti-tubercular activity.

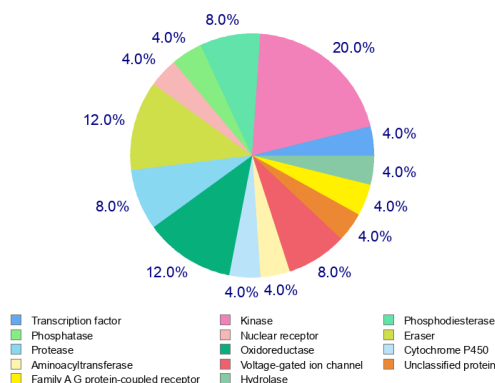


Figure 4 Represents the pie chart of isatin therapeutic targets

ii. Mannich base of isatin–tuberculosis target overlap

The Venn diagram shows the intersection between the predicted targets of the isatin Mannich-base derivatives and tuberculosis-associated targets. Of the 97 predicted targets, 77 overlapped with tuberculosis-related targets, while 20 were unique to the Mannich-base target set. The 77 common targets suggest that the isatin Mannich scaffold may interact with multiple proteins involved in tuberculosis-related processes.

The isatin core provides carbonyl and heteroatom functionalities that support hydrogen-bonding and π -mediated interactions, while the Mannich substituent adds structural and electronic diversity. The secondary amine may further influence polarity, basicity, hydrogen-bonding, and target recognition. Thus, the 77 overlapping targets were considered potential candidates for subsequent network and protein–protein interaction analyses. Overall, the target overlap indicates a possible multi-target mechanism underlying the anti-tubercular activity of the synthesized derivatives, requiring experimental validation.

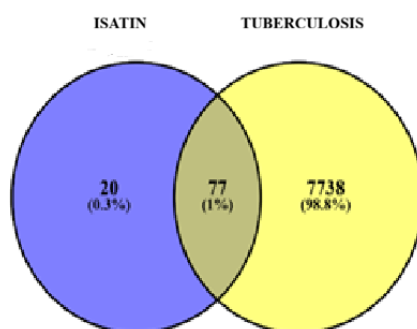


Figure 5 Venn diagram showing overlapping targets between isatin and tuberculosis related genes.

iii. STRING protein–protein interaction network

The STRING protein–protein interaction network demonstrates the functional associations among the common tuberculosis-associated targets identified from the target-overlap analysis. The highly interconnected network indicates that several of the predicted targets participate in related cellular and signaling pathways rather than functioning as isolated proteins. Prominent

interconnected proteins include JAK1, JAK2, JAK3, TYK2, EGFR, AKT1, SRC, PIK3CA, PIK3CD, GSK3B, MAPK14, LYN and LCK. The JAK1/JAK2/JAK3/TYK2 group is primarily associated with cytokine and immune-signaling processes, whereas EGFR, AKT1, SRC and PI3K-related proteins participate in cellular signaling, proliferation and survival pathways.

Additional proteins such as NOD2 and PARP1 are associated with cellular and immune responses, while CA2, CA9, CA12, F2 and F10 represent distinct enzyme-related functional groups within the predicted target set. The functional interconnectivity of these proteins provides further support for a possible multi-target mechanism of the isatin Mannich derivatives. From a structural perspective, the isatin nucleus serves as the conserved pharmacophoric scaffold, while changes in the Mannich amine may alter interactions with different protein targets. Importantly, STRING associations represent known or predicted functional relationships and do not demonstrate that the synthesized compounds directly bind to every protein represented in the network.

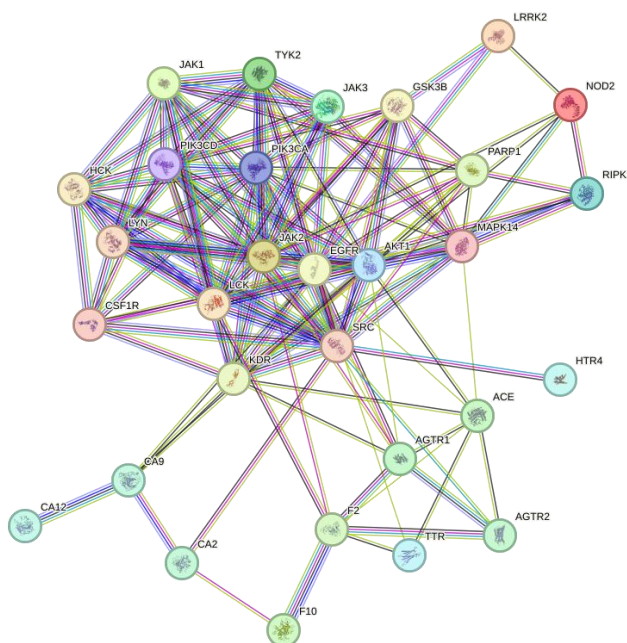


Figure 6 Protein-protein interaction network

iv. Compound–target interaction network

The compound–target interaction network illustrates the predicted relationships between the isatin Mannich-base scaffold and tuberculosis-associated protein targets. The dense connectivity observed within the network indicates that the compounds are potentially associated with multiple biological targets rather than a single molecular target. Highly connected proteins, including EGFR, PIK3CA, PIK3CD, PARP1, JAK1, JAK2, JAK3, AKT1, SRC, GSK3B and HDAC1, occupy prominent positions within the network and may represent network-important hub targets. These proteins are associated with diverse biological functions, including kinase-mediated signaling, inflammatory regulation, cellular survival and enzymatic processes.

involved in pantothenate and CoA biosynthesis, its inhibition may disrupt metabolic processes required for bacterial growth and persistence. Overall, the KEGG findings provide a systems-level view supporting the potential biological activity of the isatin scaffold, although experimental validation is necessary to establish direct mechanistic links between PanC inhibition and the enriched pathways.

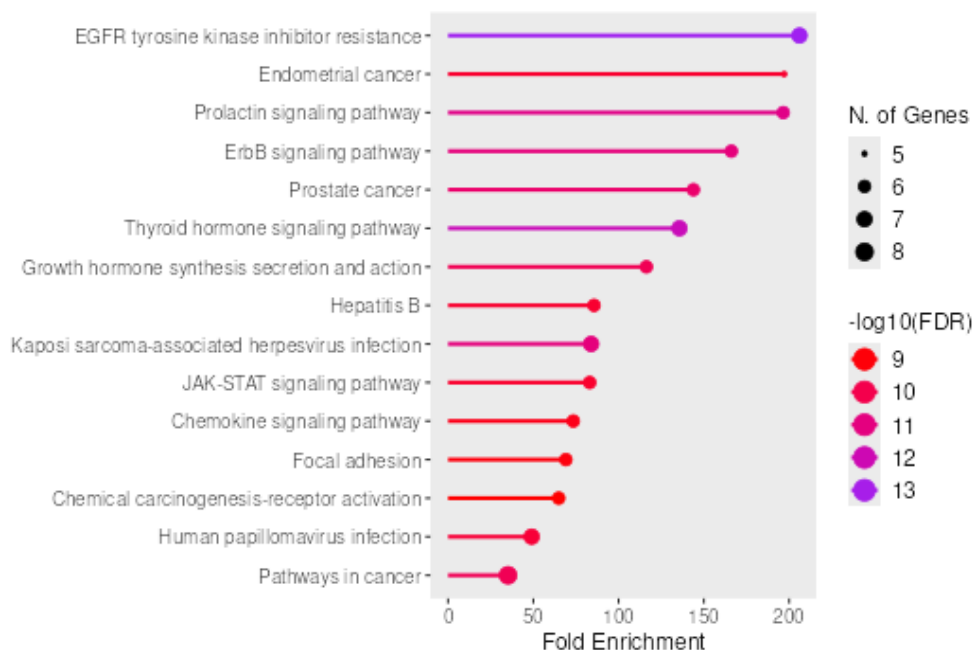


Figure 8 KEGG pathway GO analysis

vi. KEGG Tuberculosis pathway interpretation

The green-highlighted proteins represent pathway components associated with the predicted target set. The pathway involves recognition of mycobacterial components by host immune receptors, followed by intracellular signaling, cytokine production, antigen presentation, and phagosome maturation.

KEGG tuberculosis pathway analysis revealed the involvement of predicted targets in host–pathogen interaction and antimycobacterial defence. Recognition through Toll-like and NOD-like receptors activates MyD88-, IRAK-, TRAF6-, JAK–STAT-, NF-κB-, and MAPK-mediated signaling, regulating cytokine production, antigen presentation, apoptosis, antimicrobial responses, and phagosome maturation. These findings indicate functional associations with macrophage activation, inflammatory regulation, and intracellular responses to *Mycobacterium tuberculosis*. In relation to the isatin scaffold, the results provide complementary support for its proposed activity against the essential metabolic enzyme PanC, involved in pantothenate and CoA biosynthesis. Although PanC is not directly part of the host immune pathway, its inhibition may disrupt essential mycobacterial metabolism and reduce bacterial fitness. However, direct PanC inhibition and antimycobacterial activity require experimental validation.

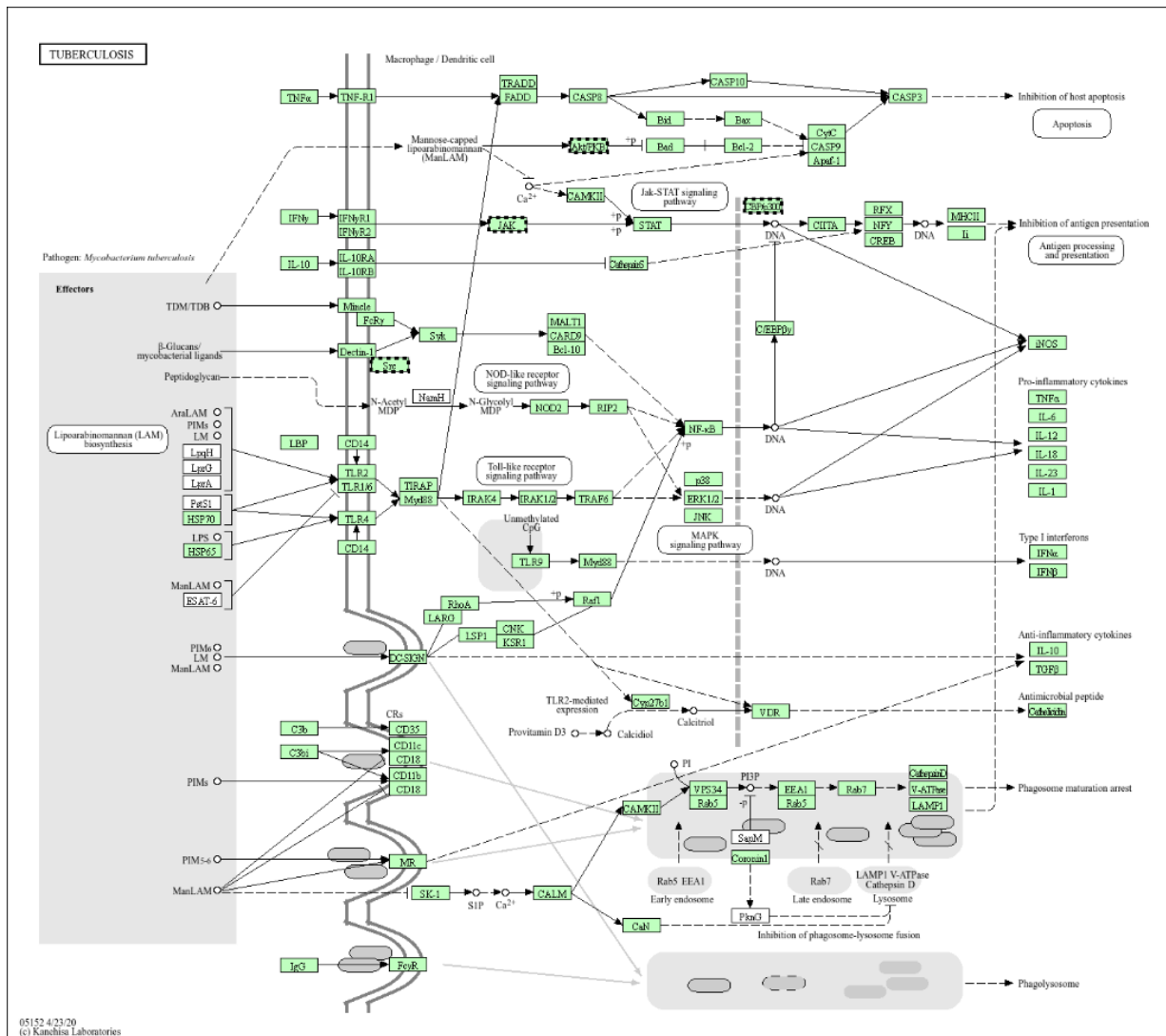


Figure 9 KEGG pathway

vii. GO Biological Process (BP) enrichment analysis

GO Biological Process enrichment analysis was performed to investigate the biological functions associated with the predicted targets of the isatin scaffold. In the context of its proposed anti-tubercular activity against pantothenate synthetase (PanC) of *Mycobacterium tuberculosis*, the enriched processes indicate associations with cellular adaptation, signaling, stress responses, and survival mechanisms.

PanC plays a key role in pantothenate biosynthesis, which supports CoA production and essential metabolic processes such as fatty-acid metabolism and energy generation. Its perturbation by isatin derivatives could therefore disrupt mycobacterial metabolism and adaptation to hostile conditions. Enrichment of protein localization, intracellular signaling, cellular responses, and programmed-cell-death-related processes further supports the functional relevance of the predicted targets. However, these GO terms represent associations within the predicted target set and do not directly establish that PanC inhibition regulates each process.

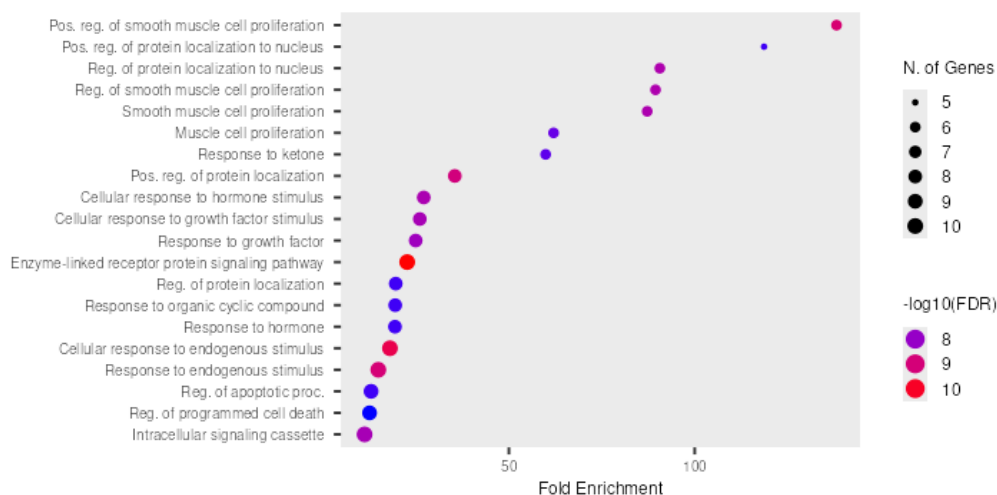


Figure 10 GO Biological Process (BP) enrichment analysis

viii. GO Cellular Component (CC) enrichment analysis

GO Cellular Component (CC) enrichment analysis was performed to identify the predominant cellular locations associated with the predicted targets of the isatin scaffold. Significant enrichment of membrane-associated structures, receptor complexes, membrane microdomains, junctional regions, and catalytic complexes indicates that many predicted targets are involved in membrane-localized signaling and regulatory machinery.

In the context of the proposed anti-tubercular activity against PanC of *M. tuberculosis*, this enrichment provides a broader cellular context, while PanC itself is an essential metabolic enzyme involved in pantothenate biosynthesis rather than a membrane-localized target. Thus, the CC results should not be interpreted as evidence that PanC is located within these mammalian membrane compartments. Together with MF enrichment for kinase, ATP-binding, nucleotide-binding, and catalytic activities, the findings suggest that the isatin scaffold may interact with proteins distributed across diverse regulatory and enzymatic compartments, supporting its broader biological relevance and potential as an anti-tubercular framework targeting essential metabolic functions such as PanC.

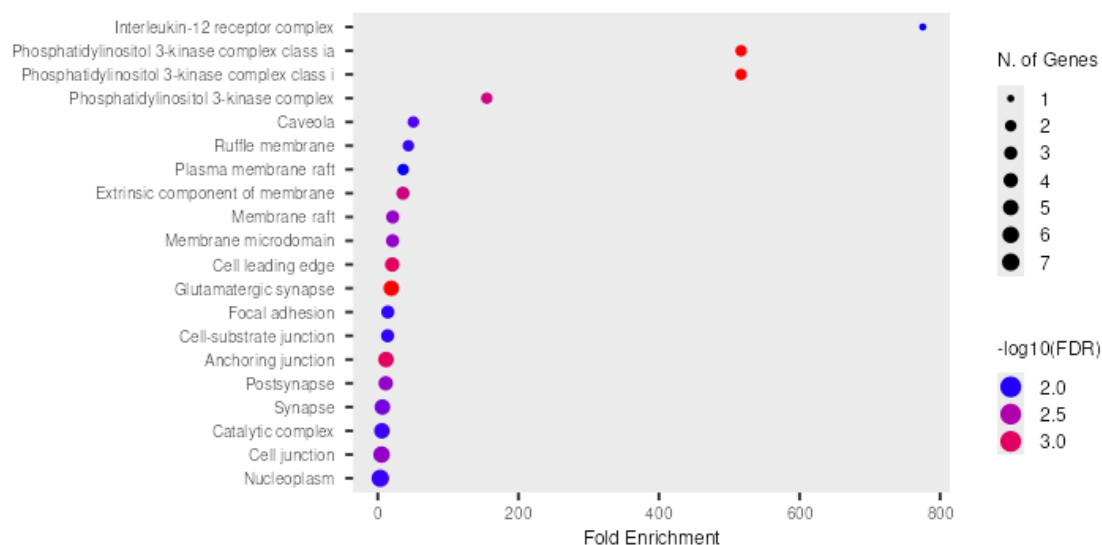


Figure 11 GO Cellular Component (CC) enrichment analysis

ix. GO Molecular Function (MF) enrichment analysis

GO Molecular Function (MF) enrichment analysis revealed significant enrichment of activities including phosphatidylinositol 3-kinase, protein kinase, phosphotransferase, transcription factor binding, adenylate/ATP and purine nucleotide binding, and protein catalytic functions. The strong enrichment of kinase, ATP-binding, nucleotide-binding, and phosphotransferase activities indicates that the predicted targets are largely involved in enzymatic and regulatory processes requiring nucleotide binding and phosphate-group transfer.

In relation to the proposed anti-tubercular activity of the isatin scaffold targeting PanC, these findings are relevant because PanC is an essential metabolic enzyme involved in pantothenate biosynthesis and subsequent CoA production. Its inhibition by isatin derivatives could disrupt essential metabolic, energy, and biosynthetic processes in *M. tuberculosis*. Overall, the enrichment supports the functional importance of enzymatic targets within the predicted target set, although it does not directly establish PanC inhibition or activity against the individual enriched functions.

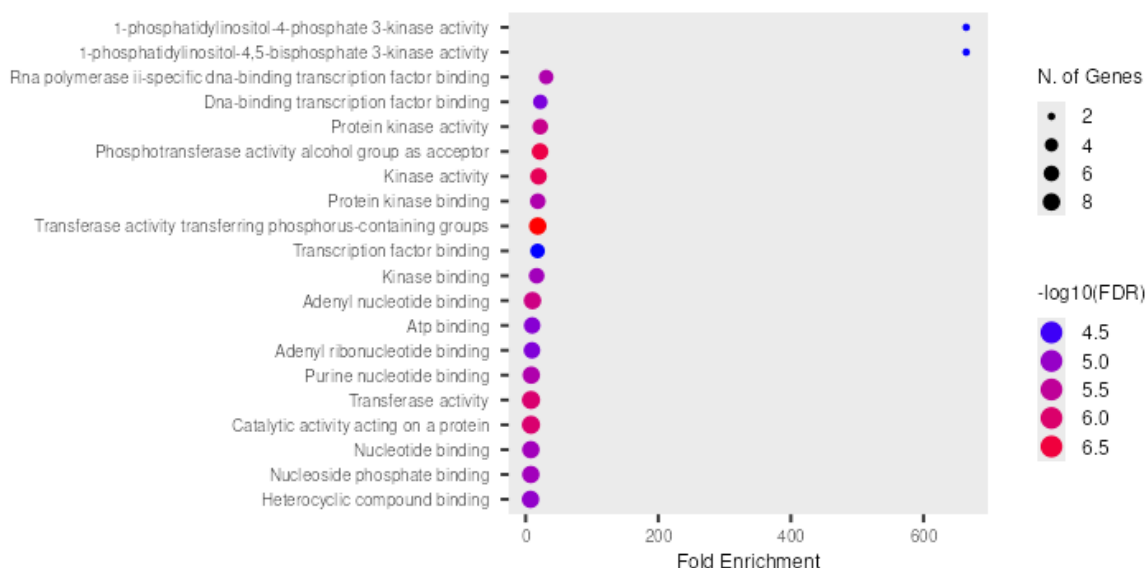


Figure 12 GO Molecular Function (MF) enrichment analysis

e) Docking-Guided Validation

Molecular docking studies were performed using the Schrödinger Suite to investigate the binding orientation and interaction pattern of the synthesized compounds (I₁–I₄) within the active site of the selected protein target, PDB ID: 3IMG. The prepared protein structure was subjected to ligand docking, and the binding affinities of the synthesized compounds were evaluated based on their Glide docking scores. The docking results indicated that all four ligands were able to accommodate within the active-site cavity of 3IMG, adopting energetically favorable binding conformations.

Among the investigated compounds, I₄ exhibited a Glide score of -7.99 kcal/mol, whereas I₁, I₂ and I₃ showed scores of -7.81, -7.0 and -6.94 kcal/mol respectively. A more negative docking score indicates a comparatively favourable predicted binding affinity toward the target. The docking poses further demonstrated that the ligands were stabilized through a combination of hydrogen-bonding, hydrophobic, π – π stacking, π –cation and other non-covalent interactions

with the active-site residues. These interactions may contribute to the proper positioning and stabilization of the ligands within the binding pocket.

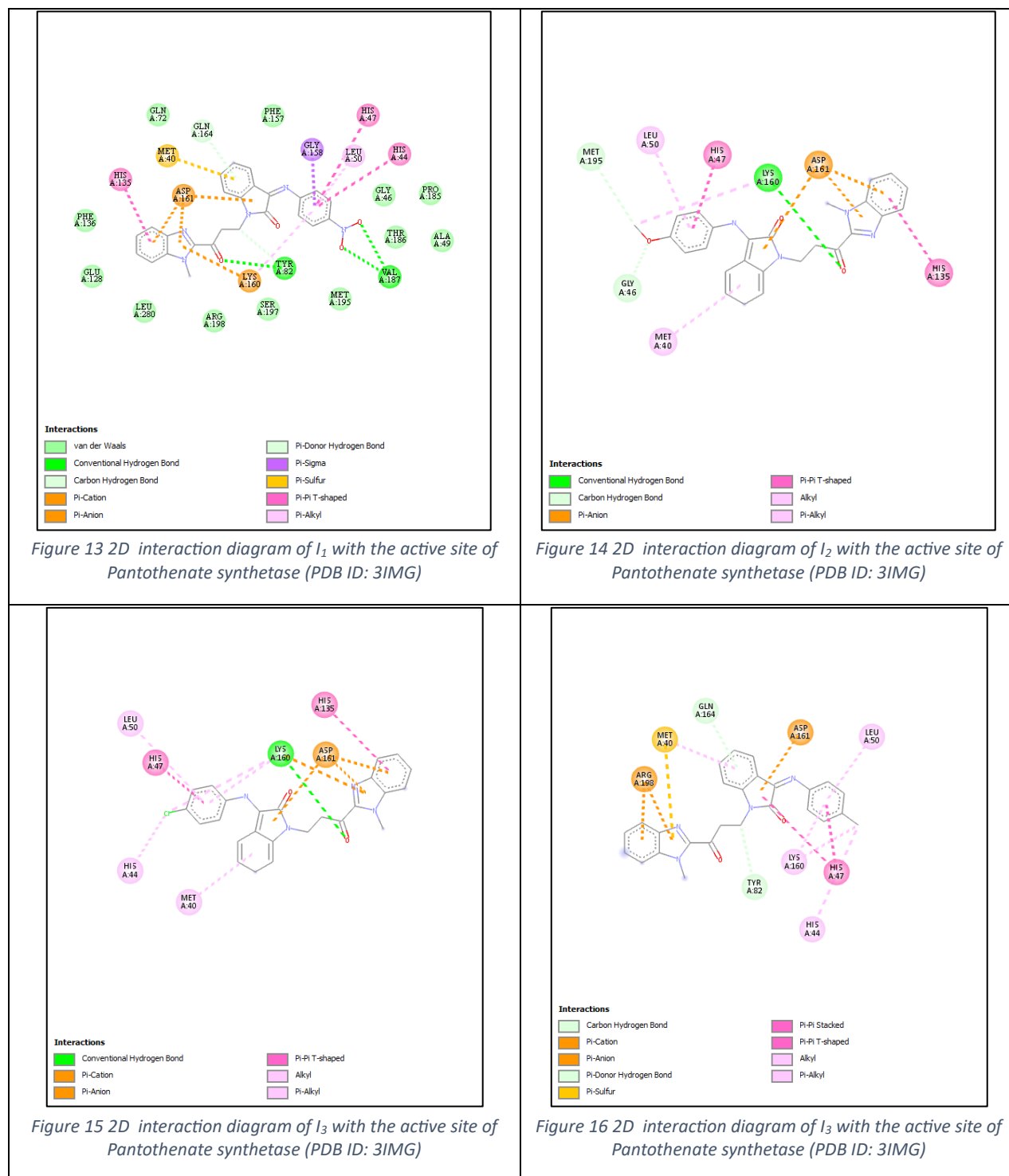


Table 5: Molecular docking scores of I₁₋₄ derivatives against pantothenate synthetase

S.No	Compound name	Docking score (Kcal/mol)	Key Interactions
1	I ₁	-7.81	His135, Asp161, Gln164, Gly158, Tyr82
2	I ₂	-7.0	Lys160, Asp161, His135, Met40, Gly46
3	I ₃	-6.94	Lys160, Met40, His135, Leu50, Asp161
4	I ₄	-7.99	Arg198, Gln164, Asp161, Lys160, Tyr82
5	Isoniazid	-6.40	Arg169, Val181, Gly183, His47, Arg198

2. Synthetic studies

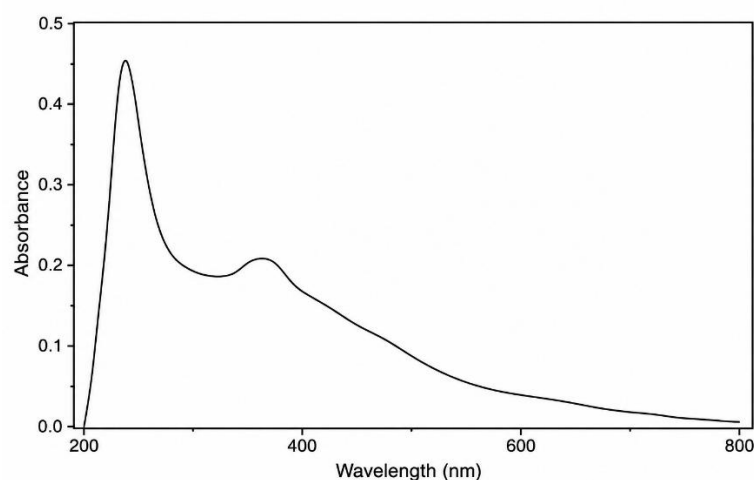
All the 4 new compounds I₁₋₄ obtained through schemes were synthesized with good yield and evaluated by their physical characterization (melting point and TLC).

Table 6: Physical data of newly synthesized compounds

S.NO	Compound code	R	Molecular formula	Molecular weight	% yield	Melting Point °C	Rf value
1	I ₁	p-nitro	C ₂₅ H ₁₉ N ₅ O ₄	453.45	84.0%	181 °C	0.79
2	I ₂	p-methoxy	C ₂₆ H ₂₂ N ₄ O ₃	438.47	83%	192°C	0.70
3	I ₃	p-chloro	C ₂₅ H ₁₉ N ₄ O ₂ Cl	442.0	81%	198°C	0.70
4	I ₄	p-methyl	C ₂₆ H ₂₂ N ₄ O ₂	422.48	88%	167°C	0.72

3. Analytical studies

The structures of synthesized compounds during the present investigation were established on the basis of chemical data IR, UV, ¹H NMR, ¹³C NMR and MASS spectra data.

*Figure 17 UV absorbance spectrum of I₄*

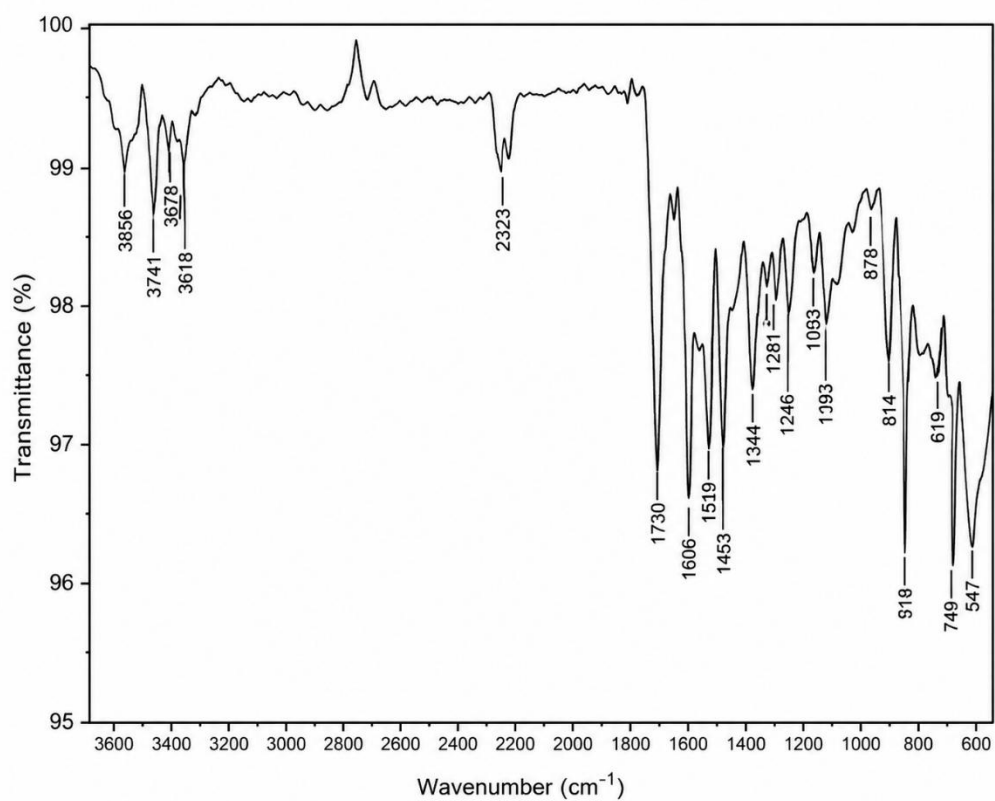


Figure 18 IR spectrum of I_4

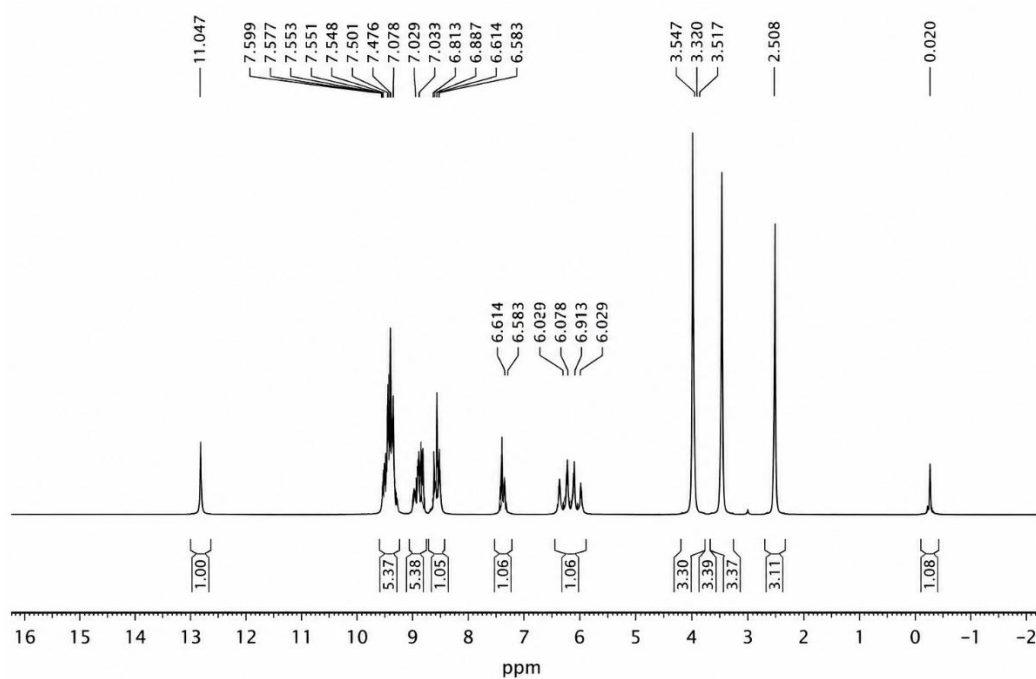
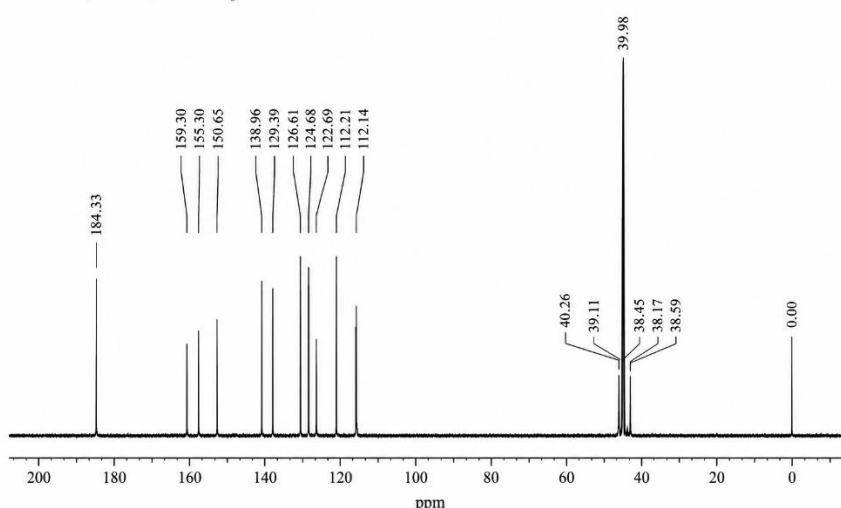
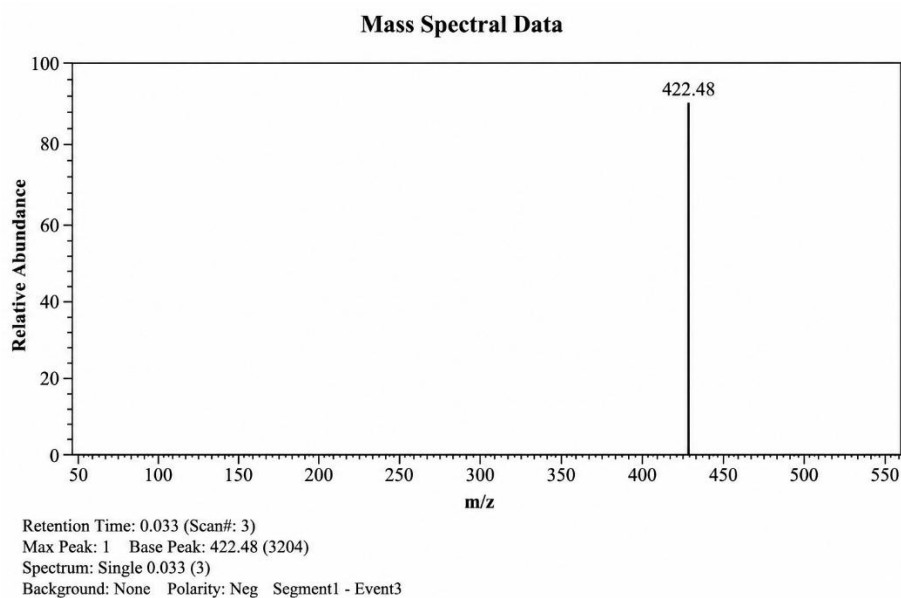


Figure 19 ^1H NMR spectrum of I_4

^{13}C NMR (75 MHz, DMSO- d_6)Figure 20 ^{13}C NMR spectrum of I_4 Figure 21 Mass spectrum of I_4

4. Anti-tubercular screening

Procedure

The anti-mycobacterial activity of compounds were assessed against *M. tuberculosis* using Microplate Alamar Blue Assay (MABA). This methodology is non-toxic, uses a thermally stable reagent, and shows a good correlation with proportional and BACTEC radiometric methods. To the outer perimeter of sterile 96 well plates, 200 μl of sterile deionized water was added to minimize evaporation of medium in the test wells during incubation. The 96 well plates received 100 μl of the Middlebrook 7H9 broth (inoculated with *Mycobacterium tuberculosis* of H37RV Strain) and serial dilution of compounds were made directly on the plate. The final drug concentrations tested were 100 to 0.195 $\mu\text{g/ml}$. Plates were covered and sealed with parafilm and incubated at 37 $^{\circ}\text{C}$ for five days. After this time, 25 μl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and

incubated for 24 hours. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as the lowest drug concentration which prevented the colour change from blue to pink. The minimum inhibitory concentrations of the newly synthesized compounds were measured and compared with that of the standard.

The anti-tubercular activity of the synthesized compounds was evaluated based on their minimum inhibitory concentrations against *Mycobacterium tuberculosis*. Among the synthesized derivatives, I₄ demonstrated the highest activity with an MIC of 6.25 µg/mL, followed by I₂ (25 µg/mL), I₁ (50 µg/mL), and I₃ (100 µg/mL). The standard drug isoniazid exhibited an MIC of 1.5625 µg/mL. Thus, the synthesized compounds displayed varying degrees of inhibitory activity, with I₄ emerging as the most active derivative. Although its activity was lower than that of isoniazid, the low MIC of I₄ indicates promising anti-tubercular potential and warrants further investigation.

Drugs used: Newly synthesized drugs (I₁₋₄)

Standard drug: Isoniazid

Solvent: DMSO

Table 7: Minimum inhibitory concentration (µg/ml)

Compound code	100	50	25	12.5	6.25	3.125	1.5625	0.7812	0.3906	0.195
I ₁	B	B	P	P	P	P	P	P	P	P
I ₂	B	B	B	P	P	P	P	P	P	P
I ₃	B	P	P	P	P	P	P	P	P	P
I ₄	B	B	B	B	B	B	P	P	P	P
Isoniazid	B	B	B	B	B	B	B	P	P	P

Where as

B – inhibits the growth of *Mycobacterium tuberculosis*

P – growth of *Mycobacterium tuberculosis*

IV. Conclusion

The present study demonstrated the potential of the isatin Mannich scaffold as a promising framework for the development of novel antitubercular agents through an integrated computational and experimental approach. Network pharmacology identified 77 common targets associated with the predicted target profile of the isatin Mannich derivatives and tuberculosis-related targets, supporting a possible multitarget mechanism. Molecular docking against pantothenate synthetase (PanC; PDB ID: 3IMG) showed favourable binding of all synthesized derivatives, with I₄ (p-methyl derivative) exhibiting the most favourable Glide score of -7.99 kcal/mol, followed by I₁ (-7.81), I₂ (-7.00), and I₃ (-6.94 kcal/mol). The predicted ADMET profiles indicated that all four derivatives complied with Lipinski's Rule of Five without violations. The compounds were successfully synthesized in good yields of 81–88% and their proposed structures were supported by the reported physicochemical and spectroscopic characterization.

The in vitro Microplate Alamar Blue Assay further confirmed the antitubercular potential of the synthesized derivatives. Based on the reported colour-change data, the calculated MIC values were 50 µg/mL for I1, 25 µg/mL for I2, 100 µg/mL for I3, and 3.125 µg/mL for I4, compared with 1.5625 µg/mL for isoniazid. Among the synthesized compounds, I4 demonstrated the highest antitubercular activity, showing a markedly lower MIC than I1–I3 and displaying a favourable correspondence with its superior docking score. Although I4 was less active than isoniazid, its combined in silico and in vitro profile identifies it as the most promising lead within the synthesized series. Overall, these findings support further structural optimization and experimental validation of the isatin Mannich scaffold, particularly the I4 analogue, toward the development of improved antitubercular candidates.

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