



Design, Synthesis, and Molecular Docking Studies of Novel Solanidine-Benzoyl Hydrazone Hybrids as Potential InhA Inhibitors

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Abstract:

Tuberculosis (TB) remains a major global health concern due to increasing drug resistance, poor bioavailability of first-line drugs, and associated hepatotoxicity. This study reports the design, synthesis, and molecular docking evaluation of novel solanidine-benzoyl hydrazone hybrid derivatives (MK-1 to MK-12) as potential inhibitors of the enoyl-acyl carrier protein reductase (InhA) enzyme of *Mycobacterium tuberculosis*. The compounds were synthesized via a multi-step process involving Vilsmeier-Haack formylation, Schiff base formation, and cyclization, and were characterized using FTIR, TLC, and mass spectrometry. Molecular docking studies using AutoDock Vina against the InhA enzyme (PDB ID: 5JFO) revealed strong binding affinities (−9.3 to −7.8 kcal/mol), exceeding that of the standard drug isoniazid (−7.5 kcal/mol). ADMET prediction using SwissADME demonstrated favorable drug-likeness, good oral bioavailability, and compliance with Lipinski's rule of five for most compounds. Toxicity profiling indicated moderate hepatotoxicity, neurotoxicity, and nephrotoxicity risks, while cardiotoxicity remained negligible. Phytochemical screening of *Solanum virginianum* confirmed the presence of bioactive constituents such as alkaloids, flavonoids, saponins, and glycosides, supporting its pharmacological relevance. The findings suggest that these hybrid derivatives exhibit promising anti-tubercular potential and may serve as effective adjuncts to existing therapies with improved efficacy and reduced toxicity.

Keywords

Tuberculosis; Solanidine derivatives; Benzoyl hydrazone; InhA inhibitors; Molecular docking; ADMET; *Mycobacterium tuberculosis*; Drug design; Phytochemicals; Anti-tubercular agents

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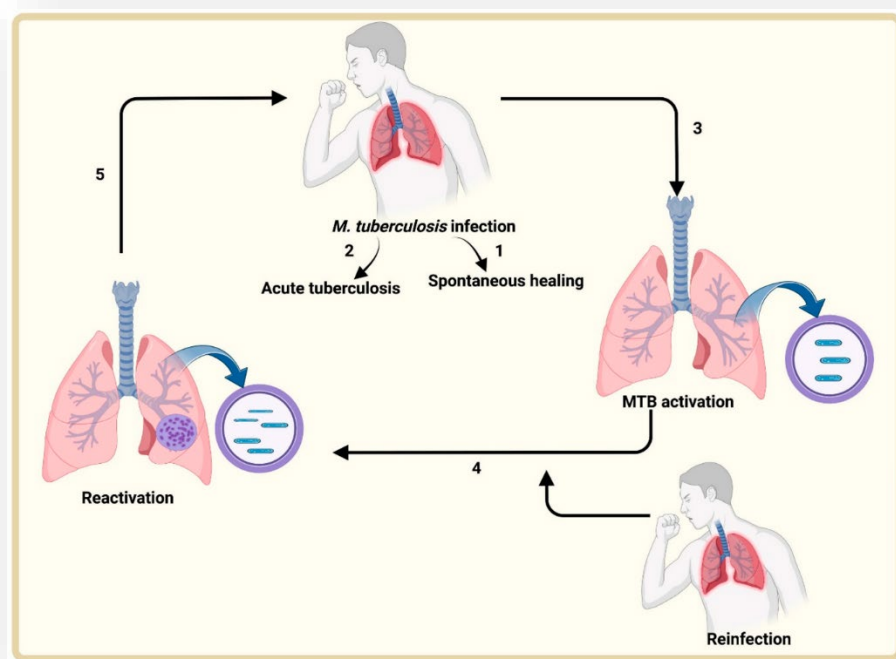
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1. INTRODUCTION

Over the years, tuberculosis has become a constant danger to human health. The 2022 Global Tuberculosis Report showed the effects of the COVID-19 pandemic, including a 4.5% rise in tuberculosis cases and deaths, which went from 1.5 million in 2020 to 1.6 million in 2020¹. The End TB Strategy 2020's goals of a 35% drop in the death rate and a 20%. The drop in the number of cases was also not a method. The Lancet Public Health, 2023) The several survival mechanisms utilized by pathogenic bacteria, *Mycobacterium tuberculosis* (Mtb), greatly complicate the treatment of tuberculosis². The principal therapy for tuberculosis depends on a standardized combination of rifampicin (RIF), isoniazid (INH), and pyrazinamide (PZ). But because these medications are not very bioavailable, they often don't work well enough in the body to stop tuberculosis and lead to the development of antimicrobial drug resistance (AMR)³. In fact, a greater minimum inhibitory concentration (MIC) of isoniazid has previously been linked to the risk of tuberculosis relapse. Drug-induced hepatotoxicity, commonly known as drug-induced liver injury (AT-DILI), represents a significant side effect contributing to the failure of anti-tubercular therapy. Ezhilarasan, 2023: Medicinal herbs are regarded as a timeless boon from nature, employed for the treatment of diverse human ailments since antiquity⁴. The World Health Organization (WHO) says that 80% of people in underdeveloped countries use traditional medicines to prevent and treat common health problems. For decades, cancer has been a prevalent health problem for people. It is caused by cells growing and multiplying in an uncontrolled way, either in a benign or malignant form. GLOBOCAN 2020 estimates say that cancer caused about 19.3 million new cases and 10 million deaths over the world⁵. For decades, cancer has been a prevalent health problem for people. It is caused by cells growing and multiplying in an uncontrolled way, either in a benign or malignant form. GLOBOCAN 2020 estimates say that cancer caused about 19.3 million new cases and 10 million deaths over the world⁶. The global cancer burden has been rising daily, reflecting contemporary epidemiological and demographic shifts. Genotypically, malignant cells exhibit seven distinct alterations in cellular physiology, resulting in their aberrant proliferation⁷. Chemicals from plants are a major source of anticancer drugs and have few side effects. Medicinal plants have been regarded as an alternative strategy for decades because of their chemoprotective and anticancer properties⁸. In the traditional Indian system of medicine, it is one of the most common herbs. Its leaves, fruits, and dried petals are used to treat many different illnesses, including hepatitis, fever, and rheumatism¹⁰. Glycosides, alkaloids, flavonoids, saponins, carbohydrates, coumarins, and phytosterols are some of the most frequent phytochemicals that are said to be responsible for their biological actions. Solanine, solamargine, uttroside B, and a few other bioactive compounds. The main goal of this study was to do early phytochemical screening and test the anti-mycobacterial activity against mc2155. This constitutes a first phase for our forthcoming research intended to clarify the anti-mycobacterial efficacy in pathogenic strains of Mtb^{11,12}. This study is part of a larger research project aimed at creating herbal medicines that may be used on their own or in addition to anti-tubercular therapy, with clear mechanisms of action and herb-drug synergy. The research delineates a phytochemical characterization of the hydro-methanolic extract derived

from *Solanum virginianum* (subsequently referred to as SVE) via HPTLC and UHPLC methodologies. The mycobacterial strain mc2155 exhibited decreased growth and viability upon treatment with ¹³. Mycobacteria have many ways to stay alive in the demanding environment of the host. The SVE treatment made mc2155 even more tolerant of varied unfavorable conditions. Different in vitro stress tests were done to put the bacilli under oxidative, hypoxic, and nutritional stress. High-resolution scanning electron microscopy (SEM) showed the deformed and abnormal ultrastructure in mc2155 when it was treated with SVE ¹⁴. The EtBr permeabilization experiment confirmed the results, demonstrating that SVE causes damage to the cell wall in mc2155. The chloroform-mediated lipid extraction process shown on the TLC plate is a traditional metabolomic method that showed a dose-dependent drop in the total lipids taken from SVE-treated mc2155. The main goal, though, is to find out how SVE could be used as an adjuvant for tuberculosis medications ¹⁵. The main goal, though, is to find out how SVE could be used as an adjuvant for tuberculosis medications. The work employed the UPLC/QToF-MS-based targeted metabolomic method to quantify and evaluate the intracellular bioavailability of INH inside the intricate biological matrix of mc2155. The combinatorial treatment also showed that mc2155 is more likely to respond to INH when SVE is present ¹⁶. The study also looked at how well INH and SVE worked together in THP-1 cells that have mc2155. Finally, it was found that SVE protects the liver from damage caused by INH and RIF. Our discovery lays the groundwork for future studies on the anti-mycobacterial properties of SVE against pathogenic *Mtb* strains (Graphical Abstract). The cumulative results indicate that SVE may serve as a formidable contender for adjunctive anti-tubercular therapy, presenting a well delineated mechanism of action. It shows that it can make INH more bioavailable, help get rid of mycobacteria when used with INH, and protect the liver ¹⁷. as shown in fig.1

Fig 1 – bioavailability of INH



This hepatoprotective effect tackles a crucial failure mechanism in conventional tuberculosis treatments, potentially enhancing patient compliance and treatment results¹⁸. The aggregated data indicates that SVE functions not only as a direct anti-mycobacterial agent but also as a multi-modal adjunct that improves the effectiveness of current medications while reducing their toxic side effects¹⁹. Subsequent research will concentrate on corroborating these results in virulent *Mycobacterium tuberculosis* strains (e.g., H37Rv) to verify the translatability of the mc²155 findings²⁰. Additionally, identifying the precise bioactive chemicals in SVE that are responsible for the reported actions is a top objective, going beyond just characterizing the crude extract²¹. Testing molecules like solamargine or uttroside B alone and with first-line medicines could lead to the discovery of powerful novel chemicals^{22–25}. The decrease in total lipid content in SVE-treated mycobacteria indicates a possible mechanism that disrupts lipid metabolism, which is essential for the virulence and persistence of Mtb^{26,27}. Focusing on this pathway could be a new technique to cut down on how long it takes to treat tuberculosis^{28–31}.

2. Review of Literature

2.1(Carl Linnaeus et al., (1753) In-silico: Studies done in-silico Using RNA sequencing technology to analyse the transcriptome of whole fruit from *Solanum virginianum* produced 18.19 million high-quality reads and put together 60,487 coding sequences. Of these, 196 were annotated CDSs that coded for 121 enzymes that are involved in making vitamin B complex, such as thiamine, riboflavin, vitamin B6, pantothenate, biotin, and folate. We might potentially use transcriptome analysis to predict antimicrobial peptides from *S. virginianum* in silico. Molecular docking studies examined the anti-cancer efficacy of nine phytochemicals derived from leaf aqueous extracts against particular apoptotic target proteins, illustrating that ritterazine A showed very high binding with a binding energy of more than -9 kcal/mol for all of the target proteins that were tested³².

2.2 (Prashith Kekuda et al. (2017). Callus Culture: Natural products, medicines, and chemical analysis. We used the maceration procedure with methanol to get shade-dried entire plant material to test its antibacterial, antiradical, and insecticidal properties. We used hydroalcoholic extraction to test the leaves' pain-relieving and fever-reducing properties. Murashige and Skoog (MS) medium with different amounts and mixes of plant hormones such TDZ, BAP, NAA, and TDZ+NAA was used to grow leaf explants for callus induction and biomass formation.

2.3(HR-LCMS/MS et al., (2020) Analysis of Phytochemicals: analysis of aqueous leaf extract disclosed the existence of over 30 phytochemicals, with nine chosen compounds identified for their capacity to bind to apoptotic target proteins²⁴. HPLC analysis of callus culture extracts indicated that a treatment of 10 mg/L TDZ resulted in the highest accumulation of coumarin, with scopoletin measuring 4.34 ± 0.20 mg/g DW and esculetin measuring 0.87 ± 0.040 mg/g DW. Conversely, a treatment of 5 mg/L TDZ produced the greatest accumulations of caffeic acid at 0.56 ± 0.021 mg/g DW and methyl caffeate at 18.62 ± 0.60 mg/g DW. Total phenolic content in leaves reached 585.15 mg GAE/100 g fresh weight, while fruit phenolics content was 105.02 mg GAE/100 g fresh weight. The amount of carotenoids in fruits was 0.024 mg/g of fresh weight.

2.4 (Abhishek Kumar Sen et al., (2008), Biological Assessment: In vitro studies: Cytotoxicity tests on MCF-7 breast cancer cell lines showed that leaf aqueous extracts were very effective, with an IC₅₀ concentration of 10.4 ± 1.13 µg/mL after 72 hours of incubation. Leaf extracts were also better than fruit extracts at stopping cell migration and colony formation. Using agar well diffusion and poisoned food techniques to test for antimicrobial activity showed that all tested bacteria were inhibited, with Gram-positive bacteria being more sensitive than Gram-negative bacteria. There was also strong antifungal activity against *Curvularia* sp. and *Alternaria* sp. The DPPH and ABTS free radical scavenging tests showed that the antiradical activity was dose-dependent, with IC₅₀ values of 45.66 µg/mL and 7.37 µg/mL, respectively ³².

2.5 (Ravishankar M. R. et al., (2011) Antioxidant Activities: The IC₅₀ values for DPPH radical scavenging in fruits were 27.90 mg/mL. Callus culture extracts showed that they might be used in cosmetics since they had antioxidant, anti-inflammatory, and anti-aging effects. Insecticidal Activity: The larvicidal test against 2nd and 3rd instar larvae of *Aedes aegypti* exhibited activity that depended on the concentration, with an LC₅₀ value of 0.82 mg/mL against 2nd instar larvae. In vivo studies ³³.

2.6 (P. Bhat, S. G. Ghane, H. V. Hegde & S. Jayagoudaret, et al., (2023). Respiratory disorders. Hydroalcoholic leaf extract's pain-relieving and fever-reducing effects were tested on rats at two doses of 100 mg/kg and 200 mg/kg body weight. The yeast-induced pyrexia and tail immersion procedures showed that the extract had strong therapeutic benefits ³⁴.

2.7 (Pradeep Meena & Surbhi Jangir et al., 2023) Traditional and Ethnobotanical Uses 2009: *Solanum virginianum* (also known as *Solanum xanthocarpum*, also known as Kantakari) has a long history in traditional medicine, especially in Ayurveda. It is a classic member of the "Dashamoola" (ten roots) group of herbs. Classical texts like the Charaka Samhita talk about how it was used in "Kantkari Ghrita" to treat coughs, colds, asthma, fevers, and heart disease. Traditionally, breastfeeding women are given tonics such as Dasmul Asava" and "Dashmularishta." The plant is used in many different local healing systems in its native range from Northern Africa to ³⁵.

2.8 (Partha Sanjana Jurashe & Pritesh Ranjan Dash et,al (2019): In the Indian subcontinent and Bangladesh, the fruit decoction is used to lower fever and ease joint pain, while fresh leaf juice or poultices are used to promote wound healing on cuts, burns, and ulcerated skin. People in Nepal's tribal groups use the root decoction to treat bladder infections and as a diuretic ³⁶.

2.9 Adaikala Raj & Kathiresan Sivakumar et,al (2019): An ethnobotanical study conducted in 2019 in the Nilgiri Biosphere Reserve (India) validated its exceptional faithfulness level of 100% and its utilization by tribal communities for gastrointestinal disorders, respiratory issues, and dental pain, with leaves and fruits being the predominant utilized components ³⁷.

2.10(Hai-Xue Kuang & Bing-You Yang et al., (2022): Diversity of Phytochemicals. *S. virginianum* has a lot of different phytochemicals that can help with health. Some of the most important bioactive compounds are steroidal alkaloids and saponins, which are found in different parts of the plant. These include solasodine, solanidine, solamargine, solasonine, and

tomatidine. In 2022, new research found eight new steroidal saponins, including the first-ever 16,26-epoxy-furostanol saponin from *S. xanthocarpum* and six new unusual cholestane saponins ³⁸.

2.11(H. V. Hegde & S. Jayagoudar et,al (2023) The plant has a lot of phenolic acids, such as chlorogenic acid, caffeic acid, and vanillic acid, as well as flavonoids, such as quercetin and kaempferol. Phytochemical screening also shows that there are terpenoids such lupeol and stigmasterol, as well as carbohydrates and proteins ³⁹.

2.12. More A., & Kharat A.et,al (2018) Pharmacological Activities 2018: Animal models have shown that leaves and fruits traditionally used for inflammatory illnesses and rheumatism have anti-inflammatory effects. In vitro tests like DPPH and superoxide radical scavenging showed that the antioxidant activity was dose-dependent. Alcoholic extracts had more polyphenols and antioxidant activities than water extracts ⁴⁰.

2.13(Surbhi Jangir et al., (2023) Plant extracts: made from leaves that have been used in poultices for cuts and ulcers show antimicrobial activity. Whole plant extracts have been shown to work as an anti-asthmatic agent in human trials, and they have been used traditionally to treat cough, asthma, and bronchitis. The antidiabetic properties are ascribed to leaf juice and fruits that are routinely employed in diabetes care. Hepatoprotective action shows that it can protect the liver in experiments. Nephroprotective and antiurolithiatic activities show that they protect the kidneys and can be utilised to treat kidney problems and urine infections. Steroidal saponins and alkaloids have anticancer and cytotoxic properties.

2.14 (Saumya Maurya et al., (2025): They are cytotoxic against a number of cancer cell lines, such as breast, colorectal, and prostate cancer. Using alcoholic seed extracts in clastogenic assays, antimutagenic activity had big effects. We saw CNS activity when we used open field and hole board tests on animal models and found that methanolic extract inhibited locomotion in a dose-dependent way. This suggests that it may have CNS depressive effect. It kills insect larvae by killing them.

3. AIM AND OBJECTIVE

To create, synthesize, and characterize a new group of benzoyl hydrazone steroid derivatives and test their potential against tuberculosis using in silico molecular docking studies against the *M. tuberculosis* enoyl-ACP reductase (InhA) enzyme.

OBJECTIVE

- To create a series of new benzoyl hydrazone steroid derivatives (MK-1 to MK-12) using a multi-step process that includes Vilsmeier-Haack formylation, Schiff base formation, and cyclisation process
- To characterize the synthesized chemicals by analytical methods, including melting point measurement, thin-layer chromatography (TLC), Fourier-transform infrared spectroscopy (FTIR), and mass spectrometry (MS).

- To use AutoDock tools to run molecular docking simulations of the synthesized chemicals against the InhA enzyme (PDB ID: 5JFO) to figure out how strongly they will bind and how they will interact.
- To use SwissADME software to figure out how drug-like the synthesized chemicals will behave in the body (ADME).
- To use ProTox-II to do an in silico toxicity profile of the synthesised compounds and look at things like how toxic they are to the liver, how likely they are to cause mutations, and how likely they are to cause cancer

4. Materials and methods

4.1 Plant Material and Extraction

The aerial parts of *Solanum virginianum* L. (syn. *Solanum xanthocarpum* Schrad.) were procured from the medicinal plant garden of the SRM Modinagar College of Pharmacy, Uttar Pradesh, INDIA. Taxonomic analysis was performed by comparing the specimen with the voat the Department of Botany, SRM Institute of Science and Technology (SRMIST), Ghaziabad, Uttar Pradesh, where its identity was confirmed. The collected plant material (250 g) was thoroughly washed with distilled water to remove adherent impurities, shade-dried at room temperature, and coarsely pulverized using a mechanical grinder. The powdered material was subjected to hot continuous extraction using 2 L of a hydroethanolic solvent system (distilled water: ethanol, 1:1 v/v) at 60–65°C for 2 hours under reflux conditions 18. The resultant extract was filtered first through a muslin cloth and then through a 10-µm filter cloth. To ensure exhaustive extraction, the marc (residual plant material) was re-extracted twice under identical conditions

4.2 Quality Control

4.2 Thin Layer Chromatography

To activate precoated TLC plates, they were run in methanol and then dried in an oven for 5 minutes to get rid of any impurities on the plate. We made samples for use as spots on the TLC plates by dissolving 30 mg of plant extract in the right solvents for each extract in water and ethanol, respectively. After testing different solvent systems by trial and error, TLC showed that the targeted compounds were present in a certain solvent system 21 In this way, a mobile phase made up of Butanol, ethyl acetate and 10% acetic acid was made in the ratio of 5:3.5:1.5 for the development of TLC fingerprints The prepared mobile phase was put in a clean twin-trough TLC chamber that had a filter paper on one of the walls to help it soak in better. It was then covered and left alone for 45 minutes. The prepared samples were put on the pre-activated TLC plates and let to develop in the pre-saturated chromatographic chamber. The plate was left to develop until the solvent front reached 80%. Then, the plate was dried Standard bio-markers were not discernible in white light, 254 nm, and 366 nm (UV inactive).The resulting plates were immersed in a freshly formulated anisaldehyde sulphuric acid reagent (10 mL glacial acetic acid + 5 mL H₂SO₄, combined with 85 mL methanol and 0.5 mL anisaldehyde, and thoroughly mixed), then dried in an oven for 5 minutes until bands became visible.

4.3 Qualitative analysis of Phytochemicals

Preliminary qualitative phytochemical screening was done for steroids (Salkowski test), terpenoids (Salkowski test), alkaloids (Wagner's test), flavonoids (alkaline reagent test, sulphuric acid test, and lead acetate test), tannins (lead acetate test and Braymer's test), glycosides (Keller-Kiliani test), and carbohydrates (Benedict's, Fehling's, and Molisch's tests) using standard protocols. Proteins and amino acids were analyzed using the Xanthoproteic Test and Ninhydrin Test. Coumarins were qualitatively determined using 10% NaOH, while saponin presence was confirmed by the frothing test.

Qualitative analysis	Phytochemicals
Steroids (Salkowski test)	Present
Saponins: (Salkowski test)	present
Alkaloids (Wagner's test)	present
Flavonoids (alkaline reagent test)	present
Carbohydrates (Benedict test)	Absent
(Cardiac) Glycosides	present
Ninhydrin Test	Absent

4.4 Salkowski Test for Steroids: In a test tube that is fully dry, mix the sample (such plant extract from *Solanum nigrum*, 1 ML) with chloroform (1 ML). Add an equal amount of concentrated sulphuric acid 1 ML to the tube very carefully. This will make two separate layers. Gently shake the mixture and look for any changes in color. Positive Result (Steroids Present) The top layer of chloroform changes from blue to red to violet. The lower acid layer becomes yellow to green and glows green.

4.5 Test for Alkaloids: 50 mg of plant material were taken and added 2 ml Methanol and then filtered. After that, 2 ml of the filtrate was taken and mixed with 1% HCL and 1 ml of steam. Wagner's reagent produced a brown precipitate, indicating the presence of alkaloids.

4.6 Test for Saponins: Approximately 0.5 ml filtered were taken and added 5 ml distilled water. Frothing persistence indicates the presence of saponins.

4.7 Test for Flavonoids: 100 mg plant material were taken and added 10 ml Ethanol, then filtered. After that 2 ml filtrates were taken and added conc HCL and magnesium ribbon .Pink, Tomato, Red colour indicate the presence of flavonoids and glycosides.

4.8 Tests for Carbohydrates: For detection of carbohydrates, 0.5 ml of all three extracts were treated with 0.5 ml of Benedict's reagent. Solutions were heated for 2 minutes on a water bath. Reddish-brown precipitate indicates the presence of carbohydrate.

4.9 Tests for (Cardiac) Glycosides; For cardiac glycoside detection, 2 ml of all three extract solutions were shaken with 2 ml of glacial acetic acid, then a few drops of concentrated sulphuric acid (H₂SO₄) and iron trichloride (FeCl₃). Brown ring formation indicated the presence of Cardiac glycosides

5. EXPERIMENTAL

We bought all chemicals and reagents (analytical grade) from Sigma-Aldrich and used them immediately. We used a Fisher-Johns apparatus to find the melting points, and they were corrected. We used coated silica gel 60 F254 plates to do the thin-layer chromatography and keep track of how the reaction was going. A 630 FTIR spectrometer (Agilent MicroLab model) was used to measure the infrared (IR) spectra using KBr in the range of 4000-400 cm⁻¹.

5.1 General procedure for synthesis of substituted 10,14,16,20-tetramethyl-22-azahexacyclo[12.10.0.0^{2,11}.0^{5,10}.0^{15,23}.0^{17,22}]tetracos-4-en-7-ol

While stirring, DMF was added drop by drop to the solution of substituted N-amino benzenes. The flask with the drying tube was then cooled to 0°C. Then, 20.2 mL of phosphorus oxychloride was added, which was 0.350 mol. The solution was held under reflux for 8 hours after 5 minutes. After that, the reaction mixture was combined with 300 mL of cold water and stirred for 40 minutes at 0–10 °C. This made substituted (3β)-3-hydroxy-22,26-iminocholest-5-ene-26-carbaldehyde form as a yellow precipitate (**Scheme-I**). The product was filtered, rinsed with distilled water, and recrystallized from ethyl acetate.

5.2 Synthesis of substituted (3β)-3-hydroxy-22,26-iminocholest-5-ene-26-carbaldehyde

In the next step, a combination of substituted (1.9 g, 0.01 mol) and NaOH (25 mL, 2 ML) and 40 mL ethanol was heated under reflux for 1 h. After the reaction, the liquid was cooled to room temperature and poured over crushed ice. This made a yellow solid of substituted Scheme-I. The solid was filtered, washed with water, and dried. The unrefined product was recrystallised from aqueous acetic acid to yield the pure component.

5.3 Synthesis of Schiff base substituted (22R,25R)-Spirosol-5-en-3β-ylidene pyridine-4-carbohydrazide (intermediate): base substituted (22R,25R)-Spirosol-5-en-3β-ylidene pyridine-4-carbohydrazide (0.12 mol) (2a-2l) in DMSO (15 mL), isoniazid (0.10 mol) in ethanol (20 mL) and glacial acetic acid (0.30 mL) was refluxed for 7-8 h. After that, the reaction mixture was left to cool to ambient temperature, which caused a solid precipitate to develop. We filtered the precipitate, washed it with water, and then recrystallised it from methanol to make the Schiff base intermediate (22R,25R)-spirosol-5-en-3β-ol 26-O-[(benzoyl)hydrazine].

7. Computational studies

Molecular docking studies: Molecular docking studies were performed to evaluate binding interactions of synthesized compounds with the M. tuberculosis Enoyl-ACP reductase (InhA) enzyme. We got the crystal structure of InhA (PDB ID, e.g., 5JFO) from the RCSB Protein Data Bank. The three-dimensional crystal structure of the enzyme was obtained from the RCSB Protein Data Bank and processed to exclude any crystallographic water molecules and co-crystallized ligands. After that, we used AutoDock Tools (v1.5.7) to add polar hydrogens and Kollman charges. Structures of ligands. We ran docking simulations with AutoDock Vina, with

the active site centered around the crystallized ligand's coordinates. The grid box was centred on the entire catalytic site⁴⁵.

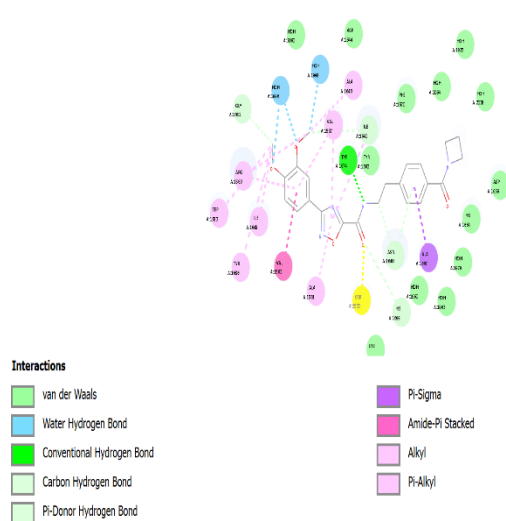


Fig. – 2 Molecular docking studies

8. RESULTS AND DISCUSSION:

The novel solandine-benzoyl hydrazone steroid derivatives (MK-1 to MK-2) were 10,14,16,20-tetra 22azahexacyclo[12.10.0.0^{2,11}.0^{4,5}.0^{15,23}.0^{17,22}]tetracos-4-en-7-ol(1a-1), obtained via Vilsmeier-Haack formylation of substituted N-phenyl acetamides using DMF/POCl₃, followed by alkaline hydrolysis to obtain the corresponding (3 β)-3-hydroxy-22,26-iminocholest-5-ene-26-carbaldehyde (2a-1). After that, isoniazid (INH) was added to DMSO/EtOH, which led to the formation of Schiff bases (3a-1). These were subsequently cyclized with benzoyl hydrazone steroid derivative to make the final derivatives (MK-1 to MK-12). All of the compounds that were made had the typical IR absorptions for OH/NH, C=O, C=N/C=C, and C-S. Their ¹H/¹³C IR spectra demonstrated that they were benzoyl hydrazone steroid derivative moieties. The MS (M⁺ peaks) also matched the expected molecular formulae, which showed that the synthesis was successful and the structure was stable.

ADME prediction:

Swiss ADME were used to guess the ADMET properties of the synthesised compounds (MK-1 to MK-12). All of the substances followed Lipinski's rule of five, which means they had good oral bioavailability (Table 3). But molecule 2 broke the Veber rule once because its topological polar surface area (TPSA) was more than 140 Å². The molecular weight (MW) of all the compounds was less than the best level of 500 Da, which means they may easily pass through membranes. The average logP values ranged from roughly 1.8 to 3.5, which means that the compound had a good combination of hydrophilic and lipophilic qualities that made it easy to absorb via the mouth. It was thought that most of the chemicals would be absorbed well by the GI tract. Molecule 2 was the only one that was expected to have poor GI absorption.

***In silico* pharmacological toxicity profiling of hybrid derivatives**

The synthesized hybrids MK-1 to MK-12, together with other analogues, exhibited a consistent profile across numerous endpoints in pharmacological toxicity experiments utilising ProTox-3.0 predictions⁴⁹. Fig. 3 illustrates that organ toxicities were primarily active for hepatotoxicity (probabilities ranging from 0.51 to 0.69), neurotoxicity (0.57 to 0.87), nephrotoxicity (0.50 to 0.90), respiratory toxicity (0.67 to 0.98), and clinical toxicity (0.53 to 0.67), whereas cardiotoxicity remained inactive (0.72 to 0.82).

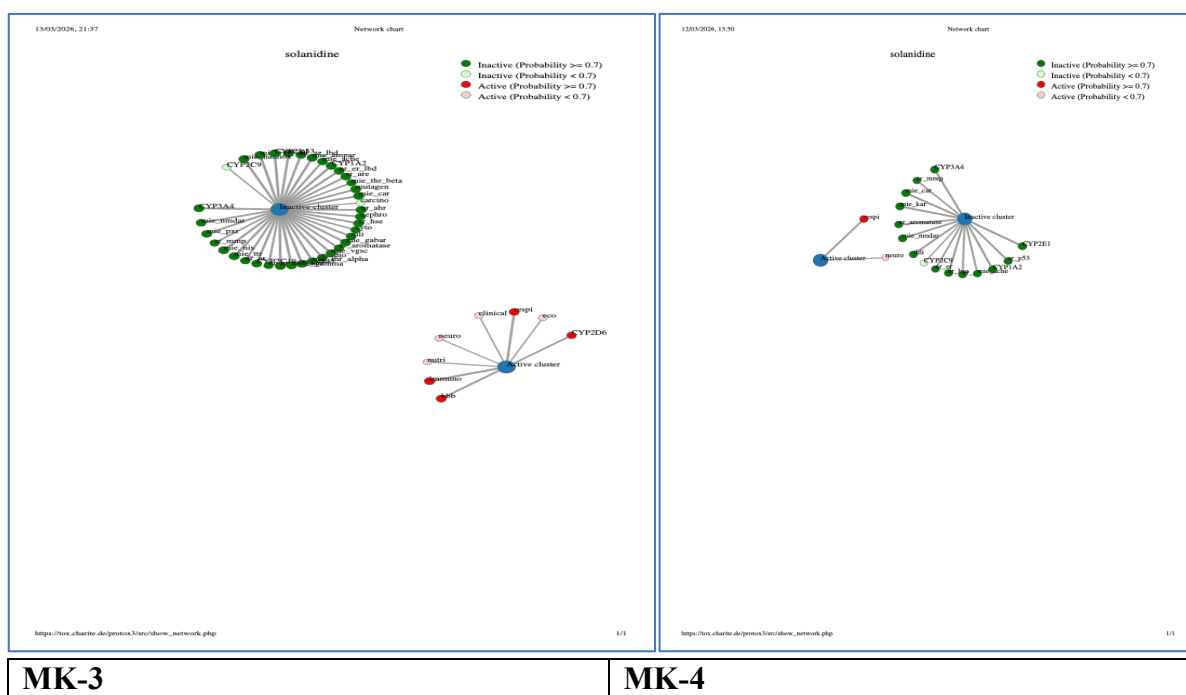
TOXICITY AND CYP450 INHIBITION PROFILE OF TEST COMPOUNDS

Fig 3 Predicted toxicological profile of Benzoyl hydrazone steroid derivative via radar chart (MK-1, MK-5)

9. Conclusion

We were able to make and characterise new benzoyl hydrazone steroid compounds (MK-1 to MK-12). These compounds exhibited enhanced binding affinities (-9.3 to -7.8 kcal/mol) for InhA in comparison to isoniazid (-7.5 kcal/mol). ADMET profiling verified drug-like characteristics, with the majority of compounds meeting Lipinski's criteria; nonetheless, toxicity assessments suggested possible hepatotoxicity and neurotoxicity issues. The study proves that *Solanum virginianum* has been used in traditional medicine and shows that it could be used as an additional treatment for tuberculosis. The combination benefits of better effectiveness and liver protection address major problems with current tuberculosis treatments. To use these results in real life, they need to be tested more in pathogenic strains and in vivo models.

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