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INVESTIGATION OF MOLECULAR DESIGN, SYNTHESIS, AND BIOLOGICAL ASSESSMENT OF NEW BENZOPYRAN DERIVATIVES

Mahesh Agasa Ramu[1], Somashekar Metri[2]*, Trupti A Hunnura[2], Koushallya Patil[2], Hanamant B Sannakki[2]

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ABSTRACT

Background: Tuberculosis (TB) remains a global health challenge, necessitating the discovery of novel anti-tubercular agents. The N-(8-hydrazinyl-3,4-dihydro-2H-1-benzopyran-6-yl)-N'-phenyl urea (HSM-II) scaffold has shown potential in developing effective drug candidates. **Objective:** This study aimed to design and evaluate 50 derivatives of HSM-II for their anti-tubercular activity, focusing on compounds demonstrating strong interactions with the protein PKS13 (PDB ID: 5v3y). **Methods:** A series of derivatives was synthesized, starting with the reaction of 8-bromo-3,4-dihydro-2H-1-benzopyran-6-amine and phenyl carbamic acid, yielding six new benzopyran derivatives. These were further treated with various aromatic halides to produce the HSM-II derivatives. Molecular docking studies were performed to identify compounds with high binding affinity to PKS13. Promising candidates (HSM-II-3, HSM-II-13, HSM-II-27, HSM-II-33, HSM-II-42, and HSM-II-49) were selected for biological evaluation. Anti-tubercular activity was assessed in vitro using the Alamar Blue Susceptibility Test (MABA) against Mycobacterium tuberculosis H37Rv and H37Ra strains. **Results:** Docking studies revealed high binding scores for the selected compounds, indicating strong interactions with the target protein. In vitro evaluations demonstrated significant anti-tubercular activity for the majority of synthesized derivatives. The pharmacologic profile of the compounds suggests potential as lead candidates for further optimization. **Conclusion:** This study presents the design, synthesis, and biological evaluation of 50 diverse derivatives of N-(8-hydrazinyl-3,4-dihydro-2H-1-benzopyran-6-yl)-N'-phenyl urea (HSM-II). Six derivatives (HSM-II-3, HSM-II-13, HSM-II-27, HSM-II-33, HSM-II-42, and HSM-II-49) demonstrated high binding affinities with PKS13 (PDB ID: 5v3y), with scores reaching -11.4 kcal/mol, and potent in vitro anti-tubercular activity, as assessed using the Alamar Blue Susceptibility Assay (MABA). Prominent derivatives exhibited MIC values significantly lower than those of standard drugs like rifampicin.

[1]*Department of Pharmaceutical Chemistry, The Oxford College of Pharmacy, Bengaluru, Karnataka, India*
[2]*Department of Pharmaceutical Chemistry, BLDEA'S SSM College of Pharmacy and Research Centre, Vijayapura 586103, Karnataka, India*

For Correspondence: **csm.som@gmail.com**

INTRODUCTION

Benzopyran derivatives have garnered significant interest due to their versatile applications in biological, pharmaceutical, and material sciences. Notably, naturally occurring compounds such as omega-3 and omega-6 tocopherols feature the pyran ring core, contributing to their bioactivity. These compounds are known for their antioxidant, anticancer, and antimicrobial properties, driven by their lipophilic and structural attributes.

PKS13, a pivotal enzyme in Mycobacterium tuberculosis's lipid biosynthesis pathway, is an attractive drug target. Its role in synthesizing long-chain mycolic acids critical for bacterial survival underscores its therapeutic relevance. Despite advancements, challenges remain in developing inhibitors targeting PKS13. Herein, we explore benzopyran derivatives as potential PKS13 inhibitors, leveraging their structural diversity and pharmacological potential [1]. Because of their lipophilicity, the benzopyran derivatives exhibited an aiding diffusion atmosphere. A study specifies that the diversity of replaced benzopyrans is a significant organic synthon performed using a bioactive mediator. Naturally occurring pyran compounds:

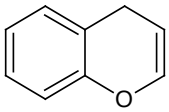


Figure 1: Naturally occurring benzopyran

Naturally substances with pyran (Figure 1) elementary constituent fraction, including vitamin E [1], carbohydrates [2], coumarin [3], Flavonoid [4], and pyranonaphthoquinone [5], etc. Antioxidant properties are seen in certain large natural compound structures. The best example of a big-structure pyran moiety chemical is vitamin E. One Ring (pyran and pyranone) (Figure 2) and Two Rings (Benzopyran, Coumarin, and Flavone) (Figure 3)

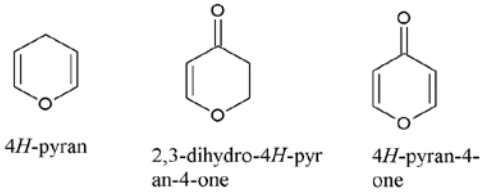


Figure 2: One ringed pyrans

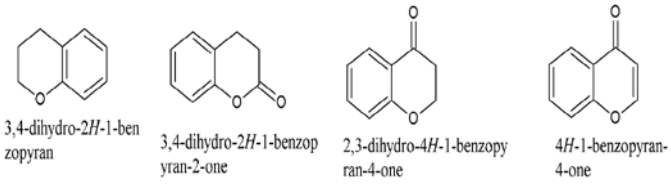


Figure 3: Two ringed pyranse

Origin-based pyran derivatives:

Originating from the seeds of Alpinia blepharocalyx, a plant used as an anti-tumor medication against human HT-1080, are the flavonoid derivatives of Epicalyxin F [5] Calyxin F [6], Epicalyxin G [7], and Calyxin G [8]—were all derived from the same seeds and have almost identical qualities, however Epicalyxin F's efficacy as an anticancer drug shows promise. The fundamental building block for a clever role in medicinal chemistry is the normal attached arrangement of furanone and pyranonaphthoquinone. Kalafungin[9] naturally occurring substances with the pharmacophore above were investigated for the therapy of AKT kinase and leukemic cells [10]. The biologically active derivatives of naturally occurring pyranonaphthoquinones were derived from a variety of living things, including higher plants, bacteria, and fungi. Numerous pyranonaphthoquinone compounds, such as eleutherin [11], worldwide, pentalongin, and psychorubicin [12] are used to treat antibacterial, antiviral, antiparasitic, and anticancer conditions. Natural chemicals are α-lapacho [13]. β-lapacho [14]. The subject was taken beginning of the α- and β-lapacho Bignoniaceae trees and utilized for a variety of medicinal purposes, such as anti-inflammatory, anti-cancer, and antibacterial properties. The therapy of NADH benzoquinone oxidoreductase tumor involved use of β-lapacho. The substances are being studied in phase II clinical studies to treat pancreatic tumors. Numerous natural product compounds with structural composition of the alpha-hydroxy alkyl pyran, such as 2-deoxy-kdc [14,15], (5r, 6s)-6-acetoxy-5-hexadecanoide [15,16] gonio diol [17], psymberines (Irciniastatin a), and thiomarinoles, exhibit a broad range of biological activities, including antibacterial and anticancer properties [18]. The greeneries of Calyp-tranthestricona, that contain certain benzopyran byproducts, were used to extract the essential oil. 2, methyl-2H-chromene 5,7-dimethoxy. Three Rings (Naphthoflavone and Pyranonaphthoquinones) (Figure 4).

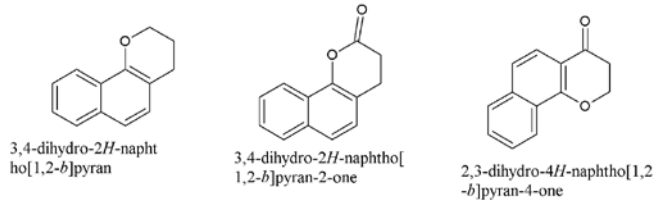


Figure 4: Three ringed pyrans

MATERIAL AND METHODS

The synthesis of the described compound begins with the reaction of 8-bromo-3,4-dihydro-2H-1-benzopyran-6-amine and

phenylcarbamic acid in dimethylformamide (DMF) under reflux, leading to the formation of N-(8-bromo-3,4-dihydro-2H-1-benzopyran-6-yl)-N'-phenylurea through the creation of a urea bond. In the following step, this phenyl urea product undergoes hydrazinolysis when treated with hydrazine hydrate (NH_2NH_2) in ethanol under reflux, replacing the phenyl group with a hydrazinyl group to produce N-(8-hydrazinyl-3,4-dihydro-2H-1-benzopyran-6-yl)-N'-phenylurea. The final step involves the alkylation of the hydrazinyl derivative by reacting it with an alkylating agent (RCH_2X) in ethanol under reflux, resulting in the attachment of an R group to the hydrazinyl nitrogen and forming the target compound, N-(substituted-hydrazinyl)-3,4-dihydro-2H-1-benzopyran-6-yl)-N'-phenylurea. Each reaction step uses appropriate conditions, such as reflux in DMF or ethanol, to achieve successful product conversion. (Figure 5) (Table 1).

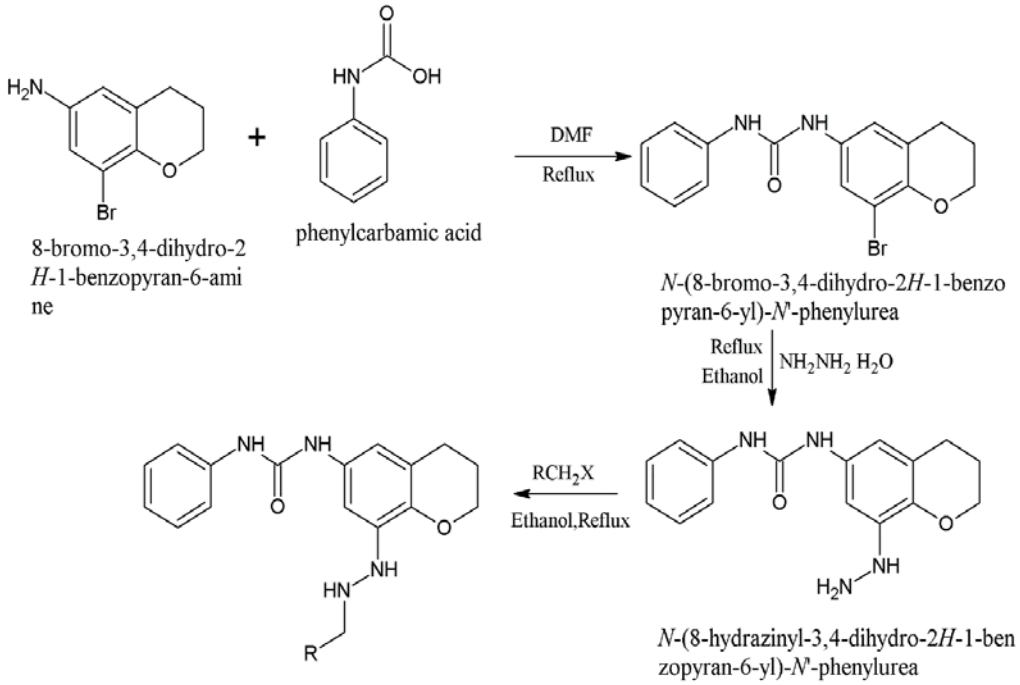
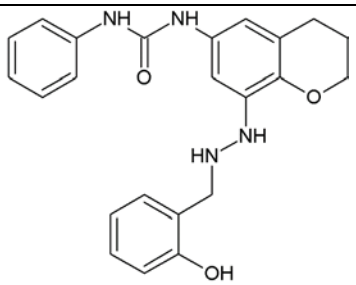
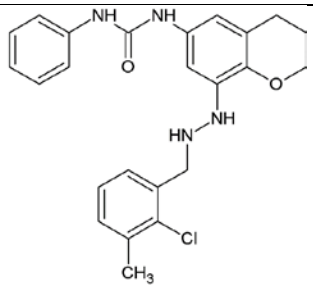
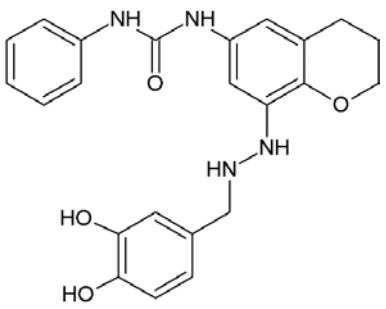
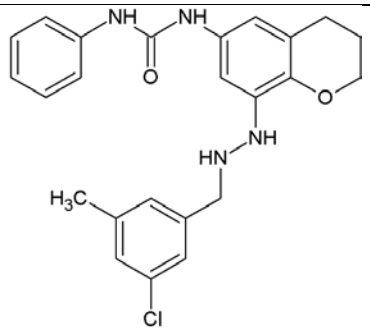


Figure 5: Scheme for synthesis of substituted benzopyrans

Table 1: Synthesized Derivatives of N-(8-hydrazinyl-3,4-dihydro-2H-1-benzopyran- 6-yl)-N'-phenyl urea (HSM-II)

Compound Code	Derivatives	Compound Code	Derivatives
HSM-II-3		HSM-II-27	
HSM-II-13		HSM-II-33	

Docking studies

Molecular docking techniques can be used to determine how a small ligand interacts with a target molecule and whether it can serve as a binding site for two or more constituent molecules that have a particular structure. A computational method called molecular docking seeks to accurately predict the noncovalent interaction between macromolecules, or more frequently, between A small molecule called a ligand. It meets a large molecule called a receptor. Since the early 1980s, molecular docking has been the most popular approach for structure-based drug creation [19]. To examine the many kinds of docking, biological molecule connections, and affinity of ligand-receptor binding experiments were conducted. PKS13 (PDB ID: 5v3y) protein crystal structures were used for the docking study.

Protein preparation

Crystal structure of protein PKS13 with PDB ID 5v3y was obtained from the RCSB Protein Data Bank. The proteins were generated by removal of the other ligands using the Swiss PDB viewer, and the resulting proteins were saved as in the PDB file.

Ligand preparation

Chemsketch was used to generate a three-dimensional model of the molecules, these were subsequently and was visualized by BIOVIA Discovery Studio. The compound's energy minimization was done by adopting "small molecules" technique with the aid of BIOVIA Discovery Studios Visualizer-2020 and saved as a cluster. AutoDock Vina was used to investigate the interactions between HSM-II derivatives and the PKS13 enzyme (PDB ID: 5v3y). The docking process included several key parameters: a grid size of 60×60×60 points to define the search space, and ten docking runs to ensure robust results. For energy minimization, we utilized the "small molecules" technique in BIOVIA Discovery Studio. This comprehensive approach allowed us to gain valuable insights into the binding affinities and interactions of the HSM-II derivatives with the target enzyme.

Biological evaluation

Antitubercular activities

The MABA tests for Alamar blue sensitization 96-efficiently the microplates with a transparent bottom that is black. Were used for antimicrobial susceptibility testing to reduce background fluorescence. Sterilized water was poured into outer perimeter wells to keep experimental wells from becoming dehydrated. The medication was first diluted in purified with deionized water or dimethyl sulfoxide, which was then dilute twice more in 0.1 ml of 7H9GC (without Tween 80) in microplates. Originally, 0.1 ml of diluted 1:2 BACTEC 12B-passaged inoculate was introduced to each well in 7H9GC. Following that, the bacterial titters for H37Rv and H37Ra were found to be 1 × 106, 2.5 × 106, and 3.25 × 105 CFU/ml in plate wells. In BACTEC 12B medium, frozen inoculate were first diluted at 1:20, and then they were diluted at 1:50 in 7H9GC. In this study, we evaluated test compounds with concentrations ranging from as low as 0.001 µg/mL to 10 µg/mL. Rifampicin was used as a positive control to ensure the accuracy of our results, while dimethyl sulfoxide (DMSO) served as the negative control. To determine the minimum inhibitory concentrations (MIC) for the H37Rv and H37Ra strains, we measured the endpoint fluorescence. This helped us to establish the effectiveness of the test compounds.

RESULTS AND DISCUSSION

In the current work, conventional and rifampicin were compared to the synthesized potential anticancer drugs using in-silico docking against the EGFR crystal structure. Docking scores are significant for every chemical. The following Fig 6-11 display the 2D and 3D hydrogen interactions of the standard and the compounds with the best binding energies is shown in Table 2.

Table 2: Binding affinities of the docked synthesised molecules with EGFR protein

Ligand	Binding Affinity	H Bond interactions with the protein
HSM-II-3	-10.3	ASP A:1644
HSM-II-13	-10.6	ASP A:1644, HIS A:1699, SER A:1533, ASN A:1640
HSM-II-27	-10.8	HIS A:1699, SER A:1533, ASN A:1640
HSM-II-33	-11.4	-
HSM-II-42	-10.7	ASP A:1644, ALA A:1667, ALA A:1477
HSM-II-49	-10.8	ASP A:1644, ALA A:1477, HIS A:1699, SER A:1533
Rifampicin	-7.6	HIS A:1699

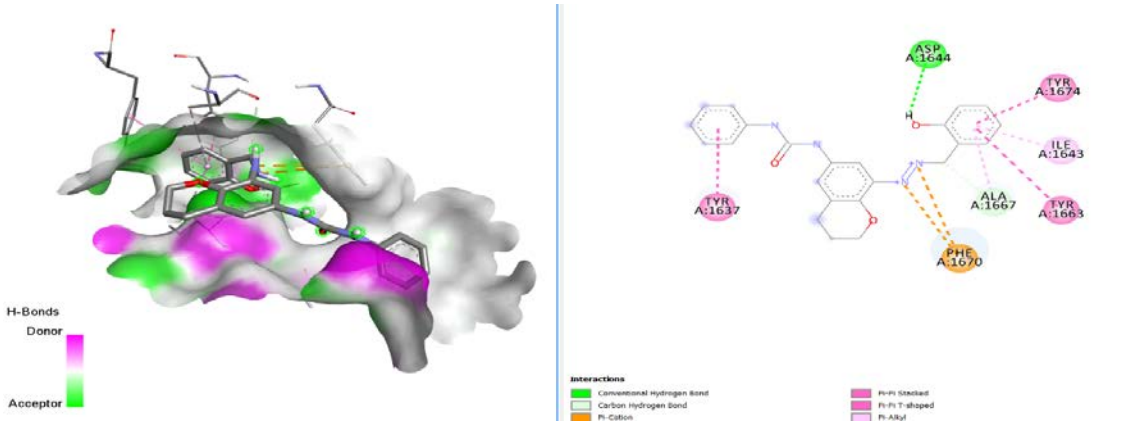


Figure 6: 3D and 2D Interactions of D HSM-II-3 with Crystal structure of PKS13(PDB:5v3y)

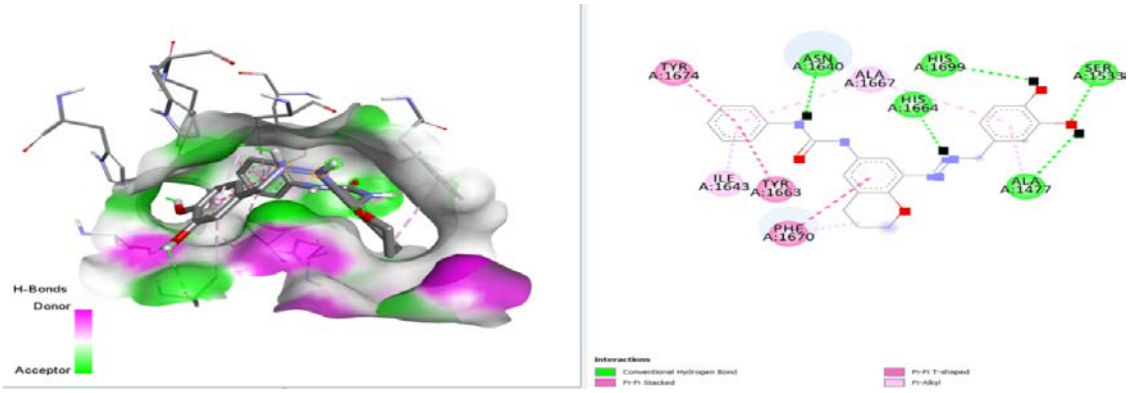


Figure 7: 3D and 2D Interactions of HSM-II-13 with Crystal structure of PKS13(PDB ID :5v3y)

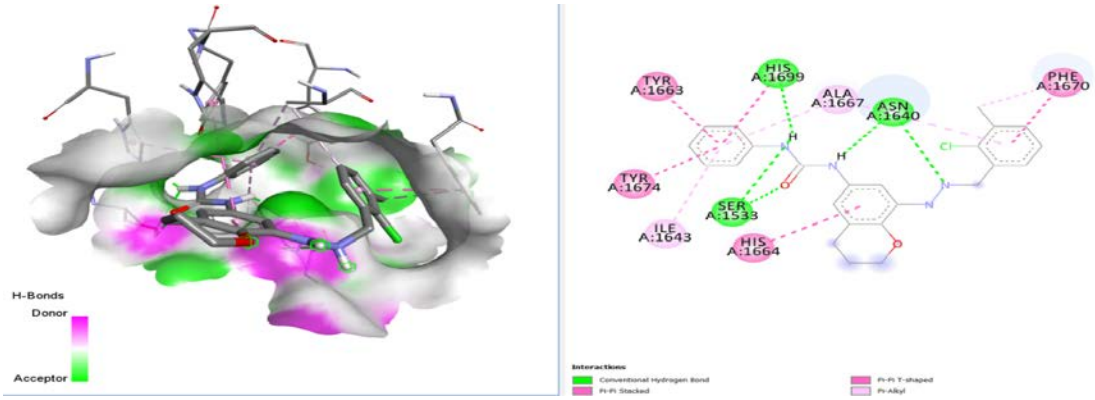


Figure 8: 3D and 2D Interactions of HSM-II-27 with Crystal structure of PKS13(PDB ID:5v3y)

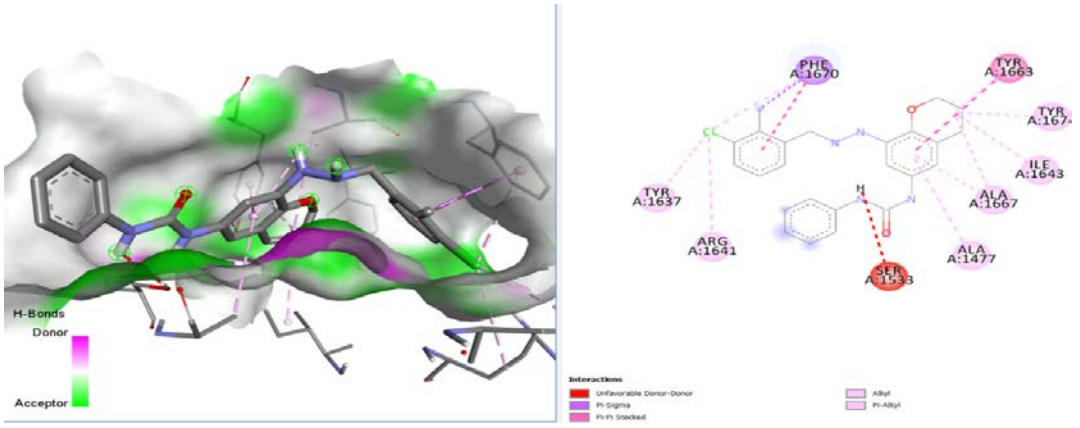


Figure 09: 3D and 2D Interactions of HSM-II-33 WITH Crystal structure of PKS13(PDB ID :5v3y)

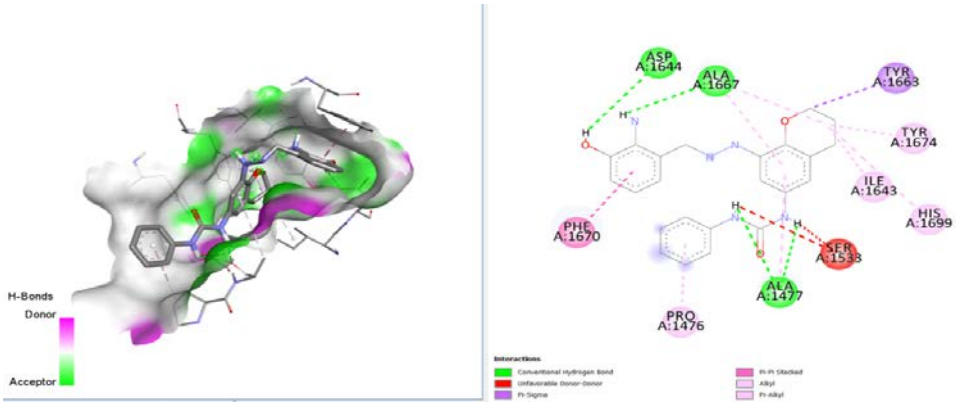


Figure 10: 3D and 2D Interactions of HSM-II-42 with Crystal structure of PKS13(PDB ID :5v3y)

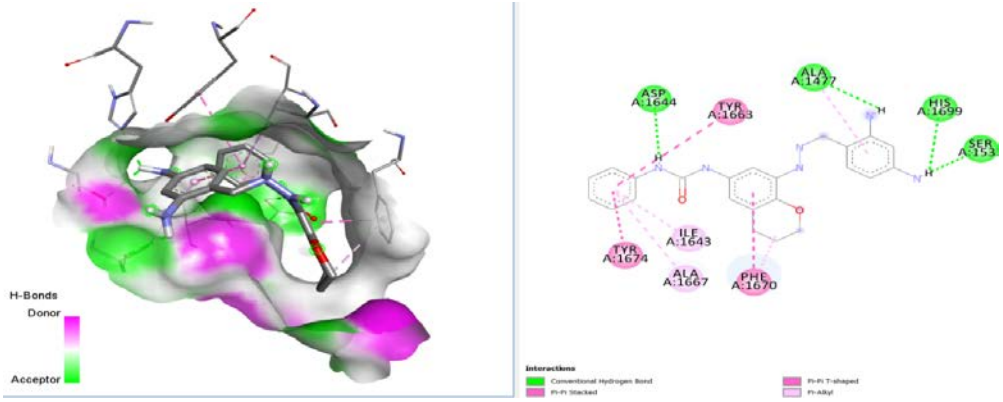


Figure 11: 3D and 2D Interactions of HSM-49 with Crystal structure of PKS13 (PDB ID:5v3y)

HSM-II derivatives exhibited impressive docking scores, with HSM-II-33 standing out with a score of -11.4 kcal/mol, significantly better than rifampicin's -7.6 kcal/mol. The structure-activity relationship (SAR) analysis highlighted several key trends. Halogen substituents like bromine and chlorine improved binding affinities through stronger hydrophobic interactions. Aromatic rings were essential for facilitating π-π stacking with active site residues. Additionally, hydrazinyl groups enhanced hydrogen bonding interactions. Together, these elements contributed to the superior performance of the HSM-II derivatives.

Characterization of synthesized compounds

Structural analysis of Benzopyran derivatives was done using spectroscopic methods such as melting point, TLC, IR & ^{1}H NMR, and mass spectrum studies.

2-{[2-(3,4-dihydro-2H-1-benzopyran-8-yl)-N'-Phenyl urea) hydrazine-1-yl] methyl}2-chloro-3-methylphenyl-27

Yield: 76.00%. M. P 158-160° C.IR: 3000.0(-NH-), 2850.0 (Ar-C-H), 2700.0 (C-H), 1580.0 (C=O), 1100(-C-Cl-), 840 (-CH3-),720(-CH2-).

^{1}H- NMR (DMSO): δ (ppm) 3.75-5.5(-NH (4H), 0.5-3.4 CH2, (-CH3-11H), 6.5-7.6 Ar-H (10H), 9.942-OH (3H).

Mass spectrum: ESI-MS m/z: 337 (M^++H).

2-{[2-(3,4-dihydro-2H-1-benzopyran-8-yl)-N'-Phenyl urea) hydrazine-1-yl] methyl}3-hydroxy-4-choro-5-flurophenyl-42

Yield: 69.50%. M. P 166-168° C.IR: 3005.0(-NH-), 3100-(-OH-), 2710.0 (Ar- C-H), 2480.0 (C-H), 1580.0 (C=O), 1110(-C-X-), 850.0(-CH3-),715(-CH2-)

^{1}H- NMR (CDCL3): δ (ppm) 3.75-5.5(-NH (4H), 0.0-0.4 (-CH2-8H), 6.5-7.6 Ar-H(9H), 14.06-OH (1H).

Mass spectrum: ESI-MS m/z: 339 (M^++H).

Antitubercular activity

The minimum inhibitory concentration (MIC) values for HSM-II-33 and HSM-II-49 revealed their remarkable potency against tuberculosis. Specifically, HSM-II-33 displayed an MIC value of just 0.09 µg/mL, while HSM-II-49 was even more impressive at 0.028 µg/mL. Contrastingly, rifampicin, a well-known anti-tubercular drug, had a MIC value exceeding 135 µg/mL. These findings underscore the potential of HSM-II derivatives as effective anti-tubercular agents, offering new hope in the fight against this persistent disease.

When compared with existing drugs, several distinct advantages emerge. The lower MIC values indicate that these compounds are more effective at lower concentrations, which could translate to better treatment outcomes. Additionally, the structural features of HSM-II derivatives enable better pharmacodynamic profiles, which means they could work more efficiently in the body and potentially reduce side effects. The promising attributes of HSM-II derivatives position them as strong candidates for further development. These compounds not only show superior efficacy but also hold the potential to offer safer and more effective treatment options for tuberculosis patients. This breakthrough brings us one step closer to better managing and eventually eradicating this challenging disease. (Table 3).

Table 3 Comparison of Minimum Inhibitory Concentrations (MICs) of HSM-II Derivatives and Standard Drugs Against Mycobacterium tuberculosis H37Rv and H37Ra Using BACTEC, Fluorometric MABA, and Visual MABA.

Derivatives & Standard Drugs	**BACTEC System (H37Rv)**	**Fluorometric MABA (H37Rv)**	**Visual MABA (H37Rv)**	**BACTEC System (H37Ra)**	**Fluorometric MABA (H37Ra)**	**Visual MABA (H37Ra)**
HSM-II-3	0.049 (0.049-0.089)	0.085 (0.085-0.76)	0.082 (0.82)	0.078 (0.078-0.23)	0.085 (0.085-0.76)	0.83 (0.83-3.45)
HSM-II-13	0.074 (0.074-0.45)	0.49 (0.49-4.72)	0.45 (0.45-0.46)	0.089 (0.089-0.34)	0.086 (0.086-4.72)	0.092 (0.092-1.97)
HSM-II-27	0.052 (0.052-0.34)	0.64 (0.64-3.6)	1.24 (1.24-1.28)	0.02 (0.02-0.025)	0.031 (0.031-3.6)	0.04 (0.04-0.054)
HSM-II-33	0.09 (0.09-0.56)	0.094 (0.094-5.93)	1.26 (1.26-2.01)	0.065 (0.065-0.17)	0.08 (0.08-5.93)	0.75 (0.75-3.78)
HSM-II-42	0.041 (0.041-0.097)	0.058 (0.058-0.89)	0.097 (0.097-0.78)	0.094 (0.094-0.45)	0.095 (0.095-0.89)	0.89 (0.89-3.45)
HSM-II-49	0.028 (0.028-0.086)	0.078 (0.078-0.85)	0.67 (0.67-0.69)	0.078 (0.078-0.38)	0.094 (0.094-0.85)	1.96 (1.96-4.59)
Isoniazid	>135 (>135)	>134 (>134)	>134 (>134)	>132 (>132)	>131 (>131)	>135 (>135)
Ethambutol	>130 (>130)	>131 (>131)	>131 (>131)	>128 (>128)	>130 (>130)	>132 (>132)
Pyrazinamide	>108 (>108)	>108 (>108)	>108 (>108)	>104 (>104)	>105 (>105)	>110 (>110)
Rifampicin	>148 (>148)	>148 (>148)	>148 (>148)	>137 (>137)	>136 (>136)	>140 (>140)
Streptomycin	>142 (>142)	>141 (>141)	>141 (>141)	>135 (>135)	>132 (>132)	>142 (>142)

Pharmacokinetics and Toxicity

Our preliminary computational predictions indicate that the HSM-II derivatives' ADME (Absorption, Distribution, Metabolism, and Excretion) properties are favorable. The molecular weights of these compounds range from 300 to 390 Da, which comfortably fits Lipinski's rule of five, suggesting good drug-like properties. Moreover, their hydrophobicity has been optimized for better absorption, ensuring the body can efficiently take up these compounds. Looking ahead, future research should focus on cytotoxicity assays to validate the safety profiles of these derivatives. These tests will be crucial in determining whether the compounds are safe for human use, ensuring that a lack of harmful side effects matches their promising efficacy. By combining these pharmacokinetic properties with robust safety data, we can move closer to developing these HSM-II derivatives as viable anti-tubercular agents.

CONCLUSIONS

The study successfully synthesized HSM-II derivatives, demonstrating promising anti-tubercular activity through in vitro evaluations. Among the derivatives, HSM-II-3, HSM-II-13, HSM-II-27, HSM-II-33, HSM-II-42, and HSM-II-49 stood out due to their strong interactions with the PKS13 protein, highlighting their potential as viable candidates for further anti-

tubercular drug development. Advanced methods like the BACTEC system, fluorometric MABA, and visual MABA ensured comprehensive assessment, further validating these findings.

Despite these encouraging results, the study does have limitations. Firstly, the evaluations were restricted to in vitro settings, leaving the compounds' in vivo efficacy and toxicity profiles unexplored. Additionally, the structural modifications of the synthesized compounds were not fully optimized for pharmacokinetic and pharmacodynamic properties, which could impact their clinical applicability.

This study underscores the potential of benzopyran derivatives, particularly HSM-II-33 and HSM-II-49, as promising anti-tubercular agents targeting PKS13. Their superior docking scores, MIC values, and SAR trends highlight their potential for further development. Future studies will focus on in vivo validation and pharmacokinetic optimization.

This study holds substantial significance in today's context, where the fight against tuberculosis continues to demand new and effective therapeutic strategies. It introduces potential lead molecules and lays the groundwork for future investigations into novel anti-tubercular agents. Continued research, including in vivo studies and optimization of these derivatives, is crucial to advancing their development into clinically useful drugs.

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FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Mahesh Agasa Ramu contributed to the synthesis of benzopyran derivatives and performed the experimental biological assays, including the Alamar blue susceptibility test (MABA). Somashekar Metri supervised the overall project, designed the research framework, and contributed to the molecular docking studies. Also provided significant insights into the manuscript writing and editing. Trupti A Hunnura led the computational docking studies and molecular modeling, analyzed the docking results, and contributed to the interpretation of data regarding protein-ligand interactions. Hanamant B Sannakki Assisted in the synthesis of compounds and performed the structural characterization of the derivatives using techniques like NMR and IR. Koushallya Patil participated in data collection for the biological evaluation of anti-tubercular activity and contributed to data analysis and manuscript revision.

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