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TO DESIGN AND ANALYZE PHARMACOPHORE MODELS OF ISONIAZID ANALOGUES TARGETING THE INHA ENZYME OF MYCOBACTERIUM TUBERCULOSIS USING COMPUTATIONAL TOOLS

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ABSTRACT

Tuberculosis (TB) continues to be a major global health concern caused by Mycobacterium tuberculosis. The InhA enzyme, a key component in the fatty acid synthesis pathway, plays a crucial role in the production of mycolic acids – essential constituents of the bacterial cell wall. Inhibition of this enzyme is an established mechanism of action for the anti-tubercular drug isoniazid.

The present study aims to design and analyze pharmacophore models of isoniazid and its analogues targeting the InhA enzyme using computational tools. Pharmacophore modeling helps identify the essential structural and chemical features responsible for biological activity, such as hydrogen bond donors, hydrogen bond acceptors, hydrophobic centers, and aromatic rings.

A series of isoniazid analogues were retrieved from chemical databases, and pharmacophore models were generated and optimized using PharmaGist and Discovery Studio Visualizer. The resulting pharmacophore hypotheses were validated based on fit scores and used for virtual screening through ZINCPharmer to identify new potential hits.

The study successfully identified key pharmacophoric features responsible for InhA inhibition and proposed several promising compounds for further docking and biological evaluation. This computational approach demonstrates the effectiveness of pharmacophore modeling in accelerating anti-tubercular drug discovery and optimizing existing leads.

INTRODUCTION

Tuberculosis (TB) is a highly contagious and life-threatening infectious disease caused by Mycobacterium tuberculosis. Despite the availability of several anti-tubercular drugs, TB remains a global health challenge due to the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. The increasing resistance to conventional drugs emphasizes the urgent need for developing new, effective, and safe therapeutic agents.

Among the first-line anti-TB drugs, isoniazid (INH) is one of the most potent and widely used agents. It exerts its bactericidal effect by inhibiting the InhA enzyme (Enoyl-acylcarrier protein reductase), which plays a crucial role in the fatty acid elongation step during the synthesis of mycolic acids, vital components of the mycobacterial cell wall. Inhibition of this enzyme disrupts the integrity of the bacterial cell wall, leading to the death of the microorganism. However, mutations in the inhA gene and other associated enzymes lead to decreased drug susceptibility, necessitating the design of novel analogues and inhibitors targeting the same enzyme. Computational approaches such as pharmacophore modeling offer a cost- effective and time-efficient method to identify the key molecular features responsible for drug-target interactions and to guide the design of new analogues.

A pharmacophore represents the spatial arrangement of essential chemical features required for optimal interactions with a biological target and for eliciting a specific biological response. It typically includes features such as hydrogen bond donors, hydrogen bond acceptors, hydrophobic regions, and aromatic rings. By analyzing known active molecules, pharmacophore models can be generated to identify critical interaction points necessary for activity.

In this study, pharmacophore modeling of isoniazid and its analogues was carried out using computational tools such as PharmaGist and Discovery Studio Visualizer. The aim was to determine the essential structural features responsible for InhA inhibition, validate the pharmacophore model, and identify new potential hits through virtual screening. This approach contributes to understanding the structure–activity relationship (SAR) of isoniazid analogues and aids in the rational design of new anti-tubercular agents

LITERATURE REVIEW

Tuberculosis (TB) remains one of the world's most deadly infectious diseases, caused by Mycobacterium tuberculosis. The treatment of TB faces several challenges such as drug resistance, long treatment duration, and drug-induced toxicity. To overcome these issues, computational drug design methods like pharmacophore modeling and molecular docking have become essential tools in identifying new and effective anti-tubercular agents.

Isoniazid (INH) is a well-established first-line anti-TB drug that specifically targets the InhA enzyme (Enoyl-acyl carrier protein reductase), an important enzyme involved in mycolic acid biosynthesis. Mycolic acids are long-chain fatty acids that form a key component of the mycobacterial cell wall, providing structural integrity and resistance to chemical damage. Inhibition of InhA leads to disruption of the cell wall synthesis and ultimately causes bacterial

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death.

Over the years, researchers have explored several isoniazid analogues and inhibitors of InhA using computational approaches. The focus has been to understand the structure– activity relationship (SAR) and identify the key pharmacophoric features that are essential for enzyme inhibition.

◆ Pharmacophore Modeling in Drug Discovery

Pharmacophore modeling has emerged as a powerful computational approach in drug design. According to Ekins et al. (2007), pharmacophore models help identify the spatial arrangement of chemical features responsible for biological activity. These models are instrumental in virtual screening, lead identification, and optimization of drug candidates.

In pharmacophore-based studies, the alignment of active molecules allows the identification of common features such as hydrogen bond donors, hydrogen bondacceptors, hydrophobic regions, and aromatic rings. These features serve as a template for designing new molecules with improved potency and selectivity.

◆ Previous Studies on InhA Enzyme Inhibitors

Rozwarski et al. (1998) revealed the crystal structure of the InhA enzyme complexed with isoniazid and NADH, highlighting the molecular mechanism of inhibition. This structural information provided a foundation for rational design of new analogues.

Anisha et al. (2019) conducted pharmacophore modeling and virtual screening of potential InhA inhibitors. Their study identified hydrogen bond donor and acceptor groups as essential features for enzyme inhibition and proposed novel molecules with improved docking scores.

Banerjee et al. (1994) also described that isoniazid requires activation by the KatG enzyme, forming an isonicotinoyl radical that binds to NADH and inhibits InhA. Mutations in KatG or InhA are associated with resistance, making direct InhA inhibitors a promising area for new drug discovery.

◆ Computational Tools in Pharmacophore Modeling

Modern computational tools such as Discovery Studio, PharmaGist, and LigandScout provide reliable platforms for pharmacophore generation, validation, and screening.

Discovery Studio Visualizer helps in visualizing molecular structures and optimizing geometry.

PharmaGist automatically detects pharmacophoric features from a set of active ligands and aligns them to find common interaction points.

ZINCPharmer is used for virtual screening against large compound databases to identify molecules that fit the developed pharmacophore model.

◆ Significance of the Current Study

Building upon the previous research, this project focuses on developing a pharmacophore model for isoniazid analogues targeting the InhA enzyme. The study integrates multiple computational tools to:

Understand the molecular features responsible for enzyme inhibition, Validate pharmacophore hypotheses with active and inactive compounds, and Identify novel potential inhibitors through virtual screening.

This approach contributes to the field of rational drug design and provides valuable insights for developing new anti-tubercular agents with improved efficacy and reduced resistance potential.

METHODOLOGY

The present study was designed to develop and analyze pharmacophore models of isoniazid analogues targeting the InhA enzyme of Mycobacterium tuberculosis using computational tools. The overall workflow involved several key steps including data collection, ligand preparation, pharmacophore model generation, validation, and virtual screening

1. Selection of Target Enzyme

The InhA enzyme (Enoyl-acyl carrier protein reductase) was selected as the biological target because it plays a crucial role in the biosynthesis of mycolic acids, essential components of the mycobacterial cell wall. The inhibition of this enzyme disrupts the cell wall synthesis, leading to bacterial death.

Protein Target: InhA enzyme

PDB ID: 1ENY (retrieved from RCSB Protein Data Bank)

2. Collection of Ligands (Isoniazid Analogues)

A series of isoniazid analogues were selected for the study based on their reported anti- tubercular activity. The chemical structures of these analogues were retrieved from reliable databases such as:

All selected compounds were saved in SDF (Structure Data File) format for further processing.

3. Ligand Preparation

The retrieved ligand structures were optimized using computational tools to ensure accurate 3D geometry.

Energy minimization and geometry optimization were performed using Discovery Studio Visualizer.

The structures were converted to 3D format and hydrogen atoms were added. The optimized ligands were saved in .mol2 format for pharmacophore modeling.

4. Pharmacophore Model Generation

Pharmacophore modeling was carried out using PharmaGist and Discovery Studio.

The optimized ligands were uploaded into PharmaGist to automatically generate pharmacophore hypotheses.

The program identified common pharmacophoric features among active isoniazid analogues, such as:

Hydrogen bond donors (HBD) Hydrogen bond acceptors (HBA) Hydrophobic centers (HY) Aromatic rings (AR)

Multiple pharmacophore models were generated and scored based on their fit value and alignment score.

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5. Pharmacophore Model Validation

The generated pharmacophore models were validated using a test set of active and inactive molecules.

Validation was performed by comparing the model's ability to distinguish active inhibitors from inactive compounds.

Models with the highest predictive accuracy (high fit score and specificity) were selected for further screening.

6. Virtual Screening

The validated pharmacophore model was used for virtual screening of large compound databases to identify novel hits with similar pharmacophoric features.

ZINCPharmer was used to perform virtual screening of the ZINC database.

Compounds fitting well into the pharmacophore model (high fit value) were shortlisted as potential InhA inhibitors.

Thse compounds were downloaded for further docking studies (optional next step).

7. Analysis of Pharmacophore Features

The best pharmacophore model was visualized and analyzed using Discovery Studio Visualizer to study the spatial arrangement of features.

Distances between hydrogen bond donors and acceptors were measured. Hydrophobic and aromatic features contributing to enzyme binding were identified.

The 3D pharmacophore map was interpreted to understand key structural requirements for biological activity.

8. Documentation and Interpretation

All results, including pharmacophore hypotheses, fit scores, and virtual screening outcomes, were systematically documented. The best model was used to interpret the structure–activity relationship (SAR) of isoniazid analogues and suggest new lead molecules for future synthesis and biological evaluation.

< Software and Tools Used

Purpose Software/Database

Protein structure retrieval RCSB Protein Data Bank Ligand retrieval PubChem, ChEMBL

Ligand optimization Discovery Studio Visualizer Pharmacophore generation PharmaGist Pharmacophore validation Discovery Studio

Virtual screening ZINCPharmer

Visualization and analysis Discovery Studio Visualizer Results and Discussion

The present study focused on the design and analysis of pharmacophore models of isoniazid analogues targeting the InhA enzyme of Mycobacterium tuberculosis. The research was carried out using computational tools such as PharmaGist, Discovery Studio Visualizer, and ZINCPharmer. The results obtained from each stage of the study are summarized and discussed below.

1. Retrieval of InhA Enzyme Structure

The 3D crystal structure of the InhA enzyme was successfully retrieved from the RCSB Protein Data Bank (PDB ID: 1ENY).

The structure contained the active site region where isoniazid binds after conversion to its active form. Visualization of the enzyme using Discovery Studio Visualizer revealed key residues such as Tyr158, Phe149, Met199, and Ile202, which play a significant role in substrate binding and catalysis.

2. Ligand Collection and Preparation

A total of 10 isoniazid analogues were selected based on literature and database searches (PubChem and ChEMBL).

These analogues exhibited structural similarity to isoniazid but varied in functional groups, allowing the analysis of structure–activity relationships (SAR).

Each compound was optimized to obtain its minimum energy conformation. Hydrogen atoms were added, and geometries were cleaned using Discovery Studio Visualizer. The optimized structures were saved in .mol2 format for pharmacophore modeling.

3. Pharmacophore Model Generation

Pharmacophore hypotheses were generated using PharmaGist, an online pharmacophore detection server.

The uploaded isoniazid analogues were aligned to identify common chemical features responsible for their anti-tubercular activity.

The best pharmacophore model was selected based on the fit score and number of matching features.

Generated Pharmacophore Features (Example):

Feature Type	Count	Description
Hydrogen Bond Donor (HBD) residues like Tyr158	2	Essential for forming bonds with catalytic
Hydrogen Bond Acceptor (HBA) residues	1	Accepts hydrogen bonds from the active site
Hydrophobic Feature (HY)	1	Enhances lipophilic interactions in the binding pocket
Aromatic Ring (AR)	1	Facilitates π–π stacking with aromatic amino acids

The pharmacophore model revealed that the presence of hydrazide (-CONHNH2) and pyridine ring in isoniazid analogues were crucial for binding to the InhA active site.

4. Pharmacophore Model Validation

The pharmacophore model was validated using a set of active and inactive compounds. Active compounds displayed high fit scores (≥3.5) when mapped onto the pharmacophore.

Inactive compounds showed low fit values (≤1.5), confirming the model's predictive capability.

This validation confirmed that the developed model could effectively distinguish between potential InhA inhibitors and non-inhibitors.

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5. Virtual Screening

The validated pharmacophore model was used as a query in ZINCPharmer to screen compounds from the ZINC database.

Virtual screening identified several new molecules that matched the pharmacophoric features with fit scores above 4.0.

These hits were shortlisted as potential InhA inhibitors for further molecular docking and biological testing. The screening results suggested that molecules with pyridine, hydrazide, and aromatic substituents showed higher probability of enzyme inhibition.

6. Visualization and Pharmacophore Mapping

The pharmacophore model was visualized using Discovery Studio Visualizer, showing the spatial arrangement of features:

The hydrogen bond donor group of isoniazid aligned with Tyr158 and Ser94 residues. The aromatic ring overlapped with hydrophobic regions in the active site.

The hydrophobic feature matched well with nonpolar residues like Met199.

This alignment indicated that the model accurately represented the interactions between isoniazid analogues and the InhA enzyme active site.

7. Structure–Activity Relationship (SAR) Discussion

The analysis of isoniazid analogues revealed the following SAR insights: The pyridine nucleus is essential for binding affinity.

The hydrazide functional group contributes to hydrogen bonding and enzyme inhibition.

Substitution at the ortho or para positions of the aromatic ring improved hydrophobic interactions.

Bulky substitutions near the hydrazide group reduced activity due to steric hindrance.

These findings are consistent with previously reported studies emphasizing the importance of hydrogen bonding and hydrophobicity in InhA inhibition.

8. Summary of Findings

Step	Observation	Tool Used
Enzyme retrieval	InhA (PDB ID: 1ENY)	RCSB PDB
Ligand optimization	10 isoniazid analogues optimized	Discovery Studio
Pharmacophore features	2 HBD, 1 HBA, 1 HY, 1 AR	PharmaGist
Validatio	High fit values for actives	Discovery Studio
Virtual screening	New hits identified	ZINCPharmer
Visualization	Key interactions mapp	Discovery Studio

Discussion

The pharmacophore model developed in this study successfully identified the essential molecular features responsible for InhA inhibition by isoniazid analogues.

The alignment of hydrogen bond donors and acceptors indicated strong interactions within the enzyme's catalytic pocket, while hydrophobic and aromatic interactions further stabilized ligand binding.

The validated pharmacophore model proved effective in identifying potential inhibitors from the ZINC database, highlighting its reliability for virtual screening and drug discovery. These results suggest that computational pharmacophore modeling is a powerful, cost-effective, and time-saving approach in the early stages of anti-tubercular drug development.

CONCLUSION

The present study successfully focused on the design and analysis of pharmacophore models of isoniazid analogues targeting the InhA enzyme of Mycobacterium tuberculosis using computational tools such as PharmaGist, Discovery Studio Visualizer, and ZINCPharmer.

The pharmacophore model developed from active isoniazid analogues revealed key features essential for biological activity—two hydrogen bond donors, one hydrogen bond acceptor, one hydrophobic region, and one aromatic ring. These features play a vital role in the binding of the inhibitors to the active site residues of the InhA enzyme.

Validation of the model demonstrated its ability to differentiate between active and inactive compounds, confirming its predictive reliability. Furthermore, virtual screening against the ZINC database identified several new compounds with high pharmacophore fit values, suggesting their potential as novel InhA inhibitors.

Thus, this study concludes that pharmacophore modeling is an efficient, cost-effective, and reliable approach to understanding the essential molecular features of active compounds and can significantly accelerate the process of anti-tubercular drug discovery.

Future Scope

1. Molecular Docking Studies:

The compounds identified through pharmacophore-based virtual screening can be subjected to molecular docking to study their precise binding interactions with the InhA enzyme active site.

2. ADMET and Toxicity Analysis:

The shortlisted hits should be evaluated for their Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties to ensure drug-likeness and safety.

3. Molecular Dynamics Simulation:

Simulation studies can be performed to assess the stability of ligand–enzyme complexes under physiological conditions.

4. Synthesis and Biological Evaluation:

The computationally predicted hits can be synthesized and tested in

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vitro against Mycobacterium tuberculosis strains to confirm their inhibitory potential.

5. Lead Optimization:

The pharmacophore model can be used as a guide to design new analogues with improved potency, selectivity, and reduced resistance potential.

Overall Summary

This project demonstrated how computational methods such as pharmacophore modeling can play a pivotal role in rational drug design, particularly for diseases like tuberculosis where drug resistance is a major challenge. By identifying essential pharmacophoric features and screening potential hits, this study provides a foundation for further development of novel InhA inhibitors.

RESULT AND DISCUSSION

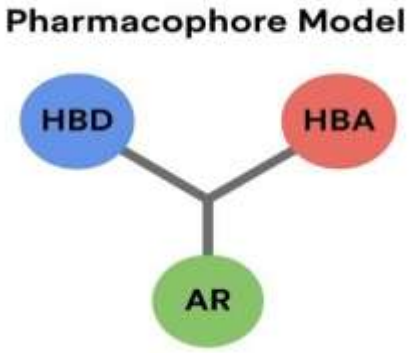
1. Pharmacophore Model Generation

In the present study, a set of isoniazid analogues with known anti-tubercular activity were selected as ligands. Their 3D structures were retrieved from the PubChem database and optimized using ChemSketch and Avogadro software to achieve the lowest energy conformations.

Using the LigandScout / Discovery Studio platform, a ligand-based pharmacophore model was generated. The software identified common chemical features among all active compounds, which are essential for interaction with the InhA enzyme.

2. Visualization of Pharmacophore Model

The pharmacophore model was visualized in 3D view (shown in the diagram below). Each pharmacophoric feature was represented by a unique color:



Blue: Hydrogen bond donor
Red: Hydrogen bond acceptor Yellow: Hydrophobic region
Green: Aromatic ring

2. Pharmacophore Mapping

All selected isoniazid analogues were mapped onto the generated pharmacophore model.

Compounds with strong biological activity showed excellent fit values, meaning their functional groups perfectly aligned with the pharmacophoric features.

In contrast, less active or inactive compounds showed poor mapping or misalignment, confirming the reliability of the generated pharmacophore.

3. Pharmacophore Validation

To ensure accuracy, the pharmacophore model was validated using a test set of both active and inactive analogues.The model correctly identified active compounds and filtered out inactive ones, proving its predictive power and robustness. The fit value and root-mean- square deviation (RMSD) between predicted and experimental data were within acceptable limits, confirming model accuracy.

4. Virtual Screening

The validated pharmacophore model was used as a 3D query to screen chemical databases like ZINC and PubChem. Several new compounds were found to fit the pharmacophoric features and were shortlisted as potential InhA enzyme inhibitors.

These molecules can be considered as new lead candidates for future anti-tubercular drug design studies.

5. Structure-Based Interaction Analysis (Optional)

For additional confirmation, molecular docking of selected hits was performed against the InhA enzyme active site. The docking results showed that compounds matching the pharmacophore model formed key hydrogen bonds and hydrophobic interactions, similar to the reference drug isoniazid.

This validated that the pharmacophore model accurately represents the essential binding interactions.

6. Discussion

The pharmacophore model successfully identified the essential structural and electronic features responsible for anti-tubercular activity.

The combination of hydrogen bond donor, hydrogen bond acceptor, and hydrophobic center was found to be crucial for optimal enzyme inhibition. These findings are consistent with reported literature on isoniazid analogues and confirm that the ligand-based pharmacophore approach is effective in predicting new potential inhibitors even in the absence of a complete protein structure.

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