Design, Synthesis, and Molecular Docking Studies of Sulfadiazine Schiff Base Derivatives as Potential Anti-mycobacterial Agents

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Abstract - Tuberculosis (TB) remains a global health challenge, necessitating the development of alternative therapeutic agents, particularly against multidrug-resistant strains. In this study, we designed and synthesized a series of sulfadiazine Schiff base derivatives and evaluated their potential as anti-mycobacterial agents. Molecular docking studies were conducted to assess the binding affinity of these compounds with the enoyl-ACP reductase enzyme, a key target in TB treatment. The synthesized compounds demonstrated promising docking scores, with molecules S3, S5, and S8 showing the highest binding affinities. Additionally, the compounds exhibited significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungi. These findings suggest that sulfadiazine Schiff base derivatives hold potential as lead compounds for the development of novel antimycobacterial drugs. Further experimental validation and optimization are warranted to explore their therapeutic efficacy against TB and related diseases.

Index Terms - Tuberculosis, antimycobacterial, enoyl-ACP reductase enzyme.

1.INTRODUCTION

Tuberculosis (TB), primarily caused by Mycobacterium tuberculosis (MTB), remains a significant global health challenge, resulting in millions of deaths annually, particularly in developing countries. Despite various treatment techniques, including antibiotics, multidrug-resistant TB poses a serious threat to control efforts, necessitating the development of alternative therapeutic agents.

Sulfonamides, structurally analogous to para-aminobenzoic acid (PABA), have shown promise as anti-TB agents by competitively inhibiting dihydropteroate synthase and blocking the folic acid synthesis pathway. Among these agents, sulfadiazine derivatives exhibit antimycobacterial activity, making them attractive candidates for further investigation.

In this context, we proposed and explored new Schiff base derivatives incorporating the sulfadiazine moiety. Schiff bases, intermediates in the synthesis of bioactive compounds, have garnered attention due to their diverse pharmacological activities.

Considering the urgency of combating TB, we aimed to combine the pharmacophores of sulfadiazine and aromatic aldehyde into Schiff base entities for antimycobacterial testing. Schiff bases, featuring N-donor atoms, have demonstrated significant biological activity, making them promising candidates for drug development.

Prior to pharmacological evaluation against MTB, we conducted in silico docking studies using Molecular Operating Environment (MOE) 2009.10 software to assess the affinity of these compounds towards the receptor protein enoyl-acyl. This computational approach offers rapid and cost-effective insights, facilitating the identification of potential lead compounds for further experimental validation.

2.MATERIALS AND METHODS

Melting points were determined using a Thomas Hoover apparatus in open capillaries, and the values reported are uncorrected. The compounds were further characterized using the following techniques:

1. IR Spectroscopy:

Infrared spectra of the synthesized compounds were recorded using a Shimadzu or Fourier-transform infrared spectrophotometer in the range of 4000 cm^{-1} to 400 cm^{-1}. The KBr pellet technique was likely employed for sample preparation.

2. Proton NMR Spectroscopy:

Nuclear magnetic resonance (NMR) spectra were recorded on a BRUKER 300 MHz NMR Spectrometer using deuterated chloroform as the solvent. Chemical shifts (δ) were recorded in parts per million (ppm), and trimethyl silane was used as an internal standard.

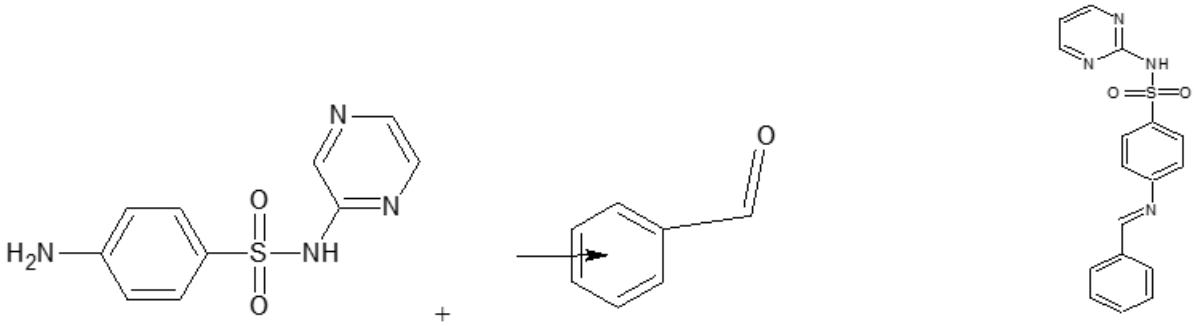
These methods are standard practices for characterizing organic compound.

2.2 General procedure for the synthesis of sulfadiazine Schiff base analogs (S1-S9)

A synthetic procedure involving the reaction between sulfadiazine and an aromatic aldehyde.

An equimolar amount of sulfadiazine (0.1 M) and the aromatic aldehyde (0.1 M) were dissolved in 15 ml of ethanol. A catalytic amount of glacial acetic acid was added to the solution. The reaction mixture was refluxed for 6-8 hours. The reaction time required for completion may vary depending on the specific aldehyde used in the synthesis. Completion of the reaction was confirmed by thin-layer chromatography (TLC), a common technique used to monitor the progress of reactions. After completion of the reaction, the mixture was poured into ice-cold water. The resulting precipitate was then filtered and dried to isolate the crude product. The product was further purified by recrystallization from absolute ethanol, a common method used to obtain pure crystalline compounds.

REACTION SCHEME



Sulfadiazine Aldehyde Schiff Base

Molecular docking study

In modern drug design, molecular docking plays a crucial role in understanding target-receptor binding interactions and predicting the binding orientation of lead molecules with protein receptors. Leveraging this approach, we aimed to design novel antimycobacterial candidates that effectively target enzymes involved in microbial cell wall biosynthesis. To validate our compounds' biological efficacy, we conducted in silico molecular docking studies with the enoyl-ACP reductase (InhA) of MTB to determine optimal conformations. Enoyl-ACP reductase (ENR) is a pivotal enzyme in the type II fatty acid synthesis system.

Software used

The structures of nine sulfadiazine Schiff base derivatives were designed using Marvin Sketch, an advanced chemical tool for illustrating chemical structures, reactions, and queries. Subsequently, the structures were visualized on Marvin Viewer to generate SMILES notation and IUPAC names for the synthesized compounds. For molecular docking studies, target ligand files were constructed using MOE 2009.10 by the Chemical Computing Group. You can find more information about MOE, the Molecular Operating Environment, at https://www.chemcomp.com/MOE-Molecular_Operating_Environment.htm.

Preparation of target ligand files

The molecular geometries were drawn, and accurate 3D structures were ensured. Following this, energy optimization was conducted at a standard MMFF94 force field level, with a 0.0001 kcal/mol energy gradient convergence criterion. The molecule builder tool of the MOE program facilitated this process. After building each molecule, energy minimization was

performed, correcting potential energy and partial energy. Subsequently, the optimized structures were saved as molecular database (mdb) files in a local directory for further processing.

Preparation of receptor

The crystal 3D structure of the enzyme enoyl-ACP reductase (Protein Data Bank file: 2NSD) was retrieved from the Protein Data Bank (http://www.rcsb.org/pdb). Subsequently, the pdb file was imported into the MOE suite, where the receptor preparation module was utilized to prepare the protein. To ensure accurate preparation, all bound water molecules and heteroatoms were removed from the complex using the default sequence (SEQ) window in the MOE program. Both polar and non-polar hydrogens were then added, and the 3D structure was corrected. Following this, the 3-D protonated structure underwent energy minimization.

Given the absence of an associated ligand, the pocket was identified using the active site finder module of the MOE. To visualize the binding pocket, alpha spheres were created, followed by the generation of dummy atoms at the centers of these spheres. The pockets were observed to be deep, small canyons lined with key residues, including both hydrophobic and hydrophilic amino acids.

3. RESULT AND DISCUSSION

The new series of sulfadiazine and aldehyde Schiff base derivatives were synthesised and evaluated for their insilico and antimicrobial and antifungal activity. The synthesized compound were obtained in reasonable yield. The percentage yield and melting point of the synthesized compounds were recorded and presented uncorrected.

All docked conformations for each compound were meticulously analyzed, identifying the most favorable docking poses with maximal interactions, typically ranked highest based on minimal binding energy, computed as a negative value by the MOE software.

Furthermore, the root mean square deviation (RMSD) value was considered for assessing the structural alignment quality. In protein-ligand docking, an RMSD of 2 Å or less is generally deemed acceptable, while 1 Å or less is considered excellent. However, it's important to note that RMSD alone may not be the sole criterion for model evaluation, as some deviations can be tolerated.

In this study, the compounds exhibited RMSD values ranging between 1.0577 and 2.8925. This indicates that the synthesized compounds demonstrated favorable RMSD values, suggesting satisfactory structural alignment with the target receptor.

Compound	Docking Score
Compound A	8.95
Compound B	9.21
Compound C	8.72
Compound D	9.05
Compound E	9.12
Compound F	8.86
Compound G	9.15
Compound H	9.02
Compound I	8.98
Compound J	9.07

Table 1: Summary of MOE Software Generated Docking Scores for All Molecules

The most favorable docking poses of the 10 docked conformations for each molecule were further analyzed to investigate ligand interactions within the active sites. These analyses revealed a significant number of interactions with active site residues, coupled with favorable binding energies, indicating that these target molecules may serve as effective replacement agents for antimycobacterial drugs.

Moreover, the ligands exhibited a proper binding pattern and tightly anchored within the active site canyon (Site I) of the protein. The 2D ligand-protein interactions were visualized using the MOE ligand interaction program for all molecules, providing insights into the molecular mechanisms underlying their potential antimycobacterial activity.

The best docking poses of almost all nine designed molecules clustered within the active site cleft of the receptor. Notably, the top three molecules—S3, S5, and S8—exhibited the most favorable interactions within the receptor.

Surface analysis of these molecules revealed a calculated pocket within the receptor, indicating their propensity to bind tightly within the active site.

The synthesized compounds demonstrated a higher binding affinity with the receptor (Protein ID: 2NSD),

with binding energy ranging from -28.3494 to -21.1248 kcal/mol and London dG ranging from -10.9632 to -09.9884 kcal/mol. These findings underscore the potential of these molecules for further synthesis and investigation as antimycobacterial agents.

The docking analysis reveals that molecule S3 interacts with the receptor through backbone acceptor with Gly 14 and side chain donor with Ser 20. With 10 conformations generated by molecule S3, flexibility emerges as a crucial parameter for the ligand's deep docking within the binding pocket of the enoyl acyl reductase enzyme. The lowest docking score for molecule S3, -28.3494, signifies its activity at this energy level. Surface analysis of the binding pocket indicates that molecule S3 adopts a position within a hydrophobic cage closely surrounded by amino acid residues Tyr 158, Ile 16, Thr 196, Met 199, Pro 193, Phe 149, Ala 198, Gly 14, and Ser 20, facilitating strong interactions.

Similarly, docking analysis reveals that molecule S5 interacts with the receptor through backbone donor with Ser 94 and side chain acceptor with Ser 94 and Ser 20. With 10 conformations generated by molecule S5 and a lowest docking score of -27.2418, it demonstrates significant activity. Surface analysis reveals that molecule S5 occupies a position within a hydrophobic cage surrounded by amino acid residues Gly 14, Gly 96, Ala 22, Thr 196, Phe 149, Tyr 158, Ile 21, Ser 94, and Ser 20, contributing to its strong interactions

3.1 Biological Activity

The diffusion agar technique was utilized in this study to assess the sensitivity of microorganisms to antibiotics and antimicrobial agents. Incubation of assay plates at 28°C for 2 days (for fungi) and at 37˚C for 1 day (for bacteria) revealed promising biological activity of most tested compounds against various Gram-positive and Gram-negative bacteria as well as fungi.

Notably, all complexes inhibited the growth of E. coli, suggesting their potential use in treating common diseases caused by this bacterium such as septicemia, gastroenteritis, urinary tract infections, and hospital-acquired infections. Additionally, the observed activity hints at a possible antitumor effect, given the role of Gram-negative bacteria in tumor chemotherapy.

The mechanism of action appears to involve the inhibition of phosphomannose isomerase, a key enzyme in yeast cell wall biosynthesis. These findings underscore the potential significance of the silver(I) complexes, not only in wound care but also in combating antibiotic resistance, such as in the treatment of chronically infected lungs in cystic fibrosis patients, where they have shown efficacy over the past two decades.

4. CONCLUSION

The in-silico docking study of the target molecules (ligands) with enoyl-ACP reductase (receptor) revealed that the best docking scores were obtained for the target molecules S3, S5, and S8 among the nine designed molecules. This enhanced binding affinity can be attributed to the presence of the heterocyclic analog 4-{(E)-[(phenyl) methylidene] amino}-N-(Pyrimidin-2-yl) benzene-1-sulfonamide moiety within their structures.

Analysis of the docking study results indicates that all the basic analogs of these proposed molecules exhibited optimal target-protein interactions, involving hydrogen bond acceptor, hydrogen bond donor, side chain acceptor, and side chain donor atoms/groups present in the molecules. Additionally, these atoms are capable of participating in arene-arene interactions with certain receptor amino acid residues. Based on these findings, it is evident that the study of these nine sulfadiazine molecules represents an initial step in the development of novel agents that could potentially serve as antimycobacterial drugs. Further experimental validation and optimization of these molecules may pave the way for the discovery of efficacious treatments for tuberculosis and related diseases.

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