INTRODUCTION

Tuberculosis (TB), which is an airborne infectious disease caused predominantly by Mycobacterium tuberculosis (MTB), is a global health problem and a leading cause of death among adults in the developing country. At present among the 8 million TB infected individual, around 2 million people will die due to this active TB. To defeat this situation, now a day there are several treatment techniques has been followed to fight TB. Besides the antibiotic treatment is one of the best methods to fight this TB condition [1-3]. Owing to the complicated shape and cell wall constituents of the mycobacterium, this will make the many antibiotics into ineffective and hinders the entry of drugs. Multidrug resistance TB [4] is a serious threat to TB control and prevention. The emergence of multidrug resistance has forced to the development of new class of therapeutic agents. Therefore, there is an interest in search of alternative antibiotics used as anti-TB agents. Among such agents, sulfonamides are structural analogs of para-aminobenzoic acid (PABA), inhibiting competitively the dihydropteroate synthase and blocking the folic acid synthesis pathway and cell division [5-7]. The most of the compounds having sulfonamide moiety in its structure may have the antibacterial [8,9], carboxic anhydride inhibitor [10], antiviral [11], antifungal [12], anti-inflammatory activities [13,14], antitumor [15], and antithyroid activity [16,17]. Schiff base of sulfonamides entity has also been shown to exhibit an antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties [18]. Sulfadiazine is one of the sulfanilamide derivatives that are used as an antibacterial as well as an antimalarial drug and thought to act as PABA competitive inhibitor [19]. Moreover, it possesses SO₂NH moiety as an important group for their antimycobacterial activity. Based on these facts, new Schiff base derivatives tagged with sulfadiazine moiety were proposed and presented in this article.

Due to the global impact of this overwhelming disease, several different strategies such as targeting bacterial virulence, high-throughput screening, structure-based drug discovery, chemical modifications of the known drugs, and combinatorial chemistry have been explored to search for novel biologically important molecules [20]. Computer-based (in silico) molecular modeling (bioinformatics and cheminformatics) is quite useful for this purpose because they are extremely fast and cost-efficient and can be applied even when a compound is not physically available.

Keeping this point discussed in above paragraph, and based on previously reported research work [21], we have decided to combine the two pharmacophores for testing their antimycobacterial activity, namely, sulfadiazine with an aromatic aldehyde into one molecule, i.e., Schiff base entity. Many kinds of literature revealed that the Schiff bases are the key intermediates for the synthesis of numerous bioactive medicinal compounds from the primary amine [22]. In recent years, the chemistry of Schiff bases contains N-donor atom which has been extensively studied and has acquired a great interest because of the azomethine RHC=N-R1 linkage, where R and R1 are alkyl, aryl, cycloalkyl, or heterocyclic groups which is essential for its biological activity [23,24]. Before stepping into its pharmacological evaluation on MTB, we have decided to go for their in silico docking study using Molecular Operating Environment (MOE) 2009.10 software for its affinity toward the receptor protein enoyl-acyl carrier protein (ACP) reductase, which is isolated from MTB pathogen.

METHODS

All the chemicals in this synthesis were of AR and LR grade and were obtained from Merck, Hi-Media, and Sigma-Aldrich, SD Fine chem, Mumbai. All the chemicals were used as received without further purification.

**Experimental section**

Melting points were determined in open capillaries on a Thomas Hoover apparatus and are uncorrected. The synthesized compounds were characterized by the following methods: IR spectra of synthesized compounds were recorded on Shimadzu (8300, Kyoto, Japan) and Fourier-transform infrared spectrophotometer in the range of 4000 cm⁻¹-400 cm⁻¹ using KBr pellet technique. Proton nuclear magnetic resonance (NMR) spectra were recorded using Bruker 300 MHz NMR Spectrometer using the solvent deuterated chloroform. Chemical shifts (δ) were recorded in parts per million (ppm) and trimethylsilane used as an internal standard.
General procedure for the synthesis of sulfadiazine Schiff base analogs (S1-S9)
An equimolar quantity of sulfadiazine (0.1 M) and aromatic aldehyde (0.1 M) was dissolved in 15 ml of ethanol and then added a catalytic amount of glacial acetic acid into it and refluxed for 6–8 h, and the reaction time may depend on the aldehyde which we used for this synthesis. Completion of the reaction was confirmed by thin-layer chromatography. The reaction mixture was then poured into ice-cold water, and the obtained precipitate was filtered and dried. The product was recrystallized from absolute ethanol. The reaction sequences for synthesized compounds leading to the formation of new compounds are outlined in Fig. 1.

| Code | R | Target compounds |
|------|---|-----------------|
| S1   |   | ![Image]       |
| S2   |   | ![Image]       |
| S3   |   | ![Image]       |
| S4   |   | ![Image]       |
| S5   |   | ![Image]       |
| S6   |   | ![Image]       |
| S7   |   | ![Image]       |
| S8   |   | ![Image]       |
| S9   |   | ![Image]       |

Molecular docking study
In modern drug designing scenario, the molecular docking is commonly used for the understanding of target-receptor binding interaction and is quite often used to predict the binding orientation of target molecule of the lead with their protein receptor to find the affinity of that target compound [25]. Keeping this in mind, we undertook to design novel antitymococcal candidates, which will target promisingly with the enzymes involved in the biosynthesis of microbial cell wall. To rationalize the biological results of our compounds, an in silico molecular docking studies with the enoyl-ACP reductase (InhA) of MTB were carried out to determine the best conformation. Enoyl-ACP reductase (ENR) is a key enzyme of the type II fatty acid synthesis system [26].

Software used
The designed structures of nine sulfadiazine Schiff base derivatives were generated using Marvin_Windows-x64_18.8 version. Marvin Sketch is an advanced chemical tool for illustrating the chemical structures, queries, reactions, etc. [27]. Then, the structures were viewed on Marvin Viewer screen to generate the SMILES notation and to IUPAC name of the synthesized compounds. The target ligand files for the molecular docking studies were built on MOE 2009.10 by Chemical Computing Group (https://www.chemcomp.com/MOE-Molecular_Operating_Environment.htm).

Preparation of target ligand files
The molecular geometries were drawn and correct 3D structures were ensured and were followed by energy optimization at a standard MMFF94 force field level, with a 0.0001 kcal/mol energy gradient convergence criterion [28]. The molecule builder of MOE program was used for this purpose, and after building the molecule, it was energy minimized, potential energy and partial energy were corrected, and then, it was saved as molecular database (mdb) file in a local directory for the further process.

Preparation of receptor
The crystal 3D structure of enzyme enoyl-ACP reductase (Protein Data Bank file: 2NSD) was obtained from Protein Data Bank (http://www.rcsb.org/pdb). The pdb file was imported to MOE suite where receptor preparation module was used to prepare the protein. All the bound water molecules and hetero atom were removed from the complex using sequence (SEQ) window which is default in MOE program. Both polar and non-polar hydrogens were added and 3D structure was corrected. The 3-D protonated structure was energy minimized. Since the protein was devoid of 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 on the centers of these spheres. The pockets were found to be deep small canyons lined with the key residues including both hydrophobic and hydrophilic amino acids.

Docking methodology
The optimized target ligands were docked with the enzymes enoyl-ACP reductase (PDB: 2NSD) protein using the MOE, 2009.10. For docking...
simulations, the placement was set as triangular match, rescoring was set as London dG, the number of retaining was set as 10, and the refinement was set as force field on MOE suite to generate 10 poses of each target ligand confirmations. As a result of docking run, the sbd. output files were created with scoring and multiple conformations of each compound. All the docked conformations were analyzed, and the best-scored pose for each compound was selected for further interaction studies. Besides, the ligand-receptor interaction followed by surface analysis of the selected best pose ligand molecule was generated on MOE suite and viewed for interpretation.  

RESULT AND DISCUSSION  
The new series of sulfadiazine aldehyde Schiff base derivatives were synthesized and evaluated for their in silico antimycobacterial activity. The synthesized compounds were obtained in reasonable yield. The percentage yield and melting point of the synthesized compounds were recorded and presented uncorrected. IR and 1H NMR spectral data of synthesized compounds were given below.

\[ \text{Table 1: Docking results for sulfadiazine derivatives with protein PDB: 2NSD} \]

| Molecule code | S | rmsd_refine | E_conf | E_place | E_score1 | E_refine |
|---------------|---|-------------|--------|---------|----------|----------|
| S1            | -27.3491 | 2.0017 | -125.3771 | -83.8810 | -10.3766 | -27.3491 |
| S2            | -24.0552 | 1.0577 | -130.1177 | -81.0930 | -9.9884 | -24.0552 |
| S3            | -28.4949 | 1.7535 | -117.72406 | -76.6933 | -12.0955 | -28.4949 |
| S4            | -25.5142 | 2.0028 | -123.90077 | -80.1463 | -10.2673 | -25.5142 |
| S5            | -27.2418 | 1.5564 | -123.5227 | -72.1410 | -10.3580 | -27.2418 |
| S6            | -24.0687 | 2.7787 | -113.0495 | -68.6075 | -10.7929 | -24.0687 |
| S7            | -26.6306 | 2.8925 | -122.4127 | -83.4326 | -10.9632 | -26.0306 |
| S8            | -28.0579 | 1.5476 | -133.8988 | -108.2688 | -10.6365 | -28.0579 |
| S9            | -21.1248 | 1.6550 | -112.9345 | -82.3756 | -10.7156 | -21.1248 |

S: The final score, rmsd_refine: The Root mean square deviation between the pose before refinement and the pose after refinement, E: conf: The energy of the conformer, E: place: Score from the placement stage, E: score1: Score from the rescoring stage (s), E: refine: Score from the refinement stage, and No. of conf: number of conformations generated by ligand, PDB: Protein data bank

S: 4-[(2E)-3-phenylprop-2-en-1-ylidene]amino]-N-[(pyrimidin-2-yl)benzene-1-sulfonamide. Yellowish solid; yield - 70%; m.p - 215–220°C.

\[ \text{Table 1: Docking results for sulfadiazine derivatives with protein PDB: 2NSD} \]
was shown in Figs. 2-4. The molecules under consideration for further synthesis have a higher binding affinity with the receptors, in All the synthesized compounds have a higher binding affinity with the receptors (Protein ID: 2NSD) in the binding energy range of −28.3494 to −21.1248 kcal/mol and the range of London dG is −10.9632 to −0.9884 kcal/mol.

Docking analysis reveals that the molecule S3 interacted with receptor through backbone acceptor with Gly 14 and side chain donor with Ser 20 (Fig. 2). The number of conformations generated by molecule S3 was 10 which indicated that flexibility is an important parameter for the ligand to dock deeply within the binding pocket of enoyl acyl reductase enzyme. The lowest docking score for molecule S3 was −28.3494, which indicates that compound is active at this energy of conformation. Further, a careful calculation of surface analysis of the binding pocket of this molecule indicated that molecule S3 adopted a position in a hydrophobic cage surrounded by the following amino acids residue such as Tyr 158, Ile 16, Thr 196, Met 199, Pro 193, Phe 149, Ala 198, Gly 14, and Ser 20 and these were approach closely to the ligand for strong interactions.

Docking analysis reveals that the molecule S5 interacted with receptor through backbone donor with Ser 94 and side chain acceptor with Ser 94 and Ser 20 which is shown in Fig. 3. The number of conformations generated by molecule S5 was 10, and the lowest docking score for molecule S5 was −27.2418. The calculation of surface analysis showed the binding pocket of this molecule indicating that this molecule S5 adopted a position in a hydrophobic cage surrounded by the following amino acids residue such as Tyr 158, Ile 16, Thr 196, Met 199, Pro 193, Phe 149, Ala 198, Gly 14, and Ser 20.
The global tuberculosis situation. Progress and susceptibility of mycobacterium thst Sulfadiazine salicylaldehyde-based Schiff et al. 5. Ameen SM, Drancourt M. 4. Espinal MA. The global situation of MDR-TB. Tuberculosis T. Sivakumar, with his docking analysis using MOE 2009.10 software. T. Prabha, who is fully contributed to prepare this whole manuscript according to the present study, it can be suggested that the study of these arene interactions with some receptor amino acid residues. Hence, according to the present study, it can be suggested that the study of these nine sulfadiazine molecules could be the first step in the development of novel agent which can act as an antimycobacterial drug.

AUTHOR’S CONTRIBUTIONS
T. Prabha, who is fully contributed to prepare this whole manuscript write up, followed by plagiarism check if any, and to check the grammar using the grammarly online tool. Moreover, she is the one who runs the docking analysis using MOE 2009.10 software. T. Sivakumar, with his guidance, the author moved further to complete this work and also the final manuscript proofreading was done by him.

CONFLICTS OF INTEREST
The authors have no conflicts of interests.

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