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# STUDY OF STRUCTURE-BASED DESIGN FOR SULFONES AS ENOYL-ACP REDUCTASE INHIBITORS

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**ABSTRACT:** *Mycobacterium leprae*, the causative agent of the disease, leprosy develops resistance against most of the drugs, so novel drug targets are required to design new drugs. Present work is aimed at understanding the inhibition of enoyl-acyl carrier protein reductase (Enoyl-ACP reductase), which is one of the receptor proteins used in drug discovery for screening anti-leprosy agents by virtually designed sulfone class of compounds. The crystal structure of the inhibited *M. leprae* InhA complex (2NTV) provides the details of protein-ligand interactions. The virtually designed series of compounds having sulfone moiety have docked well in the active site region of the protein. The prediction of ADME properties was also performed by Qikprop software. *Mycobacterium leprae*, the causative agent of the disease, leprosy develops resistance against most of the drugs, so novel drug targets are required to design new drugs. Present work is aimed at understanding the inhibition of enoyl-acyl carrier protein reductase (Enoyl-ACP reductase), which is one of the receptor proteins used in drug discovery for screening anti-leprosy agents by virtually designed sulfone class of compounds. The crystal structure of the inhibited *M. leprae* InhA complex (2NTV) provides the details of protein-ligand interactions. The virtually designed series of compounds having sulfone moiety have docked well in the active site region of the protein. The prediction of ADME properties was also performed by Qikprop software candidates.

**INTRODUCTION:** Leprosy is an infectious disease caused by an obligate intracellular micro-organism, *Mycobacterium leprae*. Leprosy currently affects approximately a quarter of a million people throughout the world, with the majority of cases being reported from India [1].

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Since ancient times, Chaulmoogra oil had been used for leprosy treatment, but its efficacy was partial, and relapse was common [2]. No effective drug was available for leprosy until the introduction of Dapsone in the early 1940s.

Soon the bacteria developed resistance for Dapsone. After 1980's multidrug therapy (MDT) was introduced in which the drugs Dapsone, Clofazimine, and Rifampicin were used in combination and found effective [3-7]. But it was expensive, and its long term treatment led to resistance. So, there is an urgent need for the development of novel antileprosy drug candidates.

The second line drugs of tuberculosis, Thioamide, Ethionamide (ETH) and Prothionamide (PTH) are effective in the treatment of *M. Leprae* and *M. Tuberculosis* infections [8]. The crystal structure of the inhibited *M. leprae* InhA complex (PDB ID: 2NTV) provides the protein-ligand interactions [8]. It is reported that Prothionamide binds with Nicotinamide adenine dinucleotide ($NAD^+$) and this adduct inhibits *Mycobacterium leprae* (InhA), the enzyme is the product of InhA gene which plays a vital role in Mycolic acid biosynthesis. This crystal structure can be used to test new inhibitors using drug design methods. The availability of three-dimensional coordinates for the target enzyme enables the use of structure-based drug design (SBDD) techniques. The 3D structure of the target protein is most often derived from X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy [9].

Using the structure of the target protein, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics. One of the key benefits of SBDD methods is its capability of providing docking of putative drug compounds in the active site of target proteins. X-ray and NMR methods can resolve the structure of proteins to a resolution of a few angstroms (about 500,000 times smaller than the diameter of a human hair). Most proteins contain pockets, cavities, surface depressions, and other geometrical regions where small-molecule compounds can easily bind. With high-resolution X-ray and NMR structures for proteins and ligands, researchers can accurately show how ligands orient themselves in active protein sites. This ability to work at high resolution with both proteins and drug compounds makes SBDD one of the potent methods in drug design [10]. Another advantage is, it helps in reducing research cost and saves the environment by avoiding wastage of a large number of chemicals during the synthesis of novel compounds in chemistry laboratory [11, 12].

**Target-Enoyl-acyl Carrier Protein Reductase Enzyme:** Fatty acids are a consequential source of energy for organisms from all taxa. Fatty acid synthesis in mammals substantially differs from that of bacteria. In mammals, the fatty acid synthesis involves a single multifunctional enzyme–acyl carrier protein (ACP) complex, while in bacteria; the synthesis utilizes several small monofunctional enzymes that operate in conjunction with ACP–associated substrates [13]. This provides an opportunity to selectively target this essential bacterial pathway without interfering with mammalian enzymes. Hence, the enzymes of the fatty acid biosynthesis pathway (FAS II) in bacteria, represent fascinating targets for antimicrobial drug design. Enoyl-acyl carrier protein reductase is a key enzyme in the bacterial FAS-II. It is a rate-controlling enzyme in the FAS-II pathway. The final and rate-determining step of chain elongation in the bacterial fatty acid biosynthesis is the reduction of enoyl–ACP to acyl–ACP, which is catalyzed by the enzyme–enoyl-acyl carrier protein reductase. Due to its essential role in metabolism and sequence conservation across many bacterial species [14,] it is an attractive target for antibacterial drug discovery. The enzyme is a member of the short-chain alcohol dehydrogenase /reductase (SDR) superfamily characterized by a catalytic triad of key tyrosine, lysine, and serine residues that reduce a double bond in the enoyl substrate with $NAD^+$ or $NADP^+$ as an acceptor, a key step in bacterial production of fatty acids [15]. The systematic name of this enzyme class is acyl-[acyl carrier-protein]: $NADP^+$ oxidoreductase (A-specific).

## MATERIALS AND METHODS:

**Preparation of Protein:** The crystal structure of selected protein target Enoyl-ACP reductase was available PDB ID: 2NTV (www.rcsb.org). The selected 3D structure of the enzyme was having intrinsic inhibitor 2-propyl-isonicotinic - acyl-nicotinamide- adenine di-nucleotide, also referred to as PTH-$NAD^+$ adducts [8]. The PDB structure of enzyme Enoyl-ACP reductase [PDB id: 2NTV] was downloaded, refined, and prepared using Schrodinger protein preparation wizard tool (Glide, version 5.9, Maestro 9.4, Schrodinger), which performed the following steps: assigning bond orders, adding hydrogens, optimization of hydrogen bonds, correction of charges, and minimization of the protein complex.

All the redundant water molecules, ligands, and cofactors were removed (preprocess) from the proteins which were taken in. mae format. The tool neutralized the side chains that were not close to

the binding cavity and did not participate in salt bridges. This step was then followed by restrained minimization of co-crystallized complex, which reoriented side-chain hydroxyl groups and alleviated potential steric clashes. The complex obtained was minimized using the OPLS_2005 force field (Kaminski and Friesner, 2001).

**Preparation of Ligands:** Structure of the Enoyl-ACP reductase inhibitors was sketched using a built panel of Maestro and taken in.mae format. Ligand Preparation is a utility of Schrodinger software suite that combines tools for generating 3D structures from 2D and searching for all possible steric isomers, tautomers, and perform a geometry minimization of the ligands. Molecular Mechanics Force Fields (OPLS_2005) with default settings were used for the ligand minimization.

All designed structures were commenced from substituted sulfones as sulfones are reported as biologically active compounds. Dapsone (4, 4'-diaminodiphenylsulphone), an antileprotic drug which is a sulfone analog, has been proved to be a powerful antimicrobial agent [16]. Sulfone derivatives provide an example of a vital class of bioactive compounds with a wide spectrum of activities, as the sulfone group is a fundamental found in diverse biologically active compounds with a vast range of biological activity including antifungal [17], herbicidal [18], anti-hepatitis [19], antitumor [20], antileprotic [21], anti-inflammatory [22], anticancer [23], anti-HIV-1 [24] and anti-tubercular [25] properties. So, our present study is aimed at studying the interaction details between Enoyl-ACP reductase and sulfone class of compounds.

**Docking Methodology:** The docking studies were carried out using the extra precision mode of Glide using default parameters. The active site was defined by the generation of a grid box such that the co-crystallized ligand occupied the center of the box. The grid-based ligand docking with energetics (glide) algorithm approximated a systematic search of positions, orientations, and conformations of the ligand in the enzyme binding pocket via a series of hierarchical filters. The shape and properties of the receptor were represented on a grid by several different sets of fields, which provided progressively more accurate scoring of the ligand pose. The inhibitor was extracted from the complex and redocked. The final docked conformation of the inhibitor was aligned to the original conformation, and root mean square deviation (RMSD) calculated. RMSD value less than 2 confirmed the accuracy of the docking program. The ligands of the dataset were docked flexibly into the receptor using default parameters. No constraints of similarity scoring were applied. The G-score value was calculated by taking into consideration factors as favorable Vander Waals, coulombic, lipophilic and hydrogen-bonding interactions and penalizing for steric and buried polar clashes.

Sulfone class of compounds was prepared and supplied to the docking software. Their G scores are depicted in **Table 1**.

**TABLE 1: DOCKING RESULTS OF STANDARD LIGAND AND DESIGNED MOLECULES**

| S. no. | Molecule | G score | S. no. | Molecule | G score |
|---|---|---|---|---|---|
| 1 | Standard Ligand | -12.1 | 12 | | -9.54 |
| 2 | | -9.64 | 13 | | -8.15 |

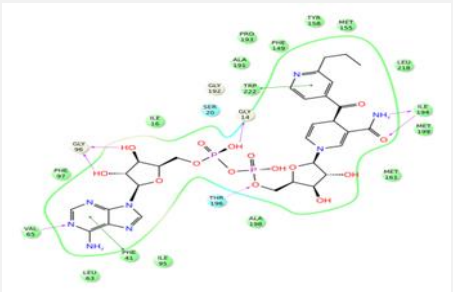
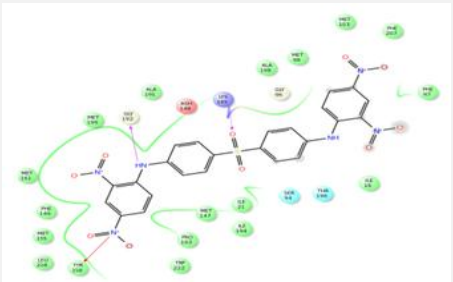
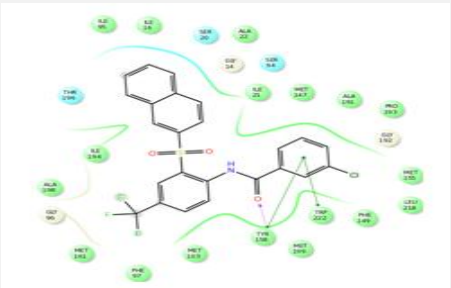
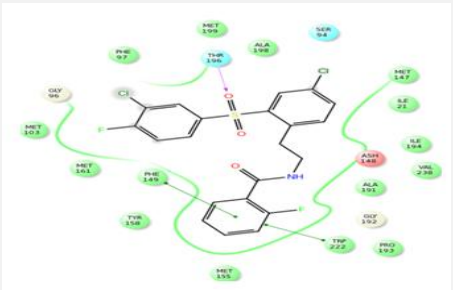
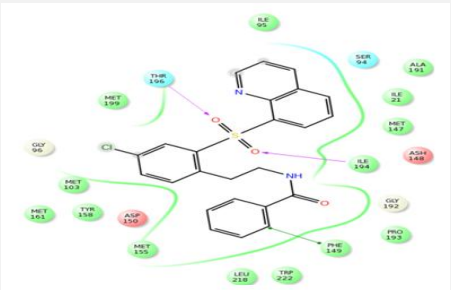
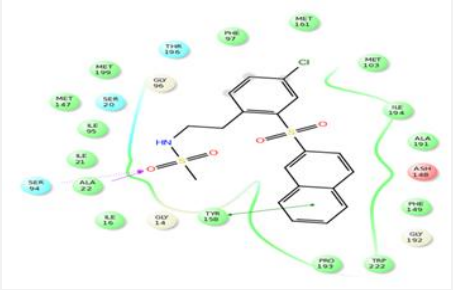
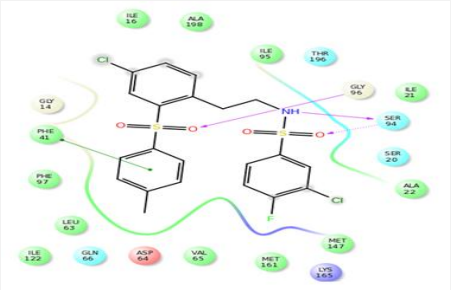
| | | | |
|---|---|---|---|
| 3 | -8.72 | 14 | -9.23 |
| 4 | -9.63 | 15 | -8.64 |
| 5 | -8.29 | 16 | -7.99 |
| 6 | -9.15 | 17 | -9.35 |
| 7 | -9.74 | 18 | -8.15 |
| 8 | -9.66 | 19 | -8.1 |
| 9 | -9.13 | 20 | -8.08 |
| 10 | -8.89 | 21 | -7.73 |
| 11 | -9.95 | | |

**QikProp Descriptors:** These molecules were also subjected to further filter via Lipinski's rule of five to identify compounds with favorable absorption, distribution, metabolism, and excretion (ADME) properties. They were calculated using QikProp. In the present study, QikProp was run in normal processing mode with default options. The molecules were analyzed for drug-likeness by assessing their physicochemical properties and by applying Lipinski's rule of five.

**RESULTS AND DISCUSSION:** The ligand fits into the active site of a protein, and so higher interactions depend on the binding affinity and possibilities of a number of hydrogen bonds between atoms of ligand and amino acids. The hydrogen bond gives stable conformation to the complex and so usually gives better drug-like properties. When the sulfone molecules were docked into the active site of the protein, it was observed that H-bonds which the actives formed were with Thr196, Ile194, Gly96. The other amino acids Gly192, Lys165, Tyr158, Ala191, Ser94, Ala22, were taking part in making H-bonds with ligand atoms. Most

Actives were able to form at least two H-bonds with the receptor. **Table 2** shows the 2D images of some of the active compounds docked into the enzyme.

**TABLE 2: 2D STRUCTURES OF STANDARD LIGAND AND SOME SULFONES**

| Molecule no. | 2D Structure | Molecule no. | 2D Structure |
|---|---|---|---|
| 1 |  | 16 |  |
| 3 |  | 17 |  |
| 7 |  | 4 |  |
| 20 |  | 21 |  |

H-bond interactions are indicated with purple lines pi-pi stacking is indicated with green line pi-cation is indicated with red line Hydrophobic interactions are indicated in green **Table 3**, given below shows amino acid interactions of all the designed sulfones.

**TABLE 3: AMINO ACID INTERACTIONS OF STANDARD LIGAND, DESIGNED MOLECULES**

| Molecule no. | Interacting residues | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | V l65 | Trp 222 | Gly 96 | Gly 14 | Phe 41 | Thr 196 | Ile 194 | Phe 149 | Tyr 158 | Gly 192 | Ile 21 | Lys 165 | Ser 94 | Ala 22 | Ala 191 |
| 1.Standard ligand | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ | | | | | | | | |
| 3 | | | | | | | | | ✔ | ✔ | | ✔ | | | |
| 4 | | | | | | ✔ | ✔ | ✔ | | | | | | | |
| 7 | | ✔ | | | | ✔ | | ✔ | | | | | | | |
| 8 | | ✔ | | | | | | ✔ | | | | | | | |
| 9 | | | | | | | | ✔ | | | | | | | |
| 10 | | | | | | | | | | | | ✔ | | | |
| 12 | | | | | | | | | | | | | | | ✔ |
| 13 | | | | | | | ✔ | | | | | | | | ✔ |
| 14 | | | | | | | | | | | | | | | ✔ |
| 15 | | ✔ | | | | | | | ✔ | | | | | | |
| 16 | | ✔ | | | | | ✔ | | ✔ | | | | | | |
| 17 | | ✔ | | | | | | | ✔ | | | | | | |
| 18 | | | | | | | | | | | | ✔ | | | |
| 19 | | ✔ | | | | | | | | | | | | | |
| 20 | | | | | | | | | ✔ | | | | ✔ | ✔ | |
| 21 | | | ✔ | | ✔ | | | | | | | | ✔ | | |

The Lipinski's rule for druglike molecules states that the molecule should have molecular weight <500 Daltons, H-bond donors <5, H-bond acceptors <10 and a logP of <5. The compound can be considered a probable drug candidate even if it violates one of the criteria mentioned above. For molecules, as given in **Table 4** the partition coefficient (QPlogPo/w) critical for estimating the absorption of drugs within the body, ranged between 2.291-6.368.

**TABLE 4: PHYSIOCHEMICAL DESCRIPTORS AND ADME PROPERTIES OF DESIGNED MOLECULES**

| Molecule[a] | QPlogPo/w[b] | QPlogS[c] | QPlogBB[d] | QPPCaco[e] | QPPMDCK[f] | % Human oral absorption[g] |
|---|---|---|---|---|---|---|
| 2 | 4.658 | -7.26 | -1.173 | 501.726 | 693.161 | 100 |
| 3 | 3.169 | -7.654 | -5.222 | 0.878 | 0.279 | **18.579** |
| 4 | 5.554 | -7.286 | -0.379 | 74.634 | 2592.71 | 80.028 |
| 5 | 3.552 | -4.443 | -0.263 | 60.181 | 3319.518 | 79.593 |
| 6 | 5.624 | -7.375 | -0.216 | 82.937 | 4692.485 | 81.26 |
| 7 | 6 | -7.83 | 0.057 | 82.937 | 10000 | 83.463 |
| 8 | 6.368 | -8.173 | -0.234 | 82.937 | 4692.488 | 85.614 |
| 9 | 8.112 | -11.447 | -0.03 | 3293.333 | 5606.829 | 100 |
| 10 | 6.837 | -9.437 | -0.124 | 1637.326 | 3265.965 | 100 |
| 11 | 5.095 | -6.165 | -0.311 | 41.233 | 2733.364 | 72.726 |
| 12 | 4.353 | -5.371 | -0.291 | 41.233 | 2733.362 | 81.342 |
| 13 | 2.291 | -2.519 | -0.319 | 29.92 | 1933.612 | 66.778 |
| 14 | 4.729 | -5.826 | -0.018 | 41.233 | 10000 | 83.545 |
| 15 | 4.77 | -6.185 | -0.43 | 780.644 | 3001.447 | 100 |
| 16 | 3.151 | -4.325 | -0.329 | 649.853 | 2797.073 | 95.737 |
| 17 | 5.558 | -6.998 | -0.363 | 896.662 | 4102.945 | 100 |
| 18 | 3.865 | -6.474 | -1.918 | 50.659 | 160.142 | 80.084 |
| 19 | 5.865 | -7.587 | -0.47 | 887.34 | 3994.782 | 100 |
| 20 | 3.58 | -5.032 | -0.361 | 65.819 | 2575.433 | 80.452 |
| 21 | 5.237 | -7.098 | -0.044 | 90.712 | 10000 | 66.732 |

[a] Molecules
[b] Predicted octanol/water partition coefficient log p (acceptable range 2.0 to 6.5)
[c] Predicted aqueous solubility; S in mol/L (acceptable range (-6.5) to 0.5)
[d] Predicted BBB permeability (acceptable range (-3) to 1.2)
[e] Predicted Caco-2 cell permeability in nm/s (acceptable range: <25 is poor and >500 is great)
[f] Predicted apparent MDCK cell permeability in nm/s (acceptable range: <25 is poor and >500 is great)
[g] Percentage of human oral absorption (acceptable range:<25 % is poor and >80 % is high)

Crossing the blood-brain barrier (BBB), which is a prerequisite for the entry of drugs to CNS, was found to be in the acceptable range ((-3)-1.2) indicating that the compounds may be considered for further development. Caco-2 cell permeability (QPPCaco), a model governing gut-blood barrier, ranged from 29.92 to 3293.333. MDCK cell permeability (QPPMDCK), a model that mimics the blood brain barrier, ranges from 160.142 to 10000. Further, the predicted percentage of human oral absorption for all molecules ranged from 66.732 to 100 %. All these pharmacokinetic parameters were found to be within the acceptable range **(Table 4 footnote)**.

**CONCLUSION:** By docking results, it can be concluded that molecules show good docking scores, the docking values indicate that some molecules like no. 4, 7, 16, 17, 3, 20, 21 are well-docked molecules, and their hydrogen bond interactions indicate the possibilities of antileprosy drug-likeness. Also, ADME properties of molecules are in an acceptable range and better than the standard ligand, which again supports the drug-likeness. It can be concluded that the designed sulfone class of molecules are positively interacting with Enoyl-ACP reductase protein and hence can be further processed as anti-leprosy drug candidates.

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**CONFLICT OF INTEREST:** Nil

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