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Role of nitrogen containing heterocyclic compounds in acyl Co-A carboxylase carboxyltransferase: Docking with dynamic simulation studies

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ABSTRACT: Acyl Co-A carboxylase carboxyltransferase (AccD5) is essential for cell wall lipid biosynthesis and its disruption leads to mycobacterial death. Acyl CoA is the key regulation point for fatty acid synthesis and therefore AccD5 has become a good target for mycobacterial disease. Herein, docking and Molecular dynamic simulations with other computational techniques and softwares has been used to select the best compound. The heterocyclic compounds were indole, n-methylpiperazine, piperidine, and pyrrolidine derivatives. Among which, the docking results showed Ib5, an indole containing heterocyclic compound, as a potent inhibitor with good binding affinity with -20.23 kcal/mol of energy as compared with the standard NCI-65828 (8-amino-5-(4'-hydroxybiphenyl-4ylazo)naphthalene-2-sulfonate molecule with -19.24 kcal/mol of binding energy. Additionally, MD simulations showed less fluctuations with depiction of root means square deviation and root mean square fluctuation graphs in 2A7S-Ib5 complex. Wherein, this molecular modeling of AccD5 with Ib5 provided an insight to use it as an anti-tubercular drug. Therefore, this method has helped to prove the nitrogen containing heterocyclic compounds can be used against *Mycobacterium tuberculosis*.

KEYWORD: Anti-tubercular; AccD5; Acyl Co-A carboxylase; carboxyltranferase; molecular docking; molecular dynamic simulation.

1. INTRODUCTION

Tuberculosis is one of the deadliest disease affecting mankind wherein one-third of the world population is infected according to World Health Organization(WHO)[1]. *Mycobacterium tuberculosis* is the pathogen causing the infection which persists intracellularly and gets reactivated with Human Immunodeficiency Virus further in life causing AIDS [2, 3]. Many moieties were introduced in the last 40 years as the first-line and second-line therapy inclusive of rifampicin, isoniazid, pyrazinamide and ethambutol wherein fluoroquinolones were effective against tuberculosis. Substituted fluoroquinolones were also introduced but multi-drug resistance developed with higher rates. This disease is spreading widely with multi-drug resistance developed and the need for new compounds has emerged. Some functions of the cell are targeted by antibiotics while treating mycobacterial infection. However, fatty acids are the precursors to many *Mycobacterium tuberculosis* lipids, including mycolic acids, the α-alkyl β-hydroxy branched long-chain fatty acids that are one of the main components of the *M. tuberculosis* cell wall. The first committed and rate-limiting step in fatty acid biosynthesis is the production of malonyl coenzyme A (malonyl-CoA) from acetyl-CoA and bicarbonate by the acetyl-CoA carboxylase (ACC) which is a regulation point for fatty acid biosynthesis[4]. In *M. tuberculosis*, this biotin- and ATP-dependent reaction consists of two catalytic steps. The first, biotin carboxylation, is catalyzed by the α-subunit, which contains both biotin carboxylase (BC) and biotin carboxylate carrier protein (BCCP) in one polypeptide chain. BC couples carbonate to a biotin residue

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covalently linked to the BCCP domain to form carboxybiotin[5]. The second step is the carboxyl transfer from carboxybiotin to acetyl-CoA, catalyzed by the β-subunit, carboxyltransferase (CT). This α-carboxylation of acetyl Co-A serves as building blocks for downstream fatty acid synthesis. Basically, acyl CoA is the key regulation point for fatty acid synthesis. Acyl-CoA carboxylase carboxyl transferase (AccD5) has become the best studied mycobacterial carboxyltransferase and the increase in its importance in metabolic regulation and the availability of ACCase sequences from different genomes, ACCases have become good targets for infectious disease, cancer[6, 7]. According to the studies carried out by Ting-Wan Lin et al., docking of NCI-65828 (8-amino-5-(4′-hydroxybiphenyl-4ylazo) naphthalene-2-sulfonate) in the acyl-CoA-binding pocket of AccD5 matches the binding motif of an acyl-CoA, in which the anionic sulfate of NCI-65828 binds the entrance of the CoA pocket and the hydrophobic moiety binds the hydrophobic interior of the CoA pocketas shown in Figure 1. NCI-65828 molecule has been validated as a reference ligand as it showed extensive enzyme inhibition [8]. Active efflux is a widespread mechanism for bacterial resistance to antibiotics, which contributes to poor intrinsic susceptibility, cross-resistance to structurally diverse classes of drugs, or selection of other mechanisms of resistance. Thus, inhibition of efflux pumps appears to be a promising strategy for restoring the activity of existing antibiotics, and a useful method to detect the presence of efflux determinants in clinical isolates. Structurally dissimilar classes of inhibitors have been patented in the last decade, some are analogs of antibiotic substrates (tetracyclines, quinolones or aminoglycosides) and others, new chemical entities (including substituted indoles, ureas, aromatic amides, piperidinecarboxylic acids, alkylamino- or alkoxyquinolines, peptidomimetics, and pyridopyrimidines). Their spectrum of activity, in terms of antibiotics and bacteria, differs significantly [9].

Literature studies show that compounds containing active hydrogen atoms yield aminobenzylated Mannich bases with secondary amines and aromatic aldehydes which are important compounds owing to their wide range of biological and industrial applications. They are also employed as intermediates in chemical synthesis. Several important therapeutic compounds have been synthesized via the Mannich reaction. They have also been found to possess pharmacological activities, such as antihypertensive, antipsychotic, anaesthetic, antitubercular, *etc*. [10]. Literature reports suggest that presently the N-containing heterocyclic scaffolds like indoles, piperazines, pyrrolidine, piperidine are evolving as promising compounds, therefore the design of potent molecules containing these moieties for docking with AccD5 can prove the affinity of these moieties as potent inhibitors. The importance of ACCase as a drug target for mycobacterial disease has been validated by bacterial physiological experiments, which found that ACCase is used as the downstream extender unit for fatty acid biosynthesiss [11, 12]. Due to multi-drug resistance (MDR-TB) and extensively drug-resistant (XDR-TB) strains are evolving that are resistant to the existing primary, secondary treatments and leave these drugs far less effective, with toxic side effects and higher death rates. Therefore, based on these evidences and the global emergency, there exists a need to design and study newer N-containing compounds as potent inhibitors of the AccD5 enzyme against tuberculosis.

2. RESULTS

2.1. Validation and energy minimization

Total 74 compounds were designed and energy was minimized using the Steepest Descent method and Universal Force Field (UFF) and all the energy minimized compounds were subjected to the docking calculations with Acyl-CoA carboxylase carboxyltransferase (AccD5).

2.2. Active site analysis

Active site consists of Gly 434, Ala 435, Gly 193, Gly 194, Cys 437, Met 439, Tyr 436, Gly 433, Ala 435, Tyr 432, Ala 431, Gly 393, Val 391, Pro 392, Phe 394, Leu 363, Val 438 present in A and C chain[8] as shown in Figure 1.

2.3. Secondary structural analysis

PROMOTIF[13] program provides information about the secondary structure of a given protein for analysis. As per the program, 2A7S consists of a total of 6 chains A, B, C, D, E, F with 529 residues, 2 sheets, 7 beta-alpha-beta units, beta hairpins, 1 beta bulge, 15 strands, 28 helices, 45 helix-helix interaction, 54 beta turns, 4 gamma turns as shown in Figure 2.

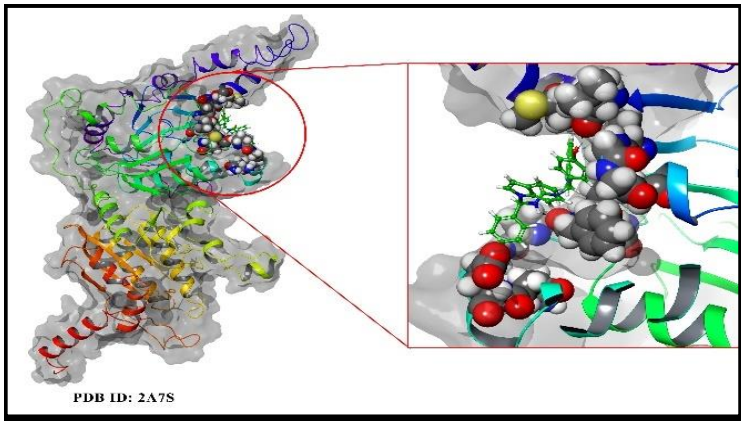

Figure 1. The binding site of 2A7S with NCI-65828 molecule binding with amino acids viz. Pro 392, Tyr 432, Gly 461, Gly 397. NCI-65828 molecule represented by ball and stick model, protein chain shown as ribbon and binding site residues represented as Van der Waals surface.

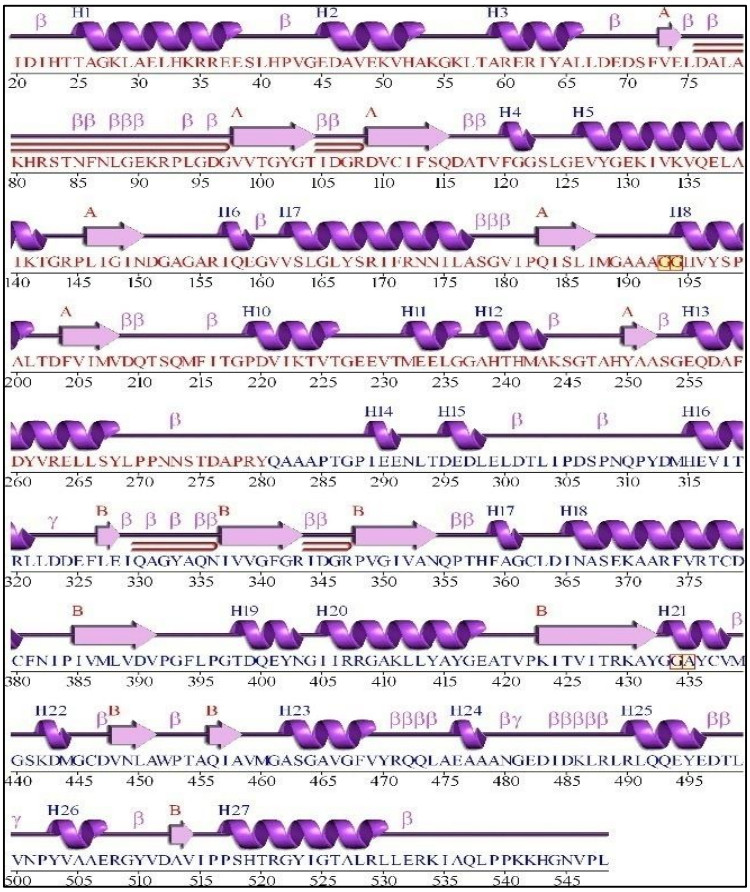

Figure 2. Secondary structure of Acyl Co-A carboxylase carboxyltransferase (PDB ID: 2A7S).

2.4. Molecular docking

Molecular docking was performed to study the binding mode of 2A7S with the heterocyclic compounds. The 2D structures for the selected heterocyclic compounds are shown in Figure 3. The 3D structures were visualized in Maestro and binding energy was calculated using FlexX Software. After running the docking calculations, all possible conformers were generated and ranked based on their docking score. Out of 74, the best 5 compounds were selected and their binding energy in the active site was speculated and was analyzed in comparison to the binding energy of NCI-65828. Their respective interacting residues, bond types and bond distances were considered. The reference ligand used was NCI-65828 (8-amino-5-(4'-hydroxybiphenyl-4ylazo) naphthalene-2-sulfonate molecule [8] shown in Figure 3.

Out of 74 compounds, 69 heterocyclic compounds were docked to the active site which showed good binding energy in comparison to NCI-65828 molecule with -19.24 kcal/mol. On the basis of the binding score comparable to NCI-65828, the best 5 molecules with favorable binding energies were selected. The binding energies with interacting amino acid residues of native ligand and test ligands are summarised in Table 1 and Figure 4.

Figure 4(a) depicts the binding interactions of NCI-65828 with AccD5 (2A7S) forming hydrogen bonds with Gly 397, Tyr 432, Pro 392, Gly 461 with bond distances 2.18Å, 1.79Å, 2.01Å, 2.07Å, and 2.01Å, respectively.

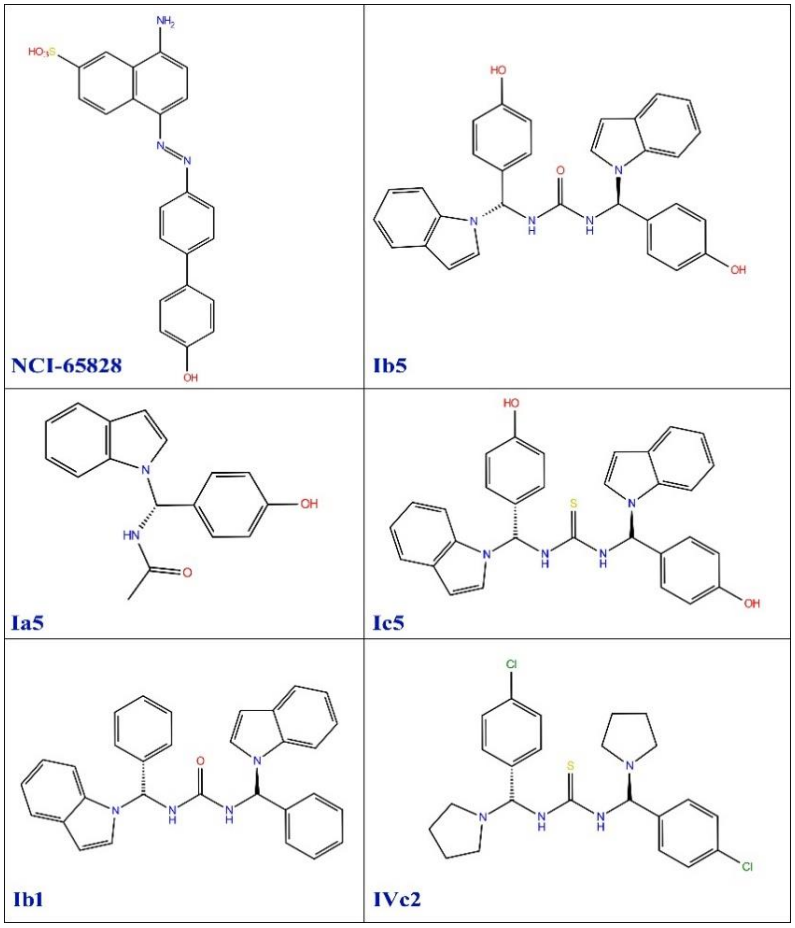

Figure 3. The 2D structures of the synthesized compounds and the reference molecule NCI-65828.

The best binding interaction with 2A7S was shown by the compound Ib5 with binding energy-20.23kcal/mol forming one hydrogen bond each with Gly 397 and Val 459 with 1.55Å, 1.90Å bond distance with the hydroxyl group of the aldehyde, two hydrogen bonds with Phe 394 with the distance of 2.27Å and 1.66Å with the amino group, and one aromatic hydrogen bond with Tyr 432 with 2.60Å of bond distance with the aldehydic ring as shown in Figure 4(b).

Figure 4(c) shows compound Ic5 with binding energy -16.85kcal/mol interacting with amino acid residues Gly 397, Ala 435 forming hydrogen bonds each with the bond distance of 1.52Å, 1.78Å with the hydroxyl group of an aldehyde, while Leu 395 and Tyr 432 forms aromatic hydrogen bonds with the aldehyde group with the bond distance of 2.72Å, 2.76Å, and 2.78Å.

Compound Ia5 has a binding energy of -16.58kcal/mol interacted with Phe 394 and Ala 435 with hydrogens of amino and hydroxyl group having a bond distance of 1.70Å and 2.16Å, whereas aldehydic aromatic hydrogen bonds were formed with Phe 394 and Pro 392 with a bond distance of 2.44Å and 2.78Å as shown in Figure 4(d).

Compound IVc2 and Ib1 formed hydrogen bonds with the amino group of thiourea and urea with bond distance 1.89Å, 2.09Å and 2.20Å, 2.01Å. The compounds have a binding affinity of -16.08 kcal/mol and -16.0358 kcal/mol as shown in Figure 4(e) and Figure 4(f). On the basis of docking, it can be concluded that indole and pyrrolidine-containing heterocyclic compounds are useful for the development of new anti-tubercular entities.

Table 1. The binding energies and intermolecular interactions of selected compounds along with reference molecule (NCI) with Acyl Co-A carboxylase carboxyltransferase.

Sr. No	Compound Name	Binding Energy (kcal/mol)	Interacting Residues	Bond Type	Bond Distance (A°)
1.	NCI-65828	-19.24	Gly397	1 H Bond	2.18
			Tyr432	1 H Bond	1.79
			Pro392	2 H Bond	2.01, 2.07
			Gly461	1 H Bond	2.01
2.	Ib5	-20.23	Gly397	1 H Bond	1.55
			Tyr432	1 Ar-H Bond	2.60
			Phe394	2 H Bond	2.27, 1.66
			Val459	1 H Bond	1.90
3.	Ic5	-16.85	Gly397	1 H Bond	1.52
			Tyr432	1 Ar-H Bond	2.72
			Phe394	1 H Bond,1 Ar-H Bond	3.45, 2.72
			Ala435	1 H Bond	1.78
			Leu395	1 H Bond,2 Ar-H Bond	2.78, 2.76, 2.78
4.	Ia5	-16.58	Phe394	1 H Bond, 1 Ar-Bond	1.70, 2.44
			Ala435	1 H Bond	2.16
			Pro392	1 Ar-H bond	2.78
5.	IVc2	-16.08	Phe394	2 H Bond, 1 Ar-H Bond	1.89, 2.09, 2.13
6.	Ib1	-16.03	Phe394	2 H Bond	2.20, 2.01

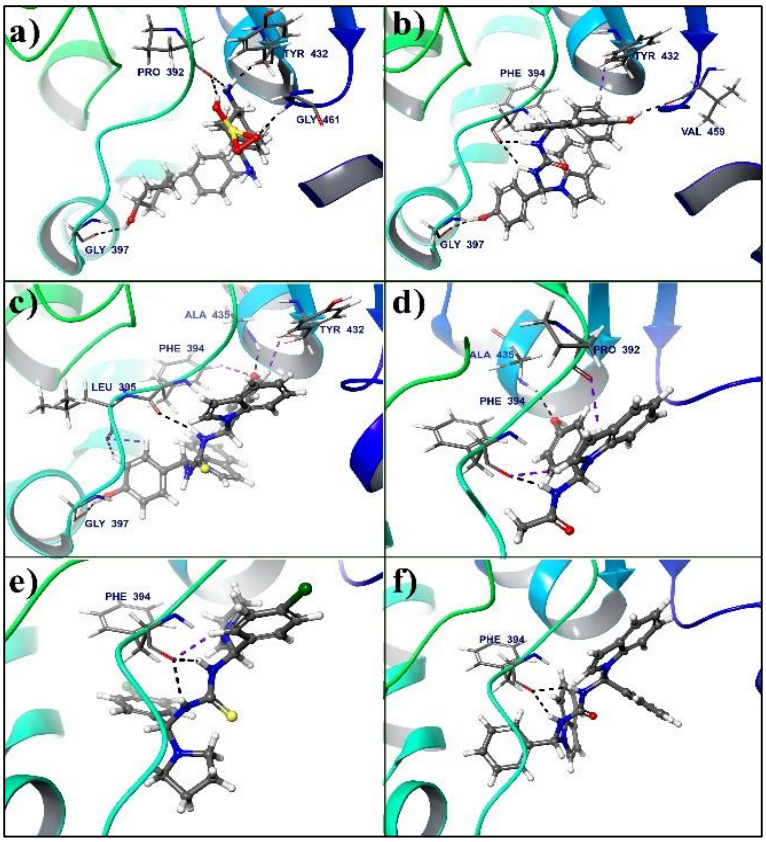

Figure 4. a) Docked pose for NCI molecule in the cavity of 2A7S; b) Docked pose of Ib5 with 2A7S; c) Docked pose of Ic5 with 2A7S; d) Docked pose of Ia5 with 2A7S; e) Docked pose of IVc2 with 2A7S; f) Docked pose of Ib1with 2A7S. Ligands are represented by ball and stick model, protein chain shown as ribbon and interacting amino acids represented as a stick model.

2.5. MD simulations

Molecular dynamics simulation is carried out to study the protein-ligand complex dynamic interactions. The stability of the system is maintained by a 50ns simulation period with decreased potential energy wherein

the receptor-ligand complex is analyzed. The conformations obtained from the simulation of 50ns of the protein-ligand complex were analyzed. The protein 2A7S was complexed with the Ib5 compound with chain A consisting of a total of 531 residues and total charge -11.

The Root Mean Square Deviation (RMSD) is used to measure the average change in displacement of a selection of atoms for a particular frame with respect to a reference frame. It is calculated for all frames in the trajectory. The RMSD and Root Mean Square Fluctuation(RMSF) plot for 2A7S-Ib5 complex is shown in Figure 5 and Figure 6 respectively, initially, conformational fluctuations are observed till 30ns and then the complex was stabilized in the production phase. The average RMSD of 4.5Å was obtained. Dynamically stable conformations were selected for docking analysis to explore the stability of top selected ligands with 2A7S. Further to check the flexibility of the 2A7S-Ib5 complex, RMSF was calculated and compared for 50ns. In the RMSF plot, the peaks indicate areas of the protein and ligand fluctuations mostly during the simulation period. The ligand RMSF is useful for characterizing changes in the ligand atom positions. The analysis revealed that Phe394 interacted with the $+NH_2$ group exhibiting stronger interaction with stable conformation.

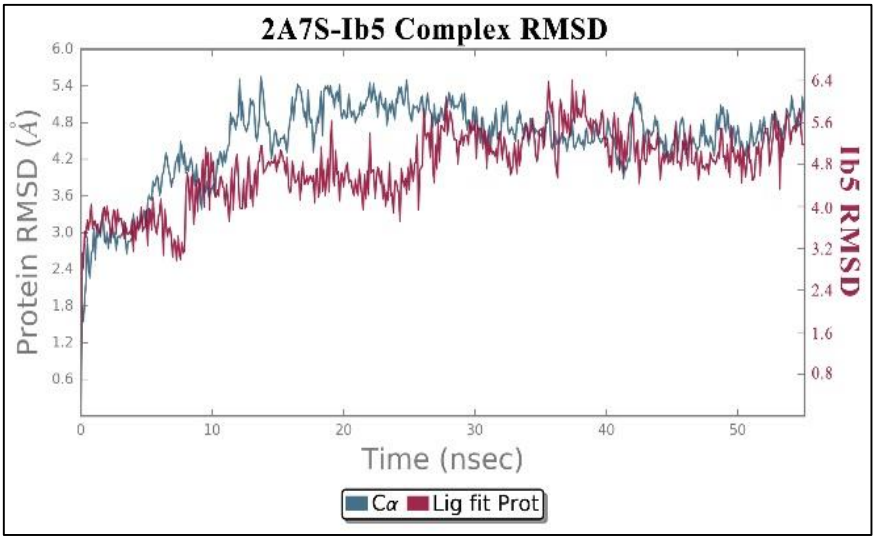

Figure 5. Root mean square deviation (RMSD) plot for 2A7S-Ib5 complex during 50 ns of molecular dynamic simulation. 2A7S shown in blue colourand compound Ib5 in brown colour.

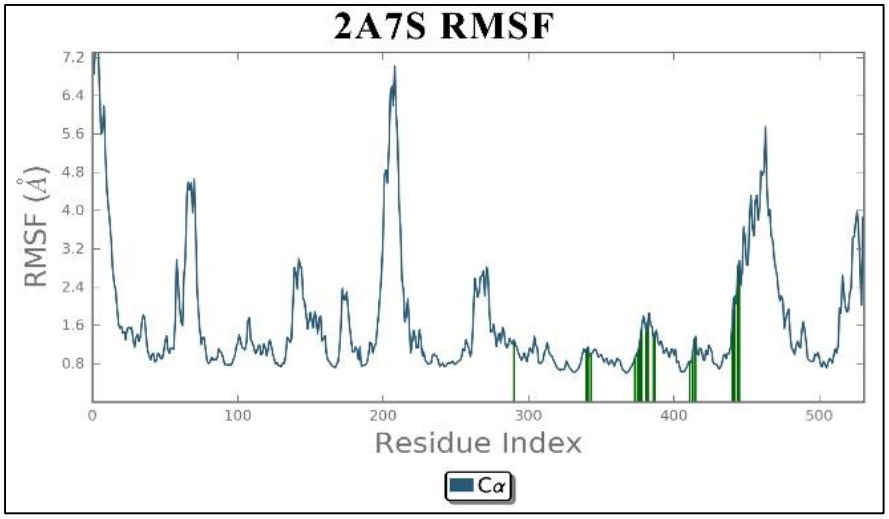

Figure 6. The Root Mean Square Fluctuation (RMSF) is useful for characterizing local changes along the protein chain.

3. CONCLUSION

The study focuses on nitrogen-containing heterocyclic compounds as potent anti-tubercular entities. Wherein we found out Gly 397, Ala 435, Leu 395, Tyr 432, Phe 394 and Pro 392 amino acid residues strongly

interact with the compounds within the cavity. From the molecular docking study, it was observed that out of 74 only 69 compounds were docked which showed good binding affinity towards the receptor, whereas the compound Ib5 showed the better binding affinity towards the 2A7S receptor with its lowest bond distance. Graph of MD simulation showing the stability of the compound Ib5 within the cavity.

Our study reveals that the compound Ib5 has good binding affinity, structural stability and is dynamically favorable to other compounds. From the docking studies, it can be concluded that the hydroxyl group and amino group forms good hydrogen bonding with the amino acid residues Gly 397, Ala 435, Leu 395, Tyr 432, Phe 394 and Pro 392 which can play an important role to access the binding pocket of the receptor by the Nitrogen-containing heterocyclic compounds. Therefore the study helps to elucidate new nitrogen-containing heterocyclic compounds as anti-tubercular agents.

4. MATERIALS AND METHODS

4.1. Selection of acyl Co-A carboxylase carboxyltransferase (AccD5)

The crystal structure of Acyl Co-A carboxylase carboxyltransferase (AccD5) with resolution 2.9Å was retrieved from Protein Data Bank [PDB ID: 2A7S][8] and was further selected for docking studies.

4.2. Preparation of acyl co-A carboxylase carboxyltransferase (AccD5) and ligands

Protein Preparation Wizard (PPW)[14] was used in order to refine the 2A7S crystal struture by applying the OPLS-2005 force field[15] with a root mean square deviation (RMSD) tolerance. The protonation and ionization state of basic and acidic group's amino acids were chosen using the PROPKA program[16] according to physiological pH 7.4.

The 2D structures of indole, n-methyl piperazine, piperidine, pyrrolidine and its derivatives were drawn on Marvin Sketch software[17] and visualized with Maestro. All the ligands were energy minimized using the OPLS-2005 force field in Maestro to get energetically favorable conformation.

4.3. Molecular docking calculations

To observe the binding pattern of selected compounds in the cavity of 2A7S, molecular docking calculations were performed. The binding cavity for 2A7S was mapped according to the reported amino acid residues[8] Gly 434, Ala 435, Gly 193, Gly 194 and also the residues within 6Å were considered which were Cys 437, Met 439, Tyr 436, Gly 433, Ala 435, Tyr 432, Ala 431, Gly 393, Val 391, Pro 392, Phe 394, Leu 363, Val 438. The cavity of the 2A7S was sufficient for ligand conformations and calculations.

All ligands were subjected to molecular docking calculations with the 2A7S crystal struture by using FlexX software[18]. All atoms from 2A7S are treated as rigid during docking. FlexX uses incremental construction algorithm [19]. The docking parameters are kept at their default value of software and numbers of possible conformation for each derivative were generated. Based on docking score and ligand interaction analysis with the 2A7S receptor, the best poses for a given ligand having greater binding affinity were selected as lead compounds.

4.4. Molecular dynamic simulations

Molecular Dynamic (MD) simulations were performed for the complex of 2A7S with lead molecule Ib5 having greater binding affinity (-20.23 kcal/mol). To determine the binding strength of ligands within the 2A7S binding pocket after docking calculation, MD simulations were run up to 50ns period using Desmond[20, 21]. This process is useful to calculate forces, compute the motion of atoms. However, Desmond incorporates a more detailed temperature, pressure, volume system and has more functionality built-in for executing protein-ligand interactions. Using the system builder of Desmond in the Maestro program[16], the 2A7S-Ib5 complex system was immersed in a water-filled cubic box containing 34581 water molecules using extended simple point charge (SPC), a three-point water model within periodic boundary conditions. The total charge of the solvent system is neutralized by adding 11 sodium ions (Na+) to the complex systems.

Energy minimization is a very essential step in MD and it is done using the steepest descent method. Cubic box type (with box size 10 Å) is considered for the purpose of minimizing edge effects in a finite system to apply periodic boundary conditions. The atoms of the system to be simulated are put into the space-filling box, which is surrounded by translated copies of itself. OPLS-2005 force field (parameters used to describe the

potential energy of a system) is chosen which is an improved force field suited for molecular dynamics simulations of proteins.

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