Homology modeling and docking studies of 3-oxoacyl synthase II protein of Neisseria meningitidis

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Abstract- Meningitis disease is the inflammation of protective membrane covering the brain and spinal cord known collectively as the meninges. The bacterial meningitis disease has repeatedly causes outbreak worldwide. Neisseria meningitidis (NM) is major causative agent of bacterial meningitis. The 3 oxoacyl (acyl carrier protein) synthase II enzyme which involved in fatty acid biosynthesis of Neisseria meningitidis. This enzyme is involved in fatty acid synthesis and target to discovery of novel antibacterial agent. In this study insilico analysis was done. Modeller was used to generate three dimensional (3D) structure modeling of 3 oxoacyl synthase II using wild type E. coli fabF (KASII) (PDB ID- 2GFW) protein 3D structure. The validation of modeled structure protein was using structural analysis and verification server (SAVES) tools. Ligands selection was performed using Drugbank and PubChem databases used for the molecular docking of protein and ligands compounds. Present investigation may show good interaction energy values of protein and ligand to understand functional aspect to development of novel drug against Meningitis disease using insilico approach.

Index term- Docking, Inflammation, Insilico, KASII, Modeller, Neisseria meningitidis.

1. Introductions

Meningitis disease is a major cause of illness, developmental damage and death during childhood in developed countries [1]. In the last 20 years about one million infected meningitis diseases cases were reported among countries of the African Meningitis Belt, including 1,00,000 deaths. In the year, 2009 approximate 80,000 of these cases, including over 4,000 deaths, occurred. Every year, about 2,500 to 3,500 people become infected with Meningitis disease in the US, with a frequency of about 1 in 100,000. Meningitis is inflammation of the coverings around the brain and spinal cord. It is usually caused by a bacterial infection. The infection occurs most often in children, teens and young adults [2, 3]. Bacterial meningitis is not as common and life threatening infection. It needs to be treated as a right away to prevent brain damage and death. Neisseria meningitidis can be classified into 13 serogroups that are based on the composition of their polysaccharide capsular antigens and more than 99% of meningococcal infections are caused by serogroup A, B, C, 29E, or W135 [4, 5]. Fatty acid biosynthesis is one of the utmost conserved mechanisms of bacterial biosynthetic machinery.

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Fatty acid synthetic (FAS) pathways are divided into two different molecular forms called types I and II. The type II FAS systems in bacteria make the enzymes of this pathway attractive targets for antibacterial and essential for bacterial viability [6, 7]. The proteins and enzymes involved in type II Fatty acid synthesis and has attracted attention as a target for drug development. The three different types of 3-ketoacyl-ACP synthase (KAS) enzymes KAS I, KAS II and KAS III from Neisseria meningitidis catalyzes the primary condensation of malonyl CoA and acyl CoA during the elongation phase [8]. The β-ketoacyl-ACP is reduced to β-hydroxyacyl-ACP by the NADPH dependent FabG. The water is removed by FabA or FabZ dehydratases forming a trans 2-enoyl-ACP which reduced by Fab-I to form Acyl-ACP. The biosynthesis of 3 oxoacyl synthase II of N. meningitidis shows in Fig. 1.

Various enzymes responsible for the Fatty acid synthesis in diverse organism including Biotin carboxyl carrier (accB), Malonyl-CoA: ACP transcylase (fabD), 3-Ketoacyl-ACP synthes II (fabF) and 3-Ketoacyl-ACP reductase (fabG) [9, 10]. The three-dimensional (3D) structure of 3 oxoacyl synthase II protein of Neisseria meningitidis H44/76 is not yet known. At present there is no vaccine and appropriate drug available against group B organisms, which are the predominant cause of meningococcal disease in developed countries [11]. The recent work was designed to model the

3D structure of 3 oxoacyl synthase II of Neisseria meningitidis and target binding site with potential drug compound through insilico studies. The docking and binding interaction of protein and ligand were studied using Hex and ArgusLab Software.

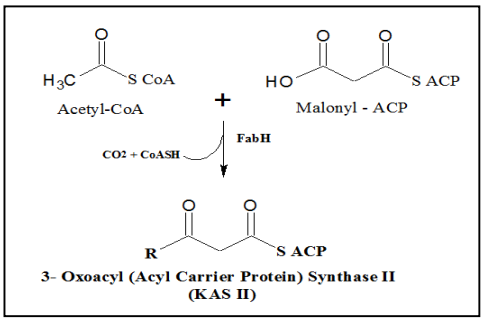


Fig. 1. Schematic representation of Fatty Acid Synthesis in N. Meningitidis showing the condensation reaction of acetyl CoA with malonyl ACP by FabH to form 3-Oxoacyl (Acyl Carrier Protein) Synthase II.

2. Materials and methods

2.1 Sequence retrieval

The three dimensional structure of 3 oxoacyl synthase II protein of N. Meningitidis, was not available. The homology modeling method was useful to modeled 3D structure of target protein. The primary sequence of 3 oxoacyl synthase II of Neisseria meningitidis serogroups B (Strain H44/76, ID: E6MWA6) was retrieved from Uniprot Protein sequence database at www.expasy.org [12]. The protein 3 oxoacyl synthase II sequence contains 415 amino acids.

2.2 Template searching

The target protein sequence 3 oxoacyl synthase II proteins were used to predict templates using BLASTp which is an online tool for searching similar sequence for structure modeling (http://blast.ncbi.nlm.nih.gov/Blast.cgi) [13]. The BlastP (protein query-protein database) comparison was done with the protein database (PDB). The wild type E. Coli Fabf 3 oxoacyl synthase II (PDB ID: 2GFW) was shows 60% sequence similarity and 4e-160 E-Value was suitable as template protein for insilico 3D structural modeling [14]. Multiple sequence alignment between the target and template sequence using Clustal W tools.

2.3 Homology modeling and model evaluation

The MODELLER V9.12 was used to generate the 3D structure model of required 3 oxoacyl synthase II protein [15, 16]. After the generated model was evaluated and verified by SAVEs server [17] which includes PROCHECK [18], ERRAT [19], Verify 3D [20], ProSA [21] and PROVE servers [22]. The Ramachandran plot generates stereochemical quality and overall G-factors of 3 oxoacyl synthase II protein [23].

2.4 Energy minimization

The energy minimization values of generated model were refined using YASARA package (Yet Another Scientific Artificial Reality Application, www.yasara.com). YASARA is particularly base on NOVA force field (protein + nucleotide optimization in vacuo) has been implemented as part of the newly developed interactive real-time molecular dynamics program. [24].

2.5 Ligand screening and molecular docking

The DrugBank [25] and PubChem compound database [26] were used to retrieve structure files of ligands compounds for the docking. Lipinski's rule of 5 is necessary screening analysis method for insilico drug design. Molecular docking analysis was carried out by HEX [27] and ArgusLab software [28]. The ligand compounds and 3 oxoacyl synthase II protein were geometrically optimized and docked using docking software of Hex6d and ArgusLab and inhibit its function [29]. The ligand compound and its analogues were docked with receptor protein using defaults parameters and message box displayed the e-total scores which showed the best score for a particular protein with ligands.

Results and discussion

3.1 Sequence analysis

The 3 oxoacyl synthase II protein of N. Meningitidis plays an important role of Fatty acid synthesis pathway which is useful for viability of bacterial cell. The homologous structure of 3 oxoacyl synthase II sequence was identified by using BLAST tool. The BlastP comparison was done with the protein database and template structure was selected. The wild type E. Coli Fabf (KasII) protein sequence shows 60% similarity was selected as a template structure for the homology modeling. The sequence alignment of target and template sequence was done by using online version ClustalW [30] as displayed in Fig. 2.

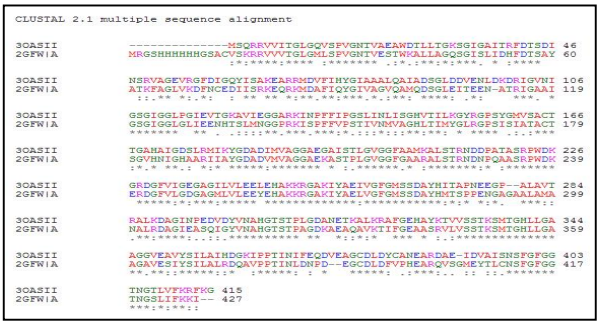


Fig. 2. Sequence Alignment of 3 oxoacyl (acyl carrier protein) synthase II protein and with template PDB ID 2GFW. The alignment shows a similarity of 60 % between the two obtained by using ClustalW program.

3.2 Structure analysis

The 3D structure of E.coli FabF protein (2GFW) was used as a template structure to developed 3D structure of 3 oxoacyl synthase II protein sequence. The alignment file of both sequences was submitted to MODELLER v9.12 to generate required model of target structure as seen in Fig. 3 [31]. The 3D structure of 3 oxoacyl synthase II protein consist of the 116 Alpha helix, 90 strands and 209 numbers of random coils. The energy minimization of generated model was refined by YASARA. Initial energy and Z-score of 3 oxoacyl synthase II protein was -60000.1 kJ/mol and -3.21 respectively, while the end energy of the model was -200402.2 kJ/mol with -0.92 Z-score (Fig. 4). The modeled protein was validate using SAVEs servers which provide Verify3D, ProSA, Prove, Errat and PROCHECK analysis. Finally, the structural and stereochemical properties of the target protein were validated using the score of Ramachandran plot which showed the percentage of residues present in core allowed and disallowed regions (Fig. 5). The good quality model would be expected to have over 90% in the most favored region in terms of Ramachandran Plot, in generated model it was found to be 91.5% and only 0.3% of residues were in disallowed region. Ramachandran plot score revealed model structure is of good quality [32]. The ERRAT was used to study overall quality factor of protein using statistics of non-bonded interaction between atoms in reported structure. In current case, the ERRAT score of overall quality factor was obtained as 86.241 as described in Fig. 6 which are very much satisfactory for high quality model [33].

The Verify 3D plot predicted 95.19% of the residues had an averaged 3D-1D score >0.2 that the model was of good quality and very less residues were found in the red region of the graph (Fig. 7). The protein volume evaluation (PROVE) analysis shows root means square (RMS) Z-score ≈1 suggesting good model quality [34]. The average z-score of generated model showed 1.480 and z-score RMS value was 36.23 (Fig. 8). ProSA tool confirm the quality and potential error of generated three dimensional structure as more reliable and within an acceptable range. The range of generated protein model was 1.48 and z-score of -8.96 which was within the range (Fig. 9). The above results of entire structure validation program indicate that the homology model is reliable for further study of drug design [35].

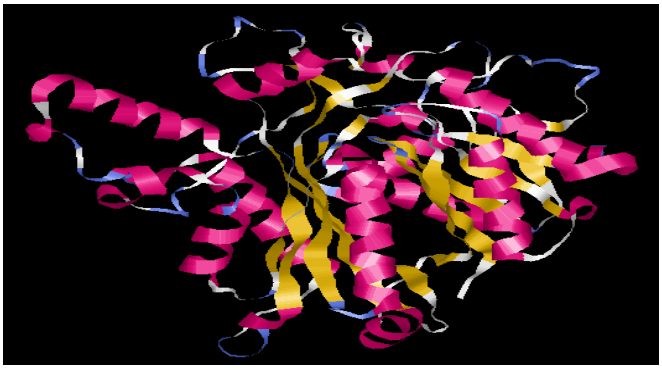


Fig. 3. 3D structure of 3OASII generated by Modeller V 9.12, on the basis of template 2GFW.

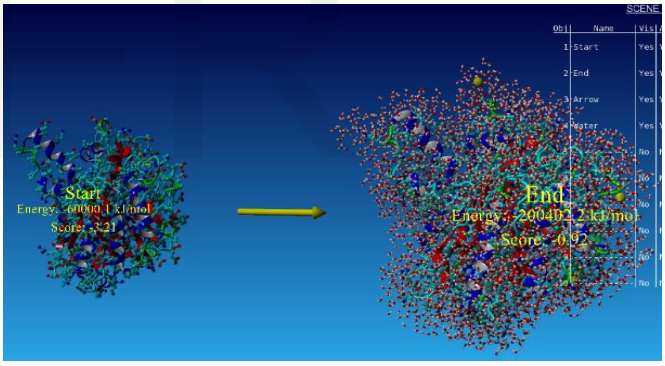


Fig. 4. Energy minimized structure analysis using YASARA of 3OASII protein of N. meningitidis.

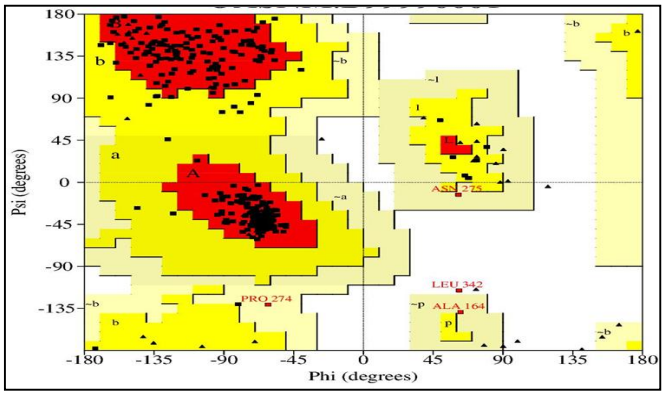


Fig. 5. Ramachandran Plot analysis of 3OASII protein which generated from Modeller v9.12. The additionally allowed, generously allowed and disallowed regions are indicated as yellow, light yellow and white fields respectively. But the most favored regions are colored red.

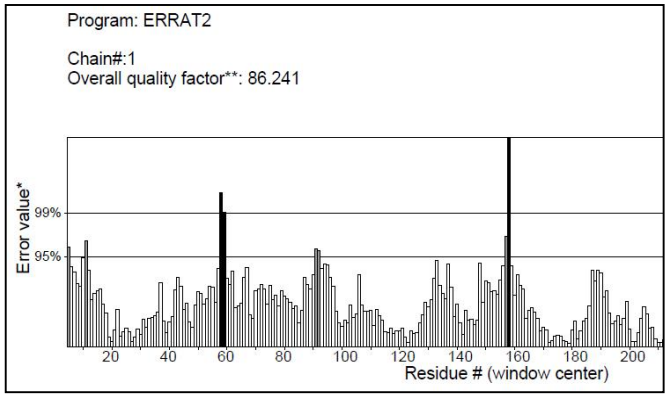


Fig. 6. The overall quality factor results in ERRAT show model quality of 86.241%.

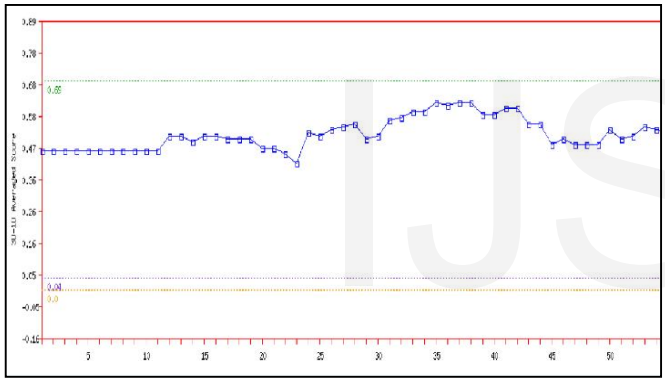


Fig. 7. The Verify3D plot predicted 95.19% of the residues had a good 3D-1D score.

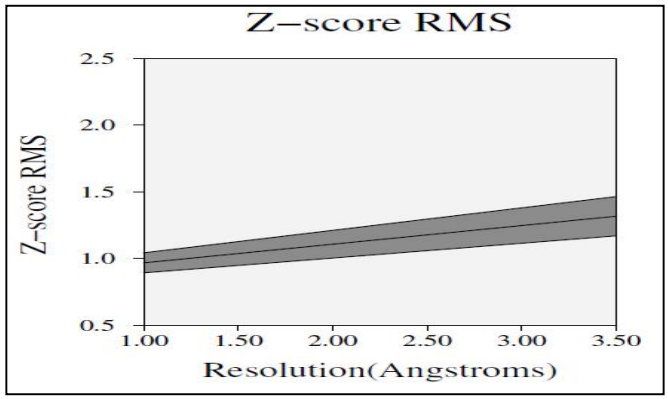


Fig. 8. The Prove result shows RMS Z-score was 1.48 which indicates the model is good.

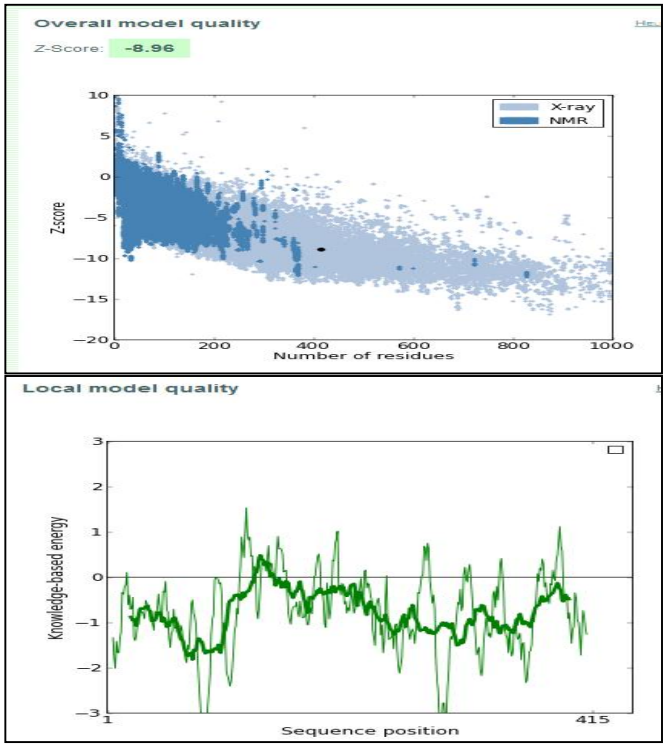
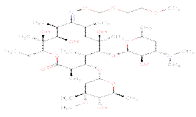


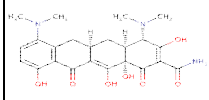
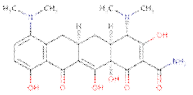
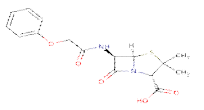
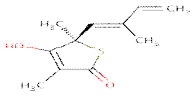
Fig. 9. ProSA server output Z score plot and Energy profile plot

3.3 Virtual screening of Lead compound

Virtual ligand screening is useful for successful strategy of structure base drug designing [36]. Five chemical compounds viz. Roxithromycin, Latamoxef, Minocycline, Penicillin V and Thiolactomycin were screened against 3 oxoacyl synthase II protein. These ligand molecules were selected from DrugBank and PubChem compound databases respectively given in Table 1 and conversion of SDF to PDB (Protein Data Bank) format carried out using Open babel program. Protein and ligands docking analysis indicates that these molecules can bind to the drug target efficiently and could be potential drug for N. meningitis.

Table 1. The chemical compound showing ID, name, formula and structure according to their Drug Bank database and PubChem Compound database.

Sr. No.	Compound ID	Compound Name	Molecular Formula	Structure	Ref No.
1	DB00778 / CID5480431	Roxithromycin	C41H76N2O15		37

2	DB04570 / CID47499	Latamoxef	C20H20N6O9S		38
3	DB01017 / CID54675783	Minocycline	C23H27N3O7		39
4	DB00417 / CID6869	Penicillin V	C16H18N2O5S		40
5	DB04302 / CID445629	Thiolactomycin	C11H14O2S		41

3.4 Lipinski's Rule of Five

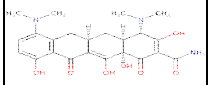
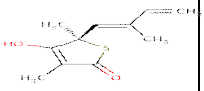
Further, screening the all five ligands for "Lipinski's rule of 5" on the drug likeliness properties is listed in table 2 [42]. This rules states that most of 'drug-like' compounds have molecular weight <500, hydrogen bond donors <5, hydrogen bond accepters <10 and logP <5 as represented in Table 2. On the basis of binding affinity and drug like properties, two ligands were finally screened.

Table 2. Lipinski's Rule of Five.

		Lipinski's Rule of Five				
Sr. No	Compound Name	hydrogen bond donors	Hydrogen bond acceptors	molecular weight	logP value	Rotational bond
1	Roxithromycin	5	16	837.04	2.9	13
2	Latamoxef	4	12	520.47	0.22	9
3	Minocycline	5	9	457.47	0.05	3
4	Penicillin V	2	5	350.39	2.09	5
5	Thiolactomycin	1	2	210.29	2.14	2

The two compounds which satisfied the "Lipinski's rule of 5" on the basis of binding affinity and drug like properties were selected as good compounds for docking process. Minocycline and thiolactomycin were selected as ligands to the docking process with 3 oxoacyl synthase II protein of Neisseria meningitidis as seen in Table 3.

Table 3. The selected compounds using Lipinski's Rule of Five.

Compound ID	Compound Name	Molecular Formula	Structure
DB01017 / CID54675783 Minocycline	(4S,4aS,5aR,12aS)-4,7bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide	C23H27N3O7	
DB04302 / CID445629 Thiolactomycin	(2R)-5-hydroxy-2,4-dimethyl-2-[(1E)-2methylbuta-1,3dienyl]thiophen-3-one	C11H14O2S	

3.5 Docking analysis

Docking studies were performed with the help of Arguslab and Hex. In this study, energy minimized 3 oxoacyl synthase II protein model of Neisseria meningitidis was docked with minocycline and thiolactomycin ligand compounds. Among the 100 different direction that were generated by Hex and arguslab, the best one was selected based on total docking energy. This investigation helps to understand interactions between protein and ligands along with their binding mode of action as depicted in Fig. 10. Docking results of Minocycline compound with 3 oxoacyl synthase II protein using ArgusLab and Hex software were -7.1865 and -236.88 respectively. The second ligand compound Thiolactomycin docking result were -7.66 and -190 respectively as shown in Table 4. The Hex software gives corresponding e-values for each docking. More negative the e-value more efficient is the docking [43]. Minocycline & Thiolactomycin showed higher affinity towards the 3 oxoacyl synthase II protein. It gave an e-value of -236.88 and -190 respectively which is the most negative among docking. In present study, it was found that several amino acid residues in 3 oxoacyl synthase II protein were observed to play an active role in the interaction with both ligand compounds.

Table 4. The chemical compounds showing interaction energy (kcal/mol) of 3 oxoacyl synthase II protein using docking software.

Compound Name	ArgusLab (e-value) kcal / mol	Hex(e-value) kcal / mol
DB01017 / Minocycline	-7.1865	-236.88
DB04302 / Thiolactomycin	-7.66	-190

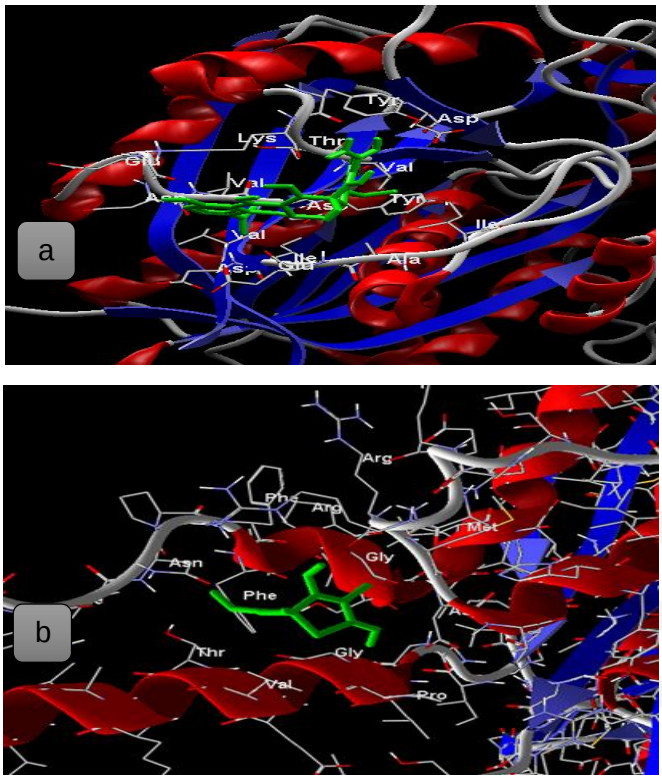


Fig. 10. Docking analysis of 3 oxoacyl synthase II protein of Neisseria meningitis with suitable ligands selected from Drugbank database using Arguslab and Hex software. (a) The Minocycline ligand docked in the active site of 3 oxoacyl synthase II protein. (b) The Thiolactomycin ligand docked in the active site of 3 oxoacyl synthase II protein.

The following amino acid residues in 3 oxoacyl synthase II protein were observed to play an important role in the interaction with the minocycline ligand which are GLU-295, ILE-391, VAL-393, ASP-296, ASP-392, VAL-297, VAL-330 and ASP-298. The following amino acid residues of protein were observed active role interaction with thiolactomycin ligand which are GLY-115, ARG-69, ALA-68, MET-71, ASP-72 and ASN-132 as shown in Fig. 11 A and B. The four and two hydrogen bonds interaction with hydrogen bond distance as shown in Table 5.

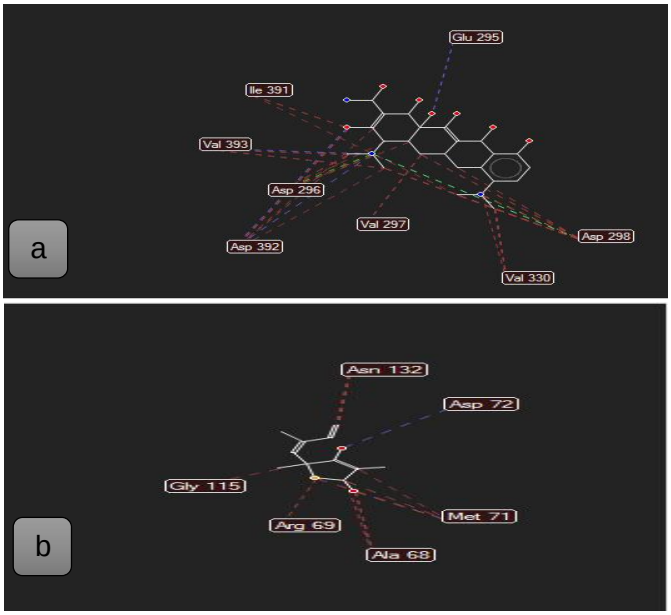


Fig. 11. Hydrogen bonds (Blue sticks) and steric interaction (Red Sticks) of amino acid and ligands compounds.(a) Minocycline (DB01017) (b) Thiolactomycin (DB04302)

Both ligands showed good binding score with hydrogen bond interaction with target protein because hydrogen bond plays an important role for the structure and function of biological molecule which work like as inhibitor. Thus, designed models in the current work could be used for further analysis by some more user friendly, visualizing and manipulating softwares like Molegro molecular viewer a displayed in Fig. 12 and 13.

Table 5 The active amino acid residues and H-bonds interaction between chemical compounds and 3 oxoacyl (acyl carrier protein) synthase II (3OASII) protein.

Compound Name	Amino acid residues in Protein	H2bonds	Distance of H2 Bonds (Ao)
DB01017 / Minocycline	GLU-295, ILE-391, VAL-393, ASP-296,ASP-392, VAL-297, VAL-330, ASP-298	GLU-295 VAL-393 ASP-392 ASP-298	2.74 2.96 2.12 3.23
DB04302 / Thiolactomycin	GLY-115, ARG-69, ALA-68, MET-71, ASP-72, ASN-132	ASP-72 MET-71	3.41 2.98

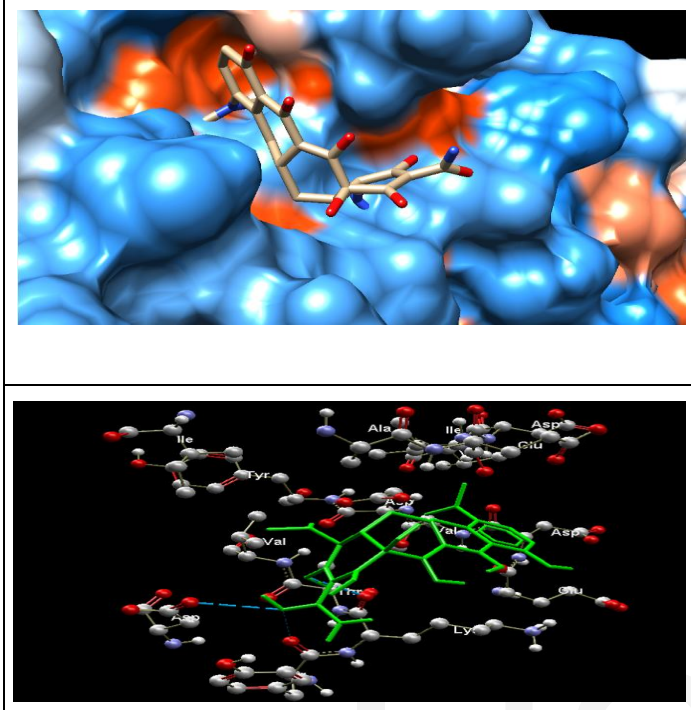


Fig. 12. Docking result for the interaction of 3OASII protein of Neisseria meningitis and ligand Minocycline which shows active site residues.

using Modeller and docking with Arguslab and Hex software.

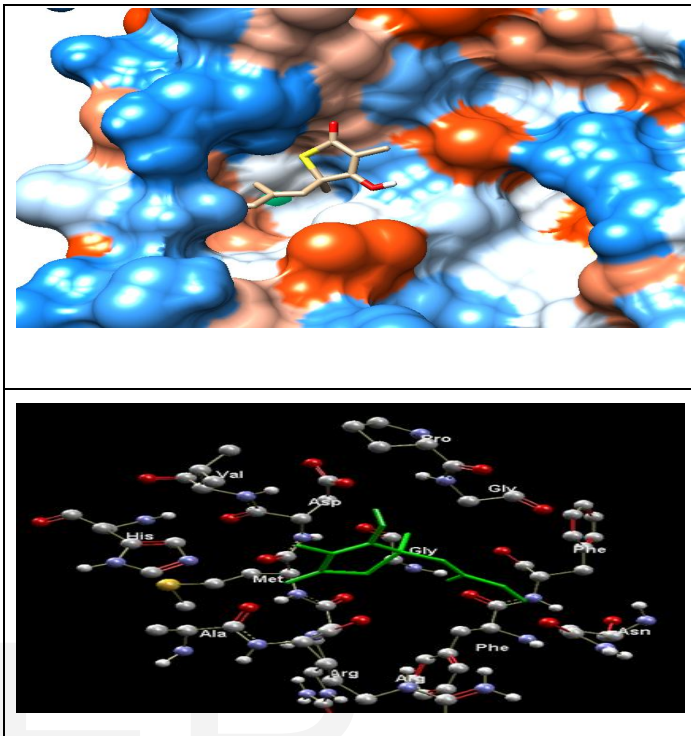


Fig. 13. Docking result for the interaction of 3OASII protein of Neisseria meningitis and ligand Thiolactomycin which shows active site residues

Conclusion

The three dimensional structure of 3 oxoacyl synthase II protein of Neisseria meningitidis serogroup B (Strain H44/76) has not been known. 3D structure of 3 oxoacyl synthase II protein was built by homology modeling, which was based on the known structure of wild type E. coli fabF (KASII) (PDB ID- 2GFW). Then SAVEs analysis verification of model using Ramachandran plot showed that most of these residues are in favored regions of the plot and energy minimization were used to refine the structure. The protein-ligand interaction plays a very significant role in structural based drug designing. The ligands which were docked with protein observed as suitable inhibitor candidates by their docking e-values. In the present work we have taken the two ligand compounds minocycline and thiolactomycin which showed significant interactions with actives sites of the drug target 3 oxoacyl synthase II protein of Neisseria meningitidis. Present study was carried out to generate the 3D structure of 3 oxoacyl synthase II protein

From this study we can conclude that some of the modified drugs are better than the existing drugs compounds. Virtual screening against these ligand compounds might be useful for the discovery of novel therapeutic compounds against Meningitis disease. In future work the ADME/T (Absorption, Distribution, Metabolism, Excretion / Toxicity) properties can be studied experimentally to identify their efficiency in binding and inhibiting the protein.

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