IN SILICO MOLECULAR MODELING AND DOCKING STUDIES OF AG85A PROTEIN WITH 3,5-DINITROBENZYLSULFANYL 1,3,4-OXIDIAZOLES COMPOUND

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ABSTRACT:
Tuberculosis (TB) caused by the bacterium *Mycobacterium tuberculosis* remains one of the most lethal infectious diseases even now. The increase in the development of multi-drug resistant by bacterium, created an urgent need for the discovery of natural novel anti-tuberculosis molecules. The aim of the current study is to evaluate the inhibitory activity of the molecules 3, 5-Dinitrobenzylsulfanyl 1, 3, 4-oxidiazoles towards tuberculosis protein Ag85A through *insilco* analysis. The 2D structure of 3, 5-Dinitrobenzylsulfanyl 1, 3, 4-oxidiazoles were retrieved through literature studies. The structure was sketched and converted in 3D structure using ACD Chemketch. The 3D structure of the protein Ag85A was predicted using molecular modeling technique because till now no proper 3D structure was documented for the protein. Further the modeled structure was validated based on Ramachandran plot using Rampage. The catalytic site of the modeled protein was predicted using Metapocket server. Finally the receptor and ligands were prepared for the step docking process. Docking studies was performed using Arguslab software and their binding interactions were visualized using PyMol. Thus the result predicted that the compound 3,5-Dinitrobenzylsulfanyl 1,3,4-oxidiazoles exhibited the least binding energy -12 Kcal/mol by the three best hydrogen bond interactions with the protein Ag85A. Hence, this study could be conclude that the molecule 3, 5-Dinitrobenzylsulfanyl 1, 3, 4-oxidiazoles might act as the potent lead candidate in designing of new drugs against tuberculosis.

Keywords: Tuberculosis, 3, 5-Dinitrobenzylsulfanyl 1, 3, 4-oxidiazoles, Ag85A, ACD Chemsketch, Homology modeling, Ramachandran plot, Docking.

1. INTRODUCTION:

Tuberculosis is the second leading disease in the world [1] and according to the World Health Organization (WHO), the highest number of deaths were caused by tuberculosis when compared with number of deaths caused by the human immune deficiency virus (HIV) [2, 3]. The latest report of WHO, stated that 9.6 million new TB cases and 2 million TB deaths were occurred between the year of 2010-2015 [4]. From the latest review on tuberculosis proteins, it was identified that the five proteins: protein 27(p27), Antigen 58A (Ag85A), PEPGRS1, PEPGRS33 and MT1866 is responsible for the TB [5]. The literature study on the protein revealed that the protein Ag85A was more virulent than other proteins [6].

Ag85A are responsible for the high affinity of mycobacteria for fibronectin (adhesive glycoprotein) which enables the attachment of *Mycobacterium tuberculosis* to murine alveolar macrophages (AMs) [7]. Ag85A also help to maintain the integrity of the cell wall by catalyzing the transfer of mycolic acids to arabinogalactan [8]. The protein AG85A responsible for the transfer of a mycoloyl residue from one molecule of α, α-trehalose monomycolate (TMM) to another molecules of TMM, leading to the formation of α, α-trehalose dimycolate (TDM) [9]. The transformation of the molecules (TMM) by the protein Ag85A was one of main reason for acquiring Tuberculosis disease [10].

Some strains present in the *Mycobacterium tuberculosis* developed resistance capacity towards the currently prescribed standard drugs through genetic changes occurred in the gene of the strains [11, 12]. The reason for the development drug resistance may be due to following reasons: 1) Incorrect or insufficient treatment with diagnosing the disease, 2) not completing the full course of medicine as prescribed by doctors. 3) Irregular intake of medication, 4) not preferring medicine due to its side effects [13, 14]. The latest study explored that the 75% of TB cases was due to resistant TB strains present in the environment which directly transmitted from an infected person to an uninfected person [15]. So there was an urgent need for the discovery of the novel TB compound without any side effects

From the last few decades, the progress in search of new anti-TB drug has been increased massively. The research on new TB drugs plays a very vital role in reducing the occurrence of TB and also to pass all the guidelines that are followed by the WHO [16]. Through literature studies, the compound 3,5-dinitrobenzylsulfanyl-1,3,4-oxadiazoles has been recognized as the promising antitubercular agents [17] and *in vitro* studies explain the strong functional activity of the compound against strains of replicating and nonreplicating *Mycobacterium tuberculosis* [18-20]. The compound showed better active against dormant mycobacteria, exhibited low toxicity, non-mutagenic activity, inhibiting the synthesis of DNA and finally revealed low cytotoxicity activity against cell line of HuH7 (human hepatocellular carcinoma), A431 (human epidermoid carcinoma), HepG2 (human hepatocellular carcinoma) and MDCK (Madin-Darby canine kidney cells).

Homology modeling technique has become as one of important choice for predicting 3D coordinates of proteins. The four consecutive steps to be followed in modeling process [21]. They are as follows: 1) template selection, 2) target-template alignment, 3) model construction and 4) model assessment. The main aim of modeling studies was to design the structure for the protein molecules which energetically act to

compound in the course of binding [22]. *In silico* modelling develop the computerized 3D structure for the protein based on the template sequence or structure related to the target protein.

The specific feature of docking studies was to execute the outcome of the structural relationship of how receptor is blocked by ligand which plays a vital role in lead optimization [23]. The latest available docking programs and algorithms are capable of predicting the activities of small molecules and protein which help the researchers to find more efficient drug candidates. In computational program 'dock' play a major role in complex formation between small molecules and structures of targets and 'score' provides their potential binding interaction between the complex which result in hit identification and lead optimization of small molecule [24]. So docking analysis were consider to very essential in selecting the specify drug lead candidate against the target.

In the present work the homology modeling was performed for the AG85A protein to predict the 3D structure for the protein. Then the modeled structure was docked with anti-tuberculosis compound 3,5-dinitrobenzylsulfanyl-1,3,4-oxadiazoles, to predict inhibitory efficiency of the compounds towards the modeled protein Ag85A.

2. MATERIALS AND METHODS

2.1 Analysis of protein sequence:

1. Uniprot Database:

For *in silico* characterization, the fasta file format of the protein sequence Ag85A of Homo was retrieved from Uniprot database (http://www.uniprot.org). The Uniprot is the Universal Protein resource of protein data created by the combination of three databases, they are as follows: the Swiss-Prot, TrEMBL and PIR-PSD databases. Uniprot database contain a large amount of information about the protein function, domain, structure derived from the various research literature

2. Prosite database:

The protein sequence retrieved from uniprot database was submitted to Prosite database (http://prosite.expasy.org) to predict the domains, families and functional sites of the protein. Prosite database comprises of various entries of amino acid patterns, signature, and profiles in the protein sequence.

3. Sopma Database:

The retrieved protein sequence was further analyzed for its secondary structures of proteins using SOPMA database (http://npsa-prabi.ibcp.fr.NPSA/npsa-sopma). SOPMA (Self-Optimized Prediction Method with Alignment) database first analyze the protein primary structure and then predict protein secondary structures.

2.2 Template selection and alignment of the target:

The first and foremost task in homology modeling was to recognition the appropriate protein structures similar to the target protein sequence. BlastP (http://www.ncbi.nlm.nih.gov/blast/) was performed for the identifications of 3D protein template structure against PDB (Protein databank database). An appropriate template structure was retrieved based on its alignment and similarity score.

2.3 Prediction of 3D structure and its validation:

1. Swiss Pdb Viewer:

In order to construct a 3D structure for the protein sequence Ag85A is achieved Homology modeling software Swiss Pdb Viewer. Homology modeling was one of the best technique to design the 3D structure of the target based on the fact that the evolutionary related proteins sequence may share a similar 3D structure. In Swiss PDB viewer software, there are 16 steps to be followed for predicting the 3D structure of known protein using protein sequence and structure. They are listed below:

Step 1: Launch SwissPdbViewer and choose the “SwissModel” from Toolbar Menu and select “Load Raw Sequence from Amino Acid” To load Fasta file of the protein.
Step 2: Choose the “Preferances” Menu and select “SwissModel” Menu and Enter Name, Email, address and select ok.
Step 3: Choose “Wind” Menu and select “Control Panel” to view the residues amino acids and select the appropriate residues.
Step 4: Choose the “Swiss Model” Menu and select “Submit Template Search Request against EXPDB from current layer” and enter the password in Dialog box.
Step 5: Choose “File” Menu and select “Open Pdb File” .
Step 6: Choose “Wind” Menu and select “Contol Panel”, select “Visible Checkbox” for the visualization of protein structure.
Step 7: Choose the “Magic Fit” item of the “Fit” Menu.
Step 8: Choose “Wind” Menu and select “Alignment”
Step 9: Choose “Select” Menu and select “Residues Making Clashes”
Step10: Choose “Tool” Menu and select “Fit Selected SideChain” → quick and dirty.
Step 11: Choose “Swiss Model” Menu and select “Submit Modelling Request for Raw Sequence” and enter the Project title in “Get String” Dialog box.
Step 12: Choose “File” Menu and select “Save” → Current Layers and Save Model structure in .pdb file format.
Step 13: View the result in PyMol.
Step 14: In PyMol, Select “file” → “Open” →.pdb file (Ex: modelprotein.pdb).
Step 15: From the model protein, predict the helix, sheet and loop by clicking “C”(Color)→by “SS”(Secondary Structure) →”Helix Sheet Loop”.
Step 16: Click “Action” → “preset”→ “Ligand sites” → “cartoon”.

2. Rampage:

The modeled 3D structure of the protein Ag85A was evaluated through Ramachandran plot via Rampage server (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php). The Ramachandran plot provides the percentage of residues that are present in either of the favored, allowed and disallowed regions [25]

2.4 Prediction of catalytic site:

The catalytic sites of modelled protein were predicted using metapocket server (http://projects.biotec.tu-dresden.de/metapocket/). The server predicts the possible catalytic regions on protein surface for protein-ligand binding.

2.5 Preparation of Ligand:

Through *invitro* study, the molecules 3,5-Dinitrobenzylsulfany 1,3,4-oxidiazoles were found to exhibit the good inhibitory activity towards *Mycobacterium tuberculosis* The 2D structure of the compound is not available in pubchem database. The 2D structure of the molecule was retrieved from the literature and was sketched, converted in to .mol file format using ACD/ChemSketch software. ACDchemsketch is chemical drawing software used to draw and modify structure of compound. The software also display the 2 & 3 dimensions structure, nature of the functional groups, structure of chemical bonds, Physiochemical properties of the molecule.

2.6 Docking studies:

Docking studies with compounds like 3, 5-Dinitrobenzylsulfany 1, 3, 4-oxidiazoles against Ag85A protein of tuberculosis were performed using Arguslab docking software [26]. In ArgusLab software, there are 11 steps to be followed for docking studies. They are listed below:

Step 1: Install ArgusLab from the web browser.
Step 2: Open ArgusLab and Select "New" from the "File" Menu.
Step 3: Select File → Open, Pdb file of protein (Eg: model.pdb).
Step 4: From the "Residues"→ "Misc" and hide the molecules.
Step 5: From the "Residues"→ "Amino acid" and select appropriate aminoacid for the given protein from the result of Metapocket.
Step 6: Select active sites of the protein → make a group from this Residues → Binding site, give a name and click ok.
Step 7: Select File → open, mol file of compound (eg: Comp.mol)
Step 8: Select Residues → Misc → Make a Ligand Group From this Residue.
Step 9: Select Calculation → Dock a Ligand
Step 10: Select GA Dock, change the size of grid and click on "Start".
Step 11: Save the result in .pdb format.

2.7 Docking Interaction:

The docking interaction between receptor and compound were performed using t PyMOl software. PyMoL is the graphical molecular visualization software, used mainly to visualize docking interaction between compound and protein. It also reveals the distance of hydrogen bond information which formed between ligands and receptors.

3. RESULT AND DISCUSSION:

3.1 Analysis of protein sequence:

The protein sequences of Antigen58A were retrieved with Accession: P9WQP3 from SwissProt database. From the prosite database, the twin arginine translacation (Tat) signal domain were predicted between the sequence length of 1 to 43. The result of SOPMA database revealed that random coil are dominant over all the other secondary structures followed by alpha helix, extended strand, and beta turns. From the result it was clearly predicted the function of protein A58A quite be unstable because functional region may fall under the secondary structure of random coils (Figure 1).

3.2 Template selection and alignment of the target:

The FASTA format of protein sequence Ag85A was submitted to BlastP (Basic Local Alignment Search Tool Protein) program. The BlastP search resulted with the best match of PDB (Protein DataBank) ID: 1SFR of chain A was selected as a template structure to predict the 3D structure of the tuberculosis protein Ag85A (Figure 2).

3.3 Prediction of 3D structure and its validation:

The three dimensional structure of antigen58A was generated using the template structure 1SFR chain A with loop region refined, water molecules and heteroatoms present in the structure were removed using PyMol Software. The RMSD values generated by modeled structure and template structure were found to be 0.19 Å after super imposing both the structures. The 3D modeled structure of Ag85A was viewed in PyMol. The Ramachandran plot was generated for the model protein from the plot, it was revealed that the majority of the amino acids are in the Phi-Psi distribution. The 95.1% of the amino acids were found within the favored regions of the plot , 4.9% of the amino acids were found within the allowed regions of the plot and no amino acid were found in outlier region of the plot (Figure 3). From the result of rampage it was confirmed that the predicted model is reliable and also good quality.

Figure1: Result of Analysis of protein sequence

Fasta sequence of the protein Ag85A: >sp|P9WQP3|A85A_MYCTU Diacylglycerol acyltransferase/mycolyltransferase Ag85A OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv) GN=fbpA PE=1 SV=1

MQLVDRVRGAVTGMSRRLVVGAVGAALVSGLVGAVGGTATAGAFSRPGLPVEYLQVPSPSMGRDIKVQFQSGGANSPALYLLDGLRAQDDFSGWDINTPAFEWYDQSGLSVVMPVGGQSSFYSDWYQPACGKAGCQTYKWETFLTSELPGWLQANRHVKPTGSAVVGLSMAASSALTLAIYHPQQFVYAGAMSGLLDPSQAMGPTLIGLAMGDAGGYKASDMWGPKEDPAWQRNDPLLNVGKLIANNTRVWVYCGNGKPSDLGGNNLPAKFLEGFVRTSNIKFQDAYNAGGGHNGVFDFPDSGTHSWEYWGAQLNAMKPDLQRALGATPNTGPAPQGG

Prosite Database

sp-P9WQP3-A85A_MYCTU (338 aa)

sp-P9WQP3-A85A_MYCTU (sp-P9WQP3-A85A_MYCTU) TAT (338 aa)

PS51318 TAT *Twin arginine translocation (Tat) signal profile*

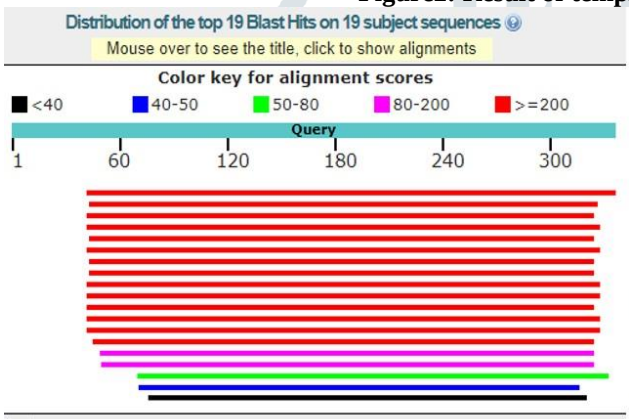
1 - 43: score = 7.684

MQLVDRVRGAvtGMSRRLVVGAVGAALVSGLVGAVGGTaTAGA

SOPMA Database

Alpha helix	(Hh) :	97 is	28.70%
3_{10} helix	(Gg) :	0 is	0.00%
Pi helix	(Ii) :	0 is	0.00%
Beta bridge	(Bb) :	0 is	0.00%
Extended strand	(Ee) :	64 is	18.93%
Beta turn	(Tt) :	38 is	11.24%
Bend region	(Ss) :	0 is	0.00%
Random coil	(Cc) :	139 is	41.12%
Ambiguous states (?)	:	0 is	0.00%
Other states	:	0 is	0.00%

Figure2: Result of template search using BlastP



Pairwise alignment between the sequence of Ag58A and 1SFR

Chain A, Crystal Structure Of The Mycobacterium Tuberculosis Antigen 85a Protein

Sequence ID: 1SFR_A Length: 304 Number of Matches: 1

▸See 2 more title(s)

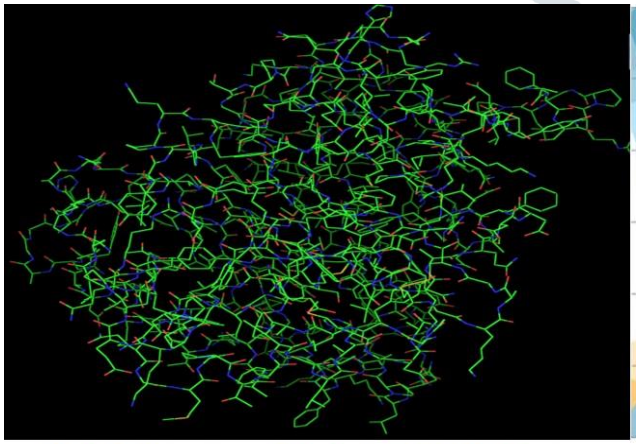
Range 1: 1 to 295 GenPept Graphics

Score	Expect	Method	Identities	Positives	Gaps
608 bits(1569)	0.0	Compositional matrix adjust.	295/295(100%)	295/295(100%)	0/295(0%)

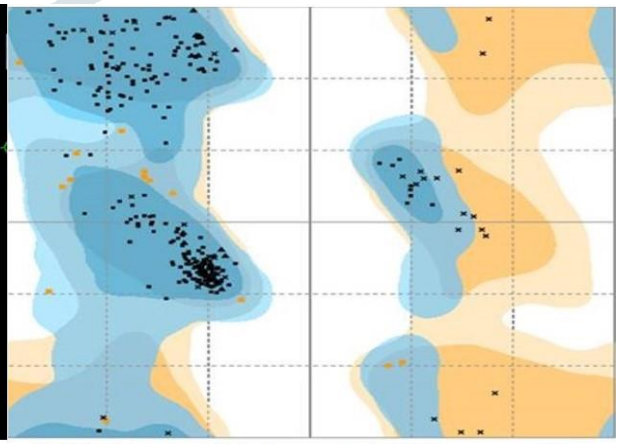
```
Query  43   AFSRPGLPVEYLQVPSPSMGRDIKVQFQSGGANSPALYLLDGLRAQDDFSGWDINTPAFE  102
            AFSRPGLPVEYLQVPSPSMGRDIKVQFQSGGANSPALYLLDGLRAQDDFSGWDINTPAFE
Sbjct  1    AFSRPGLPVEYLQVPSPSMGRDIKVQFQSGGANSPALYLLDGLRAQDDFSGWDINTPAFE  60

Query  103  WYDQSGLSVVMPVGGQSSFYSDWYQPACGKAGCQTYKWETFLTSELPGWLQANRHVKPTG  162
            WYDQSGLSVVMPVGGQSSFYSDWYQPACGKAGCQTYKWETFLTSELPGWLQANRHVKPTG
Sbjct  61   WYDQSGLSVVMPVGGQSSFYSDWYQPACGKAGCQTYKWETFLTSELPGWLQANRHVKPTG  120

Query  163  SAVVGLSMAASSALTLAIYHPQQFVYAGAMSGLLDPSQAMGPTLIGLAMGDAGGYKASDM  222
            SAVVGLSMAASSALTLAIYHPQQFVYAGAMSGLLDPSQAMGPTLIGLAMGDAGGYKASDM
Sbjct  121  SAVVGLSMAASSALTLAIYHPQQFVYAGAMSGLLDPSQAMGPTLIGLAMGDAGGYKASDM  180

Query  223  WGPKEDPAWQRNDPLLNVGKLIANNTRVWVYCGNGKPSDLGGNNLPAKFLEGFVRTSNIK  282
            WGPKEDPAWQRNDPLLNVGKLIANNTRVWVYCGNGKPSDLGGNNLPAKFLEGFVRTSNIK
Sbjct  181  WGPKEDPAWQRNDPLLNVGKLIANNTRVWVYCGNGKPSDLGGNNLPAKFLEGFVRTSNIK  240

Query  283  FQDAYNAGGGHNGVFDFPDSGTHSWEYWGAQLNAMKPDLQRALGATPNTGPAPQGG  337
            FQDAYNAGGGHNGVFDFPDSGTHSWEYWGAQLNAMKPDLQRALGATPNTGPAPQGG
Sbjct  241  FQDAYNAGGGHNGVFDFPDSGTHSWEYWGAQLNAMKPDLQRALGATPNTGPAPQGG  295
```

Description	Max score	Total score	Query cover	E value	Ident	Accession
Chain A, Crystal Structure Of The Mycobacterium Tuberculosis Antigen 85a Protein	608	608	87%	0.0	100.00%	1SFR_A
Chain A, MYCOBACTERIUM TUBERCULOSIS ANTIGEN 85B WITH TREHALOSE	502	502	84%	0.0	82.39%	1F0P_A
Chain A, Crystal Structure Of Antigen 85c In Presence Of Ebselen	411	411	83%	1e-144	69.26%	4MQM_A
Chain A, Crystal Structure Of Antigen 85c-s124a Mutant	410	410	84%	1e-144	68.99%	4QEK_A

Figure 3: Predicted modeled 3D structure and its validation of Ag85A:



Modeled Structure of the Protein "Ag85A" were viewed using PyMol Software



Validation of modeled protein Ag58A generated by RAMPAGE server.

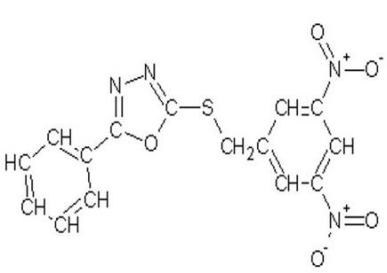
Docking Studies:

The 46 catalytic sites were predicted for the modelled protein Ag85A through metapocket database (Table 1). The predicted catalytic regions could act as the binding sites for the compound. The compound 3,5-Dinitrobenzylsulfany 1,3,4-oxidiazoles were constructed and physiochemical chemical properties of the compound were retrieved using ACD Chem Sketch and the 2D structure were optimized for docking studies (Figure 4).

Figure 4: Catalytic site of Ag85A, 2D Structure & Physiochemical Properties of 3, 5-Dinitrobenzylsulfany 1, 3, 4-oxidiazoles

RESI	SER_A^126^	MET_A^127^	SER_A^130^	GLY_A^151^	LEU_A^152^
RESI	PRO_A^155^	PHE_A^78^	ILE_A^164^	SER_A^150^	LEU_A^163^
RESI	PHE_A^228^	PHE_A^232^	VAL_A^233^	GLY_A^160^	SER_A^131^
RESI	LEU_A^229^	ALA_A^167^	HIS_A^262^	LEU_A^166^	THR_A^162^
RESI	LEU_A^42^	PRO_A^225^	GLY_A^165^	ASP_A^170^	GLY_A^41^
RESI	ARG_A^43^	TRP_A^267^	LEU_A^125^	TRP_A^264^	ALA_A^226^
RESI	ASN_A^223^	SER_A^263^	ASP_A^40^	ASN_A^54^	ALA_A^44^
RESI	GLY_A^50^	GLN_A^45^	SER_A^49^	PHE_A^48^	ILE_A^53^
RESI	ASP_A^46^	ASP_A^47^	TRP_A^180^	ASN_A^191^	SER_A^156^
RESI	TRP_A^188^				

Catalytic site of the protein Ag58A predicted by Metapocket



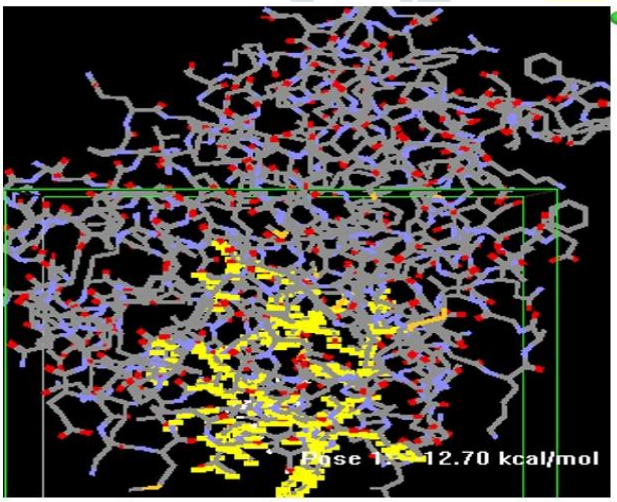
Molecular Formula	= $C_{15}H_{10}N_4O_5S$
Formula Weight	= 358.3287
Composition	= C(50.28%) H(2.81%) N(15.64%
Molar Refractivity	= 88.95 ± 0.4 cm^3
Molar Volume	= 230.9 ± 5.0 cm^3
Parachor	= 702.4 ± 6.0 cm^3
Index of Refraction	= 1.696 ± 0.03
Surface Tension	= 85.5 ± 5.0 dyne/cm
Density	= 1.55 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 35.26 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 358.037189 Da
Nominal Mass	= 358 Da
Average Mass	= 358.3287 Da

2D structure and physiological properties of the compound 3,5-Dinitrobenzylsulfany 1,3,4-oxidiazoles by ACD ChemSketch

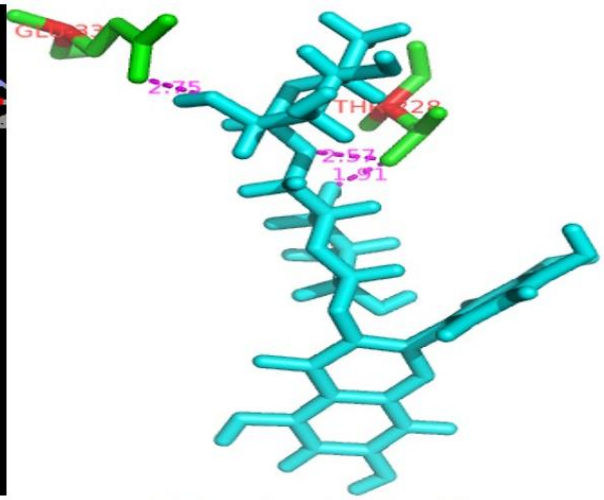
3.4 Docking analysis:

Docking studies were performed between the compounds 3, 5-Dinitrobenzylsulfany 1, 3, 4-oxidiazoles and tuberculosis protein Ag85A using Argus lab. Docking result revealed the compounds 3, 5-Dinitrobenzylsulfany 1, 3, 4-oxidiazoles exhibited the better binding energy -12.70 Kcal/mol forming three hydrogen bonds interaction with protein Ag85A. On analyzing the hydrogen bonds interaction using PyMol, the NH_2 atom present in the backbone of Glutamic acid 23 bounded to the NH_2 atom of compound with a distance of 2.35 Å. The oxygen atom of threonine 28 bounded to the Hydroxyl group of the compound with the distance of 2.57 Å and 1.91 Å (Figure 5). From the result of the docking studies it was clearly exposed that the 3,5-Dinitrobenzylsulfany 1,3,4-oxidiazoles exhibit the better binding energy and interactions with the protein Ag85A.

Figure 5: Result of docking studies between ligand and receptor



Docking Studies using Argus Lab



Docking interaction using PyMol

4. CONCLUSION:

Novel molecule should targets new functional site of the tuberculosis protein with unique inhibitory action thereby limiting the development of multi–drug resistance. In current study, the 3D structure was predicted for the tuberculosis protein antigen 85 proteins using Swiss PDB viewer. The model structure would useful in designing of drug, based on its structural studies. Result of study concluded the compound 5-Dinitrobenzylsulfany 1, 3, 4-oxidiazoles exhibited the best inhibitory activity towards modeled Ag85A protein. Finally the compound 5 Dinitrobenzylsulfany 1, 3, 4-oxidiazoles could emerge as a potent anti-tuberculosis drug molecule against antigen 85 protein and further experiential studies will enlighten the compound 5-Dinitrobenzylsulfany 1, 3, 4-oxidiazoles as TB drug.

5. CONFLICT OF INTEREST:

The authors declare they have no competing interests.

6. ACKNOWLEDGEMENT:

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