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HOMOLOGY MODELING AND E-PHARMACOPHORE MAPPING OF DIHYDROPTEROATE SYNTHASE 1 ENZYME OF *MYCOBACTERIUM LEPRAE*

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ABSTRACT

Worldwide, about 250,000 new cases of leprosy are reported each year and about two million people have leprosy related disabilities. Due to main drugs resistance and multidrug resistance there is need of crucial drug target identification and potential drugs. ***Dihydropteroate synthase 1*** enzyme coded by the gene folP1 found to have nill similarity with human genome. *Dihydropteroate synthase 1* enzyme is found to be responsible for the formation of the immediate precursor of folic acid used in biosynthesis of tetrahydrofolic acid (tetrahydrofolate, THF), the active form of folic acid (vitamin B9). Folic acid is an essential vitamin (B9), which plays a key role in the methylation cycle and in DNA biosynthesis. So inhibition of particular enzyme can lead to death of mycobacterium leprae. In present research effort homology model will be made and e-pharmacophore mapping will be done on the basis of interaction analysis of target model with pterin monophosphate an endogenous ligand. 3D model of target protein **(*Dihydropteroate synthase 1*)** can used for structure based drug designing. Potent inhibitors for target protein model can be designed on the basis of e-pharmacophore hypothetical model.

Key words: - MDR, Folic acid, *Dihydropteroate synthase 1*, pterin monophosphate, e-pharmacophore, MacroModel.

INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* . M. leprae is an acid fast bacillus with high infectivity. The appearance of drug resistance is a cause for

distress and a threat for any infectious disease. For leprosy, with social disgrace, drug resistance poses a serious barrier at a stage when there is a dramatic decline in frequency due to intensive and concerted chemotherapy intervention made by the global community. To efficiently cure the leprosy illness, multidrug resistance challenges have to be taken under consideration.

Current recommended control measures for treating leprosy with MDT are designed to prevent the spread of drug-resistant *M. leprae*. Drug resistance has been reported since 1964 for dapsone [1] 1976 for rifampin [2]. Due to emergence of multidrugresistant (MDR) leprosy [3] it has been urge to discover some new potential drug target.

Dihydropteroate synthase 1 enzyme coded by the gene folP1 has nill similarity with same gene and protein found in humans [4] and is responsible for the formation of the immediate precursor of folic acid used in biosynthesis of tetrahydrofolic acid (tetrahydrofolate, THF), the active form of folic acid (vitamin B9). Folic acid is an essential vitamin (B9), which plays a key role in the methylation cycle and in DNA biosynthesis. The folic acid derivatives are made up of a pterdine ring attached to a p-aminobenzoate and a polyglutamyl chain. Tetrahydrofolic acid has C1 units enzymically attached. These C1 units (as a formyl group) are passed on to enzymes in the purine pathway that insert the C-2 and C-8 into the purine ring. A methylene group (-CH2-) attached to tetrahydrofolate is used to convert the uracil-type pyrimidine base found in RNA into the thymine base found in DNA [4]. A further folate cofactor, i.e. 5-methyltetrahydrofolate, is involved in the remethylation of the homocysteine produced in the methylation cycle back to methionine. After activation to S-adenosylmethionine this acts as a methyl donor for the dozens of different *methyltransferases* present in all cells. Folate deficiency results in reduction of purine and pyrimidine biosynthesis and consequently DNA biosynthesis and cell division. The folate pathway represents a powerful target for combating rapidly dividing systems such as cancer cells, bacteria and malaria parasites. Therefore *Dihydropteroate synthase 1* enzyme can be a potential target in the era of MDR.

Aim of the present work is to make homology model of target protein *Dihydropteroate synthase 1* will be made including endogenous ligand of template. and active site will be predicted to identify the potential inhibitors. There e-pharmacophore mapping will be done on the basis structure- ligand descriptor identification from e-pharmacophor module of Schodinger9.2. [5-6].

MATERIALS AND METHODS

These were the following materials and methods used for the study which are mentioned as following:

Homology modeling

The amino acid sequence for *Dihydropteroate synthase 1* protein was obtained from the uniprot (http://www.uniprot.org/) a curated protein sequence database as It has been stated by Amos B and Rolf A (2000) that the UniProtKB/Swiss-ProtProtein Knowledge base is a curated protein sequence database and it provides a high level of annotation, a minimal level of redundancy and a high level of integration with other databases.

Template structure is very essentially needed for three-dimensional homology modelling of the target protein. The template structure or the homologue of the target sequence was obtained using PSI-PHI BLAST of NCBI server against PDB.

Sequence alignment was done by using prime module Schodinger9.2. It calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen.

Homology modeling was done using Prime module of Schodinger9.2 which is a powerful molecular graphics commercial tool used as a tool to predict the 3D structure of the protein by using the most identical available protein structure of the template [7-8]. The tool builds the model by transferring the 3D spatial coordinates including endogenous ligand of template model into the target protein model while modeling if identity between template and raw protein sequence is quite good.

Model validation was done using Structure analysis and Verification server (nihserver.mbi.ucla.edu/SAVES/). SAVE utilizes five different programs for doing the verification which includes PROCHECK, WHATCHECK, ERRAT, VERIFY 3D, and PROVE and the secondary structure prediction of amino acids analyzing their dihedral angles (Phi, Shi) displayed in Ramachandran plot to analyze amino acids on the basis of allowed and disallowed region.

Molecular docking

The SCHRODINGER9.2 module Glide5.5 was used to perform docking of endogenous

ligand with target protein [8-9]. It introduces the scientific methods and computational procedures used in Glide and describe the preparation of the protein and the ligands for use in Glide. Also describes the use of the Receptor Grid Generation panel to calculate the grids that represent the receptor. Flexible mode was used for docking. XP glide generates different conformations which get passed through a series of filters. Glide contains information on visualizing the results of Glide docking runs, using the Glide Pose Viewer, the Project Table, and the Glide XP Visualizer.

E- Pharmacophore

E-Pharmacophore module of schodinger9.2 can build pharmacophore hypothetical model from the co-crystal or docked pose of a known ligand in the target receptor. Fragment-mode will generate e-Pharmacophores from the energetically selected sites of docked fragment in cases where experimental information is unavailable. Both modes produce viable hypotheses, good database enrichments, and a diverse set of retrieved hits. So it is useful for rapidly screening huge compound databases. Its structure-based approaches can yield more diverse actives and lead to important target insight predictions.

RESULT AND DISCUSSION

Sequence of target protein *Dihydropteroate synthase 1* (ID: P0C0X1) retrieved from Uniprot protein database given below is composed of 284 amino acids. It belongs to pterin binding enzyme family of enzymes as shown in **Fig.1** calculated from Pfam and amino acids from 11-217 are responsible for catalytic action or interacting with ligands.

>sp|P0C0X1|DHPS1_MYCLE *Dihydropteroate synthase 1* OS=*Mycobacterium leprae (strain TN) GN=folP1 PE*=3 SV=1

MSLAPVQVIGVLNVTDNSFSDGGRYLDPDDAVQHGLAMVAEGAAIVDVGGESTRPGAIRTDPRVELSRIVP
VVKELAAQGITVSIDTTRADVARAALQSGARIVNDVSGGRADPAMAPLVAEAGVAWVLMHWRLMSAER
PYEAPNYRDVVAEVRADLLAGVDQAVAAGVDPGSLVIDPGLGFAKTGQHNWALLNALPELVATGVPILL
GASRKRFLGRLLAGADGAVRPPDGRETATAVISALAALHGAWGVRVHDVRASVDALKVVGAWLHAGPQ
IEKVRCDG

Template identification & 3-D model building of target protein

Blast was performed of target protein *Dihydropteroate synthase1* against PDB and most identical template (PDB code-1EYE) having highest identity with the *Dihydropteroate synthase1*. Template has score: 429.098, length: 284, indentity: 77.1127, similarity: 86.26 and

homology: 86.6197 with target protein selected as template to build homology 3 D model of *Dihydropteroate synthase1*.

3-D model of *Dihydropteroate synthase1* was built by prime module of Schodinger9.2 including endogenous ligand (pterin monophosphate) of template used as identity of target protein and template selected is quite more than required found beneficial for predicting active site since its alignment as shown in **Fig. 2** is preferably good. 3-D model of target protein as shown in **Fig.3** was validated from NIH server (SAVE) and the ramachandran plot as shown in **Fig.4** shows that 93.3% aminoacids are in allowed region, 0% amino acids in the disallowed region. So the model retrieved from the prime module of Schodinger9.2 can be used as target for structure-ligand based ligand designing as a good quality model would be expected to have over 90% in the most favoured regions.

Docking

Template used was also of *Dihydropteroate synthase 1* of same family mycobacterium from mtb. Endogenous ligand pterin monophosphate was docked again with target protein model active site by using extra precision docking method of glide module of schodinger9.2, which was done by grid generation of active site by clicking on ligand. Interaction of pterin monophosphate ligand was analysed from docked complex as shown in **Fig.5.** Glide score was found quite good with value **-11.505**. Amino acid residues participating in interaction with *Dihydropteroate synthase1* target model were identified as shown in **Fig.6**. Hydrogen bond acceptor and hydrogen bond donor atoms of endogenous ligand and residues of target protein model were identified as shown in **Fig.6**.

Asp177, ASN105, Lys213, Arg253, HIE255, ASN13, ASH86 found bonded with ligand forming total nine hydrogen bonds in purple colour dotted lines. Interacting residues has been found crucial residues for endogenous substrate binding for their catalytic activity found from uniproat. So pharmacophoric features can be used to design drugs or inhibitors against *Dihydropteroate synthase 1* enzyme.

E-pharmacophore mapping

Dynamic stabled protein ligand complex of target model and pterin monophosphate was taken as input for e-pharmacophore hypothesis. All important descriptors (interacting features) (shown in **Table.1**) usefull for binding with crucial amino acid residues were energetically evaluated where feature (electronegative atom, electropositive atom, hydrogen

bond acceptor and hydrogen bond donor as shown in **Table.1**) were ranked on the basis of affinity score towards target model. All features interacting with amino acid residues were found crucial for inhibitory action of ligand. So all feature (excluding electronegative and electropositive atoms) were selected for building e-pharmacophore model. Hypothetical e-pharmacophore model was retrieved as shown in **Fig.7.** Hydrogen bond donar D8, D10 in **light blue color** and hydrogen bond acceptor in **light Red color** A1, A2, A6 as shown in **Fig.7** which make hydrogen bonds with the target protein. All the hydrogen bond donor and acceptors found crucial and stable on dynamic study performed to check target protein ligand interaction stability in water as solvent medium. Distances between all the interacting pharmacophoric features (H-bond donor and acceptor) were mapped so from schodinger9.2 as shown in **Fig.8.**

Features responsible for binding with the crucial amino acids of target protein.

Table.1:- Features retrieved from e-pharmacophore module and selected for e-pharmacophore model building.

Rank	Feature_label	Score	type	source
1	P13	-2.89	Positive	Electro
2	N11	-1.16	Negative	none+HBond
3	D8	-0.84	Donor	HBond
4	A2	-0.7	Acceptor	HBond
5	A6	-0.66	Acceptor	HBond
6	D10	-0.66	Donor	HBond
7	A1	-0.64	Acceptor	HBond

Family assignment of target protein by pfam(prtein family prediction online server).

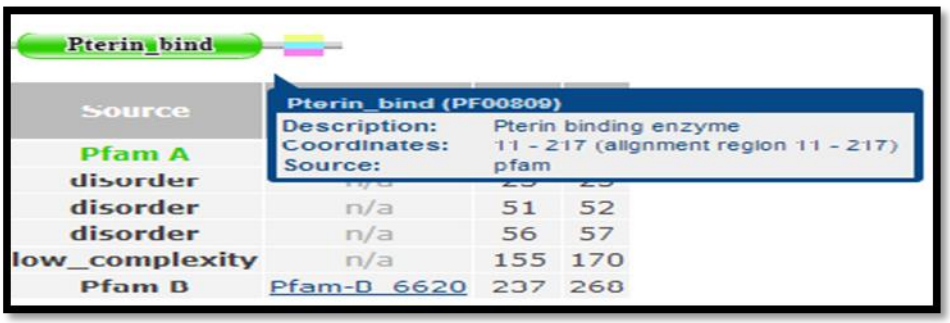

Fig.1:- **Family of target protein *Dihydropteroate synthase1*.**

Alignment of target protein and template protein by pBlast.

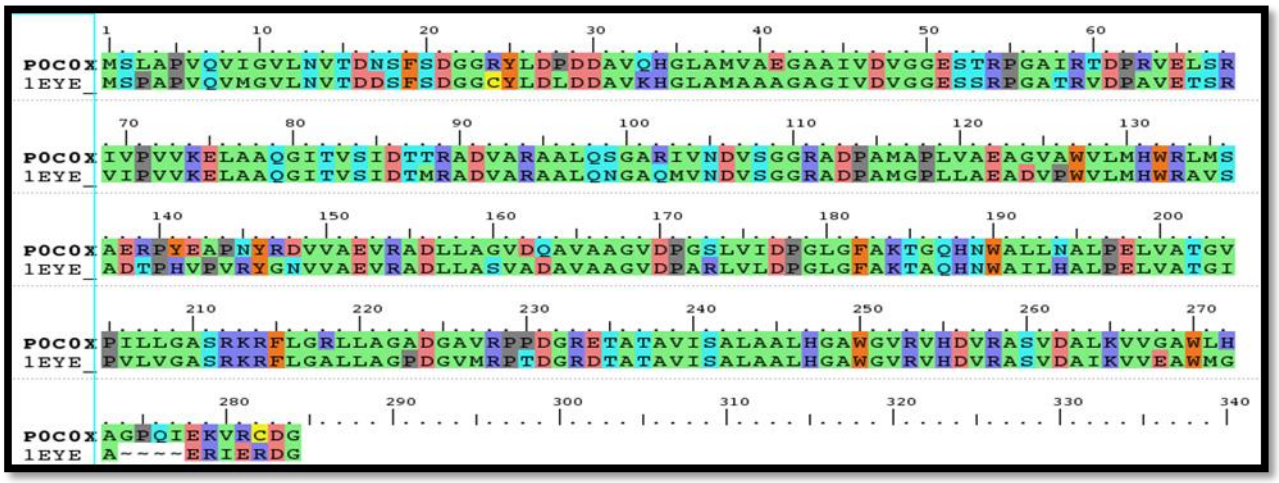

Fig.2:- Alignment of *Dihydropteroate synthase1* protein with template sequence.

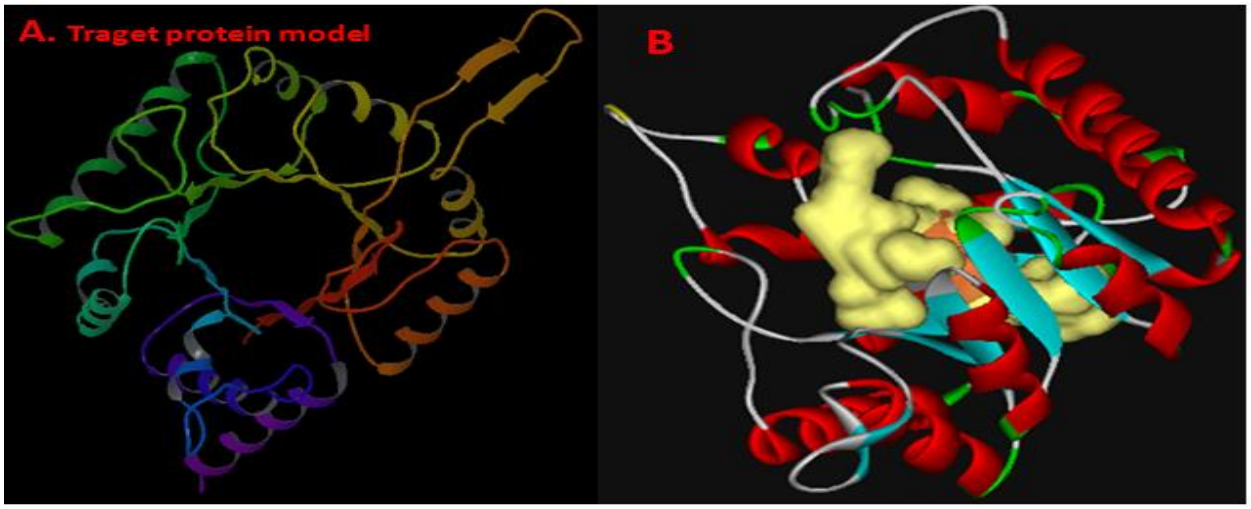

Fig.3:- A. 3D model of Dihydropteroate synthase1 and B. 3D model of Dihydropteroate synthase1 including endogenous ligand interacting pocket in solid view.

Target protein model validation from NIH server (SAVE).

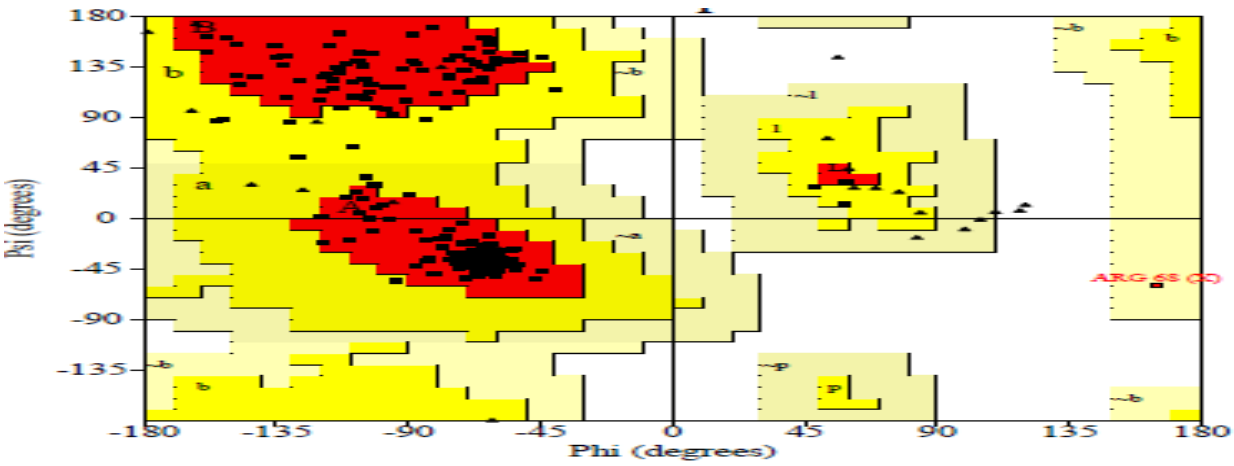

Fig.4:- Ramachandran plot of Dihydropteroate synthase1 3D model.

Interactions of target protein model with pterin monophosphate from schodinger9.2 interface.

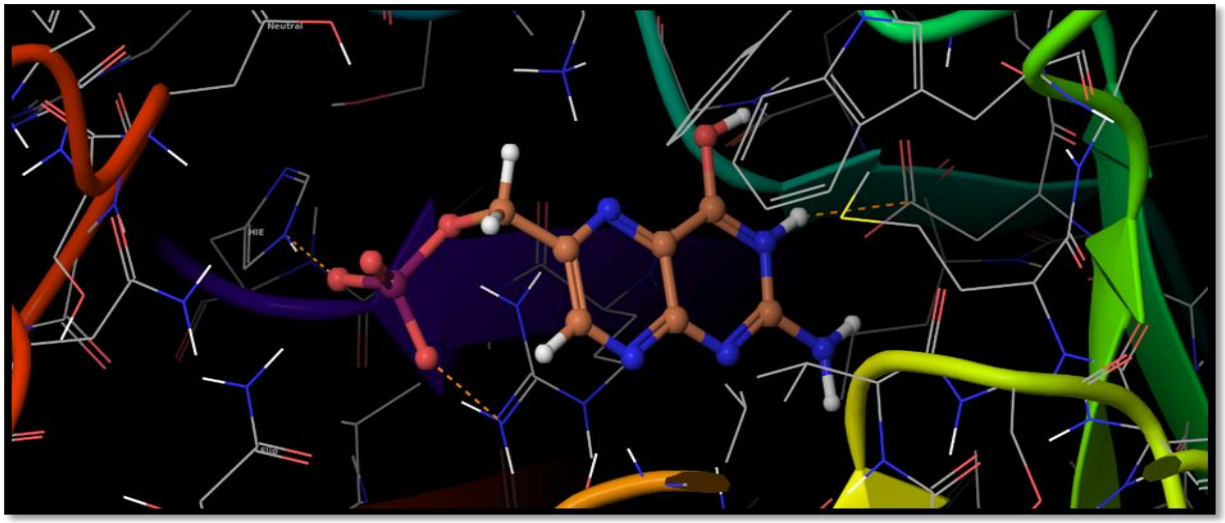

Fig.5:- Docked complex showing interacting pocket of target protein model.

Residues of target protein forming H- bonds with pterin monophosphate.

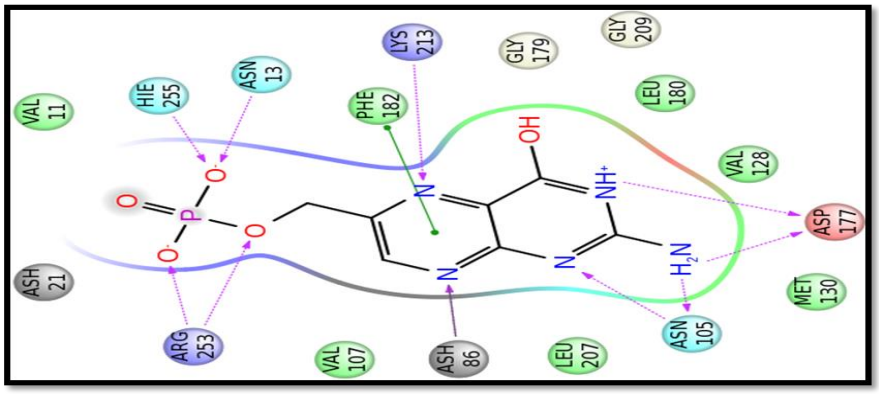

Fig.6:- Hydrogen bond acceptor and donor (hydrogen bonds in purple dotted arrows) within the binding pocket of target model.

Crucial Features responsible for interacting with target protein.

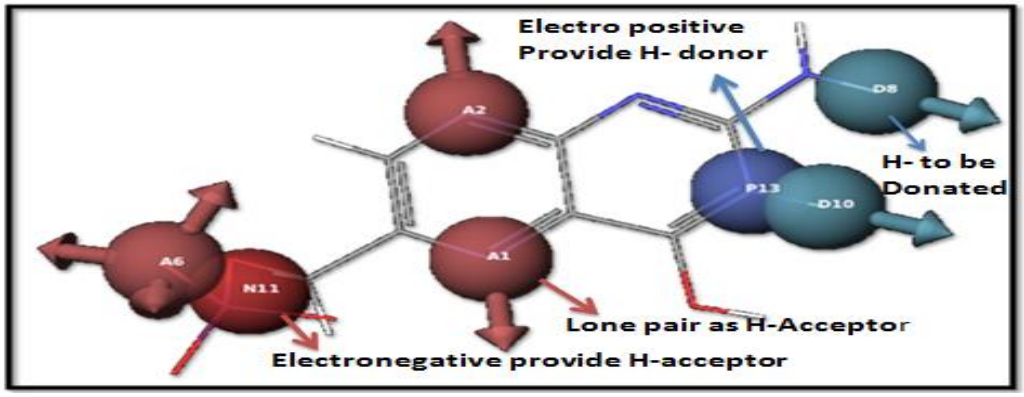

Fig.7:- E-pharmacophore model for pterin monophosphate.

Distances between features forming H-bonds mapped from schodinger9.2.

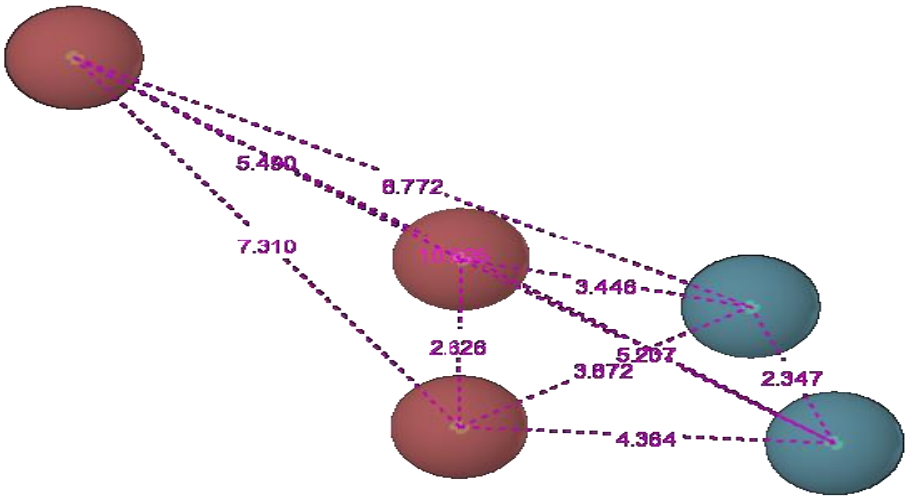

Fig.8:- **Distances between features forming H-bonds with amino acids of target model protein.**

CONCLUSION

Dihydropteroate synthase 1 enzyme has nill similarity with same genom of humans. It is responsible for the formation of the immediate precursor of folic acid used in biosynthesis of tetrahydrofolic acid (tetrahydrofolate, THF), the active form of folic acid (vitamin B9). Folic acid is an essential vitamin (B9), which plays a key role in the methylation cycle and in DNA biosynthesis. So inhibition of *dihydropteroate synthase 1* can lead to death of *mycobacterium leprae*. Homology model built found to have better identity with template selected and have no residues in disallowed region. On the basis of better similarity, model built including endogenous ligand in homology modeling. Endogenous ligand pterin monophosphate found to bind with crucial residues for substrate binding site of target prtein. The e-pharmacophore model built on basis of interacting pharmacophoric of features target protein model and pterin monophosphate. Distance model of e-pharmacophore was made which gives the distance map of the crucial pharmacophoric features of pterin monophosphate. On the basis of distance model of e-pharmacophore, compound libraries can be screened find the compounds of similar pharmacophore.

The homology model of target protein and e-pharmacophore model built both further can be used for screening and designing new potent inhibitors to inhibit *dihydropteroate synthase 1*.

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