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INHIBITION OF PROTEIN (ENZYME) DHNA BY USING MOLECULAR DOCKING

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Keywords:

DHNA enzyme, Microorganism, Molecular docking

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ABSTRACT: Aim of this study was to generate a model of DHNA using protein sequence and homology modeling and then dock the modeled protein with an inhibitor. Molecular docking is a frequently used method in structure-based rational drug design. It is used for evaluating the complex formation of small ligands with large biomolecules, predicting the strength of the bonding forces and finding the best geometrical arrangements. For inhibition of final model built from the swiss model by using target sequence of DHNA from *Shigella flexneri* and template sequence from *E. coli* choose 4 types of ligand molecule in which 2 molecules (2-amino pyrimidine and neopterin) selected for docking with the help of Autodock Vina (software). And the final result is shown in docking result 1 and 2 respectively. Docking results shows mean binding energy -3.42 by neopterin and -2.77 by 2-amino, pyrimidine. Neopterin shows high mean binding energy in both of ligands so we can use neopterin as a strong inhibitor of DHNA.

INTRODUCTION: Folate Biosynthesis Pathway: Folate cofactors are important for living systems. Most of the microorganisms synthesize folates de novo, but in mammals, folate synthesis does not occur. Hence, the folate biosynthetic pathway is a perfect target for antimicrobial agents. It required for the transfer of one-carbon units in several metabolic steps, including the key methylation of dUMP to give dTMP, an essential nucleotide for DNA synthesis. Most microorganisms can synthesize the required folates from the simple precursor GTP, p-aminobenzoate (pABA) and glutamate. But folate biosynthetic pathway absent in mammals because of lack of all three enzymes which works in the middle of folates synthesis. So mammals take folates through diet [1].

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Folate Biosynthetic Pathway:

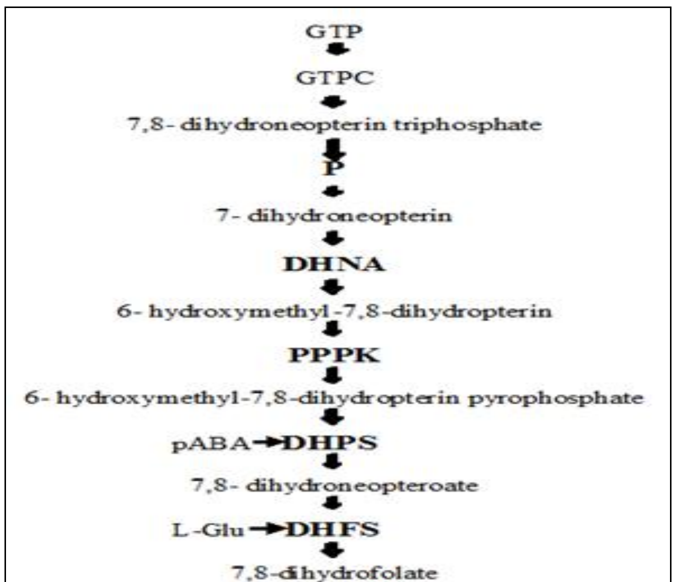


FIG. 1: IT SHOWS THE SIMPLE FOLATE BIOSYNTHETIC PATHWAY IN WHICH GUANINE TRIPHOSPHATE (GTP) THOUGH USING DIFFERENT ENZYMES IN EACH STEPS LIKE DIHYDRONEOPTERINALDOLASE (DHNA); PYRO-PHOSPHOKINASE (PPPK); PARA-AMINOBENZOIC ACID (PABA); DIHYDROPTEROATE SYNTHASE (DHPS); DIHYDROFOLATE SYNTHASE (DHPS) [1]

Enzyme: Dihydroneopterinaldolase (DHNA) is the first enzyme in the pathway and has a major role of the three enzymes that are absent in mammals and therefore an attractive target for developing antimicrobial agents [2].

Structure, Function, Reaction, Inhibitors: DHNA has a hollow cylinder structure, 70Å in height, an outer diameter of 65 Å and an inner diameter of 13Å. Two tetrameric rings are placed head to head forming an octamer of cylindrical shape. The N and C termini are located on the top and bottom of the structure.

The figure represents the DHNA crystal structure with different subunits represented as distinguished with colors. The HP molecules are shown as stick models in atomic color scheme (Carbon in black, nitrogen in blue, and oxygen in red).

DHNA is a unique enzyme which works as aldolase as well as epimerase. It works as a unique type of aldolase it requires neither the construction of a Schiff's base between the substrate and enzyme nor metal ions for catalysis. When it works as aldolase then conversion of DHNP to 6-hydroxymethyl-7, 8-dihydropterin (HP) with the generation of glycoaldehyde (GA) and the epimerization of 7, 8-dihydroneopterin (DHNP) to 7, 8-dihydromonapterin (DHMP).

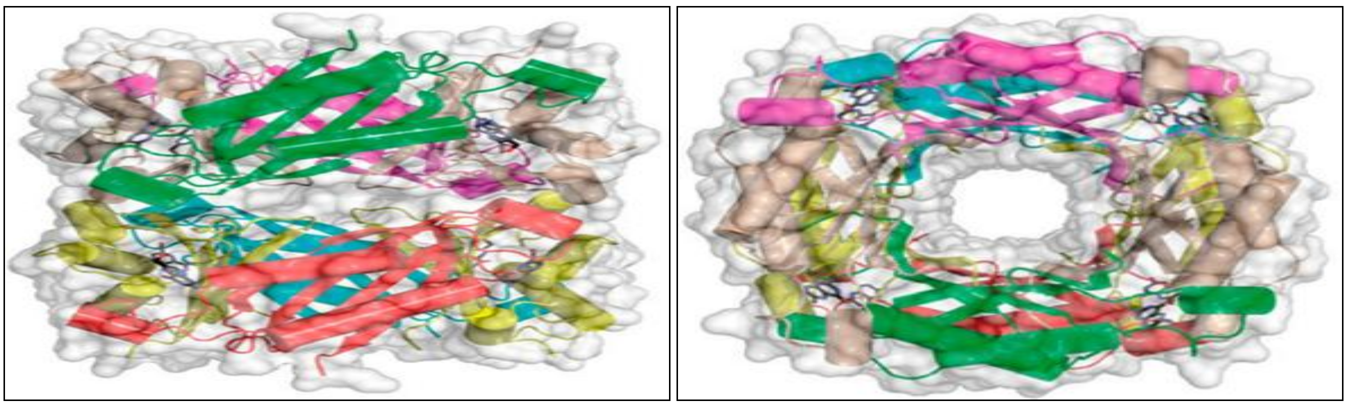


FIG. 2: TWO VIEWS (SIDE, ON THE LEFT; TOP, ON THE RIGHT) OF THE SaDHNA-HP OCTAMER (PDB ENTRY 2DHN)

The reaction of DHNA it works as aldolase and epimerase both.

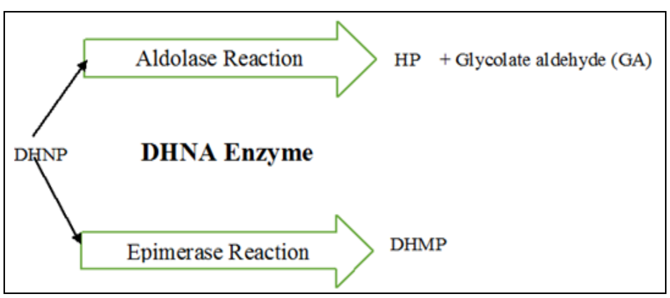


FIG. 3: IT SHOWS ONLY ONE ENZYME DHNA (DIHYDRO-NEOPTERINALDOLASE) PROCEEDS TWO TYPE OF MECHANISM FIRST ALDOLASE REACTION WHICH FORMS HP(6-HYDROXYMETHYL-7, 8-DIHYDROPTERIN) WITH GA (GLYCOLATE ALDEHYDE) BY DHNP (7,8-DIHYDRO-D-NEOPTERIN) AND WITH SIMILAR INTER-MEDIATE EPIMERASE RECTION GET PROCEEDS DHMP (7, 8-DIHYDRO-L-MONAPTERIN) MOLECULE GET FORMS [2]

Inhibitors: Neopterin (oxidized form of DHNP) and monopterin (oxidized form of DHMP) are known inhibitors of DHNA. After the oxidation of DHNP and DHMP forms a double bond between C7 and N8, which may make the protonation of N5 much harder so that NP and MP may not undergo chemical reaction catalyzed by DHNA. Thus, these two blocks DHNA catalysis. 2-amino pyrimidine, a substrate analog, forms the same hydrogen bonding with the enzyme as the substrate and is also a good inhibitor.

Organism from Where the Enzyme is taken: Scientific name of organism *Shigella flexneri* (Uniprot id-P0AC18).

Classification of Organism:

Kingdom:	Bateria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Enterobacteriales
Genus:	Shigella
Species:	flexneri [3]

Some Reported Work on DHNA in Other Organisms:

TABLE 1: SOME REPORTED WORK ON DHNAS AND ITS ORGANISM [8, 9, 10, 11, 12]

S. no.	Enzymes	Organism	Work	Reference
1	EcDHNA	*E. coli*	First identified of DHNA	Mathis and Brown 1970
2	SaDHNA	*S. aureus*	Crystal structure of DHNA complex with the product HP	Hennig and coworkers 1998
3	SaDHNA	*S. aureus*	aldolase and epimerase activities and determined the steady-state kinetic parameters for both reactions	Haussmann and coworkers 1998
4	SaDHNA	*S. aureus*	the total sequential resonance assignment of the 110-kDa homo-octomeric SaDHNA	Wu thrich 2000
5	SaDHNA	*S. aureus*	pKaof N5 of SaDHNA-bound 7,8-dihydrobiopterin by Raman spectroscopy	Deng and coworkers 2000
6	SaDHNA	*S. aureus*	protonation of the reaction intermediate prefers the pro-S position	Illarionova and coworkers 2002

The objective of Study: Objective of the study was to generate a model of DHNA using protein sequence and homology modeling and then dock the modeled protein with an inhibitor.

METHDOLOGY: Using uniprot id P0AC18 of Dihydroneopterin-aldolase from *Shigella flexneri* run FASTA for align sequence of that enzyme. Then using blasta do pBlast of fasta sequence in pdb formate. I have got chain A, atomic resolution crystal structure of *E. coli* dihydroneopterin-aldolase in complex with neopterin. It gives a maximum, total score of alignment is 246 with 100% quality cover, identity and E value 2e-84 and accession no. is 2O90_A. Using 2O90_A accession no. or PDB id in RCSB download template .pdb file. Then in swiss modeler, put the target template in FASTA format and upload a .pdb file of the template and build the model. The model obtained was docked with an appropriate ligand using Autodock Vina [4, 5].

RESULTS AND DISCUSSION:

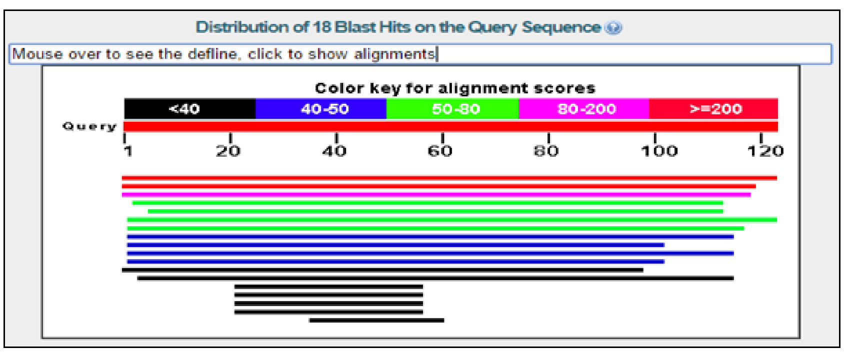
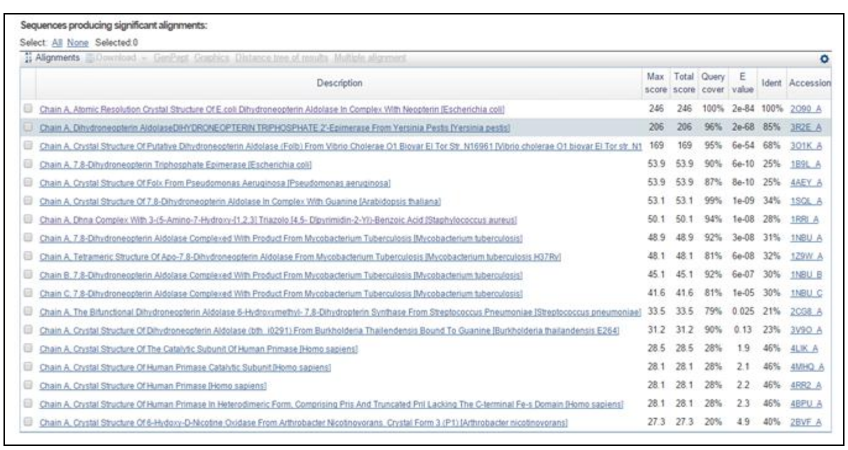


FIG. 4: FIRST RED COLOUR LINE REPRESENT 100% IDENTITY IN *E. COLI* AND 85% IDENTITY IN *YERSINIA PESTRIS*



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

Description	Max score	Total score	Query cover	E value	Ident	Accession
Chain A, Atomic Resolution Crystal Structure Of E.coli Dihydroneopterin Aldolase In Complex With Neopterin [Escherichia coli]	246	246	100%	2e-84	100%	2O90_A
Chain A, Dihydroneopterin AldolaseDIHYDRONEOPTERIN TRIPHOSPHATE 2'-Epimerase From Yersinia Pestis [Yersinia pestis]	206	206	96%	2e-68	85%	3R2E_A
Chain A, Crystal Structure Of Putative Dihydroneopterin Aldolase (Folb) From Vibrio Cholerae O1 Biovar El Tor Str. N16961 [Vibrio cholerae O1 biovar El Tor str. N1	169	169	95%	6e-54	68%	3O1K_A
Chain A, 7,8-Dihydroneopterin Triphosphate Epimerase [Escherichia coli]	53.9	53.9	90%	6e-10	25%	1B9L_A
Chain A, Crystal Structure Of Folx From Pseudomonas Aeruginosa [Pseudomonas aeruginosa]	53.9	53.9	87%	8e-10	25%	4AEY_A
Chain A, Crystal Structure Of 7,8-Dihydroneopterin Aldolase In Complex With Guanine [Arabidopsis thaliana]	53.1	53.1	99%	1e-09	34%	1SQL_A
Chain A, Dhna Complex With 3-(5-Amino-7-Hydroxy-[1,2,3] Triazolo [4,5- D]pyrimidin-2-Yl)-Benzoic Acid [Staphylococcus aureus]	50.1	50.1	94%	1e-08	28%	1RRI_A
Chain A, 7,8-Dihydroneopterin Aldolase Complexed With Product From Mycobacterium Tuberculosis [Mycobacterium tuberculosis]	48.9	48.9	92%	3e-08	31%	1NBU_A
Chain A, Tetrameric Structure Of Apo-7,8-Dihydroneopterin Aldolase From Mycobacterium Tuberculosis [Mycobacterium tuberculosis H37Rv]	48.1	48.1	81%	6e-08	32%	1Z9W_A
Chain B, 7,8-Dihydroneopterin Aldolase Complexed With Product From Mycobacterium Tuberculosis [Mycobacterium tuberculosis]	45.1	45.1	92%	6e-07	30%	1NBU_B
Chain C, 7,8-Dihydroneopterin Aldolase Complexed With Product From Mycobacterium Tuberculosis [Mycobacterium tuberculosis]	41.6	41.6	81%	1e-05	30%	1NBU_C
Chain A, The Bifunctional Dihydroneopterin Aldolase 6-Hydroxymethyl- 7,8-Dihydropterin Synthase From Streptococcus Pneumoniae [Streptococcus pneumoniae]	33.5	33.5	79%	0.025	21%	2CG8_A
Chain A, Crystal Structure Of Dihydroneopterin Aldolase (bth_i0291) From Burkholderia Thailendensis Bound To Guanine [Burkholderia thailandensis E264]	31.2	31.2	90%	0.13	23%	3V9O_A
Chain A, Crystal Structure Of The Catalytic Subunit Of Human Primase [Homo sapiens]	28.5	28.5	28%	1.9	46%	4LIK_A
Chain A, Crystal Structure Of Human Primase Catalytic Subunit [Homo sapiens]	28.1	28.1	28%	2.1	46%	4MHQ_A
Chain A, Crystal Structure Of Human Primase [Homo sapiens]	28.1	28.1	28%	2.2	46%	4RR2_A
Chain A, Crystal Structure Of Human Primase In Heterodimeric Form, Comprising Pris And Truncated Pril Lacking The C-terminal Fe-s Domain [Homo sapiens]	28.1	28.1	28%	2.3	46%	4BPU_A
Chain A, Crystal Structure Of 6-Hydoxy-D-Nicotine Oxidase From Arthrobacter Nicotinovorans, Crystal Form 3 (P1) [Arthrobacter nicotinovorans]	27.3	27.3	20%	4.9	40%	2BVF_A

FIG. 5: TOTAL 18 RESULT SPECIES HAVE FOUND WHICH SHOWS THE PRESENCE OF DHNA ENZYME

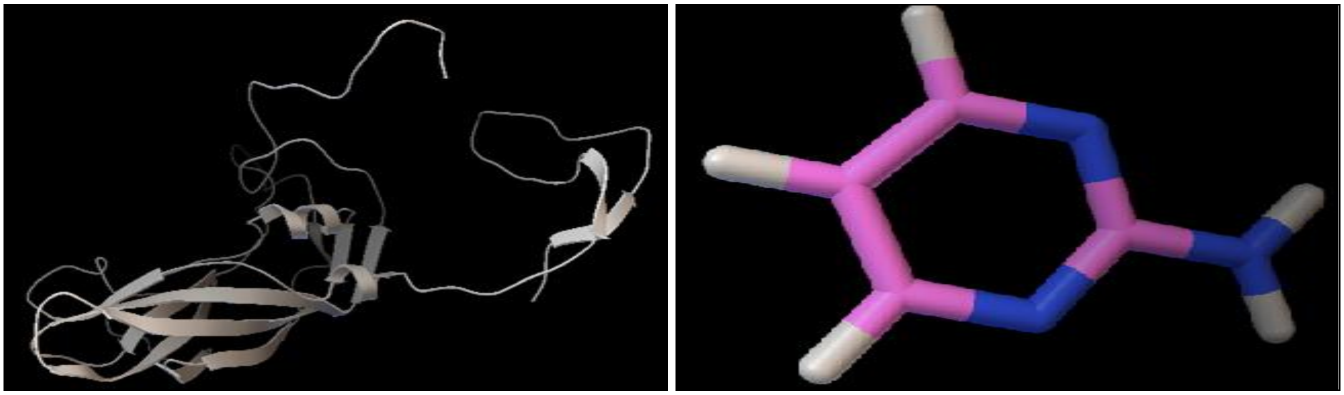


FIG. 6: FINAL MODEL BUILT FROM THE SWISS-MODEL WITH THE HELP OF TARGET SEQUENCE OF DHNA FROM *SHIGELLA FLEXNERI* AND TEMPLATE SEQUENCE FROM *E. COLI.* AND IT'S FRONT OF LIGAND (2-AMINO, PYRIMIDINE) STRUCTURE [7]

Docking Result 1 of Fig. 6 Protein and Ligand (2-amino, pyrimidine):

CLUSTERING HISTOGRAM

Clus -ter Rank	Lowest Binding Energy	Run	Mean Binding Energy	Num in Clus	Histogram 5 10 15 20 25 30 35
1	-2.82	4	-2.77	7	#######
2	-2.41	2	-2.38	2	##
3	-2.15	1	-2.15	1	#

Number of multi-member conformational clusters found = 2, out of 10 runs.

RMSD TABLE

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	4	-2.82	0.00	147.52	RANKING
1	2	3	-2.82	0.17	147.60	RANKING
1	3	5	-2.82	0.05	147.53	RANKING
1	4	8	-2.79	0.25	147.64	RANKING
1	5	9	-2.79	0.10	147.48	RANKING
1	6	6	-2.75	0.17	147.47	RANKING
1	7	7	-2.58	0.49	147.31	RANKING
2	1	2	-2.41	0.00	134.80	RANKING
2	2	10	-2.35	1.41	134.61	RANKING
3	1	1	-2.15	0.00	133.20	RANKING

INFORMATION ENTROPY ANALYSIS FOR THIS CLUSTERING

Information entropy for this clustering = 0.35 (rmstol = 2.00 Angstrom)

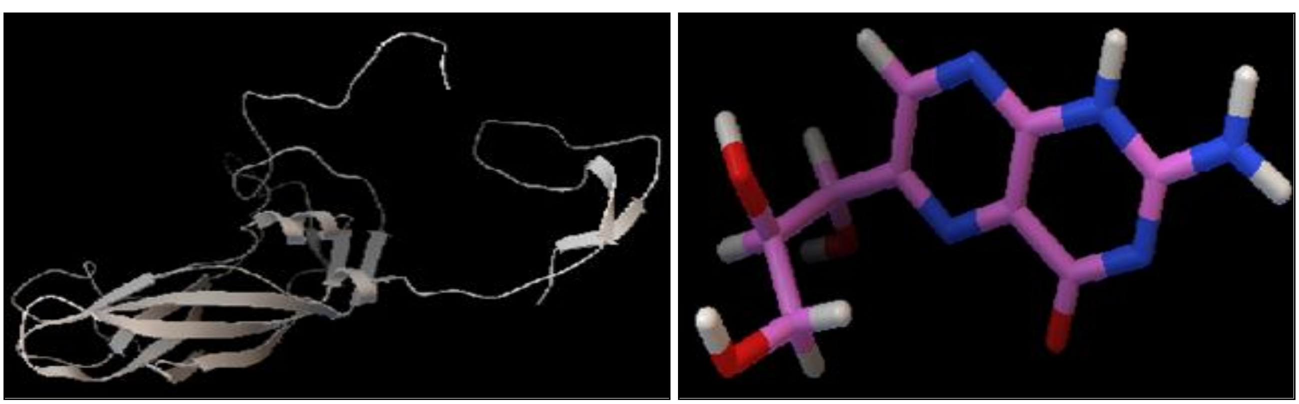


FIG. 7: FINAL MODEL BUILT FROM THE SWISS-MODEL WITH THE HELP OF TARGET SEQUENCE OF DHNA FROM *SHIGELLA FLEXNERI* AND TEMPLATE SEQUENCE FROM *E. COLI.* AND IT'S FRONT OF LIGAND (NEOPTERIN) STRUCTURE

Docking Result 2 of Fig. 7 Protein and Ligand (Neopterin):

```
CLUSTERING HISTOGRAM

     |            |     |            |     |
Clus | Lowest     | Run | Mean       | Num | Histogram
-ter | Binding    |     | Binding    | in  |
Rank | Energy     |     | Energy     | Clus|    5    10   15   20   25   30
35
_____|____________|_____|____________|_____|____:____|____:____|____:____|__
__:___
   1 |     -3.75  |   3 |     -3.42  |   7 |#######
   2 |     -3.49  |   1 |     -3.09  |   2 |##
   3 |     -2.97  |   5 |     -2.97  |   1 |#
_____|____________|_____|____________|_____|_________________________________

Number of multi-member conformational clusters found = 2, out of 10 runs.
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RMSD TABLE

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	3	-3.75	0.00	130.56	RANKING
1	2	8	-3.72	1.40	130.34	RANKING
1	3	4	-3.66	0.43	130.57	RANKING
1	4	6	-3.56	1.45	130.27	RANKING
1	5	7	-3.24	1.92	131.21	RANKING
1	6	10	-3.17	1.27	130.32	RANKING
1	7	9	-2.84	1.96	130.36	RANKING
2	1	1	-3.49	0.00	130.32	RANKING
2	2	2	-2.70	1.17	130.39	RANKING
3	1	5	-2.97	0.00	130.26	RANKING

INFORMATION ENTROPY ANALYSIS FOR THIS CLUSTERING

Information entropy for this clustering = 0.35 (rmstol = 2.00 Angstrom)

CONCLUSION: For inhibition of final model built from the swiss model by using target sequence of DHNA from *Shigella flexneri* and template sequence from *E. coli* choose 4 types of ligand molecule in which 2 molecules (2-aminopyrimidine and neopterin) selected for docking with the help of Autodock Vina (software).

And the final result is shown in docking results 1 and 2 respectively. Docking result shows mean binding energy -3.42 by neopterin and -2.77 by 2-amino, pyrimidine. Neopterin shows high mean binding energy in both of ligands so we can use neopterin as a strong inhibitor of DHNA.

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CONFLICT OF INTEREST: Nil

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