Potential leads for development of new antimalarial drugs

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Abstract:

Malaria is a dreadful disease caused by the Abstract:

parasite Plasmodium and is transmitted to humans by mosquitoes. Malaria remains an important public health problem, especially in endemic regions of India. Globally, malaria remains a leading infectious disease, and especially so in Sub-Saharan Africa and South East Asia. Global efforts are underway to eliminate malaria and to use a multi-pronged strategy where drugs play a crucial part. The most effective present day line of treatment option is based on the artemisinin-based drugs. The malarial parasites are developing resistance to the artemisinin class of drugs; it is likely that one day these drugs will be ineffective. Therefore, there is an urgent need to develop new classes of anti-malaria drugs with novel modes of action.Cladosporin (also known as asperentin), 3,4-dihydro-6,8-dihydroxy3-(6-methyl tetrahydropyran-2-ylmethyl) isocoumarin, is an important secondary metabolite isolated from Cladosporium cladosporioides in 1971. It is the major compound of C. cladosporioides, but a minor metabolite of other fungal sources including Aspergillus flavus. In Present study we have assessed the fungal metabolite-inspired molecules (Cladosporin stereoisomers) as potential lead antimalarials. Novel synthetic routes were developed in the laboratory to access this natural product. In addition, the team has synthesized all the possible stereoisomers of Cladosporin using novel synthetic organic chemistry protocols. After the successful synthesis of all eight compounds (called Cladologs), the teams tested it against malaria parasites to address their potency. Enzyme and structure-based studies were done to address mechanistic details of the drug interactions. The important cladologs were co-crystallized with the target enzyme lysyl-tRNA synthetase of malaria parasite in order to provide atomic details. The bases for wide differences in antimalarial potency between various sterioisomeric forms of cladosporin using an elegant chemistry, strong biochemisty and modern structure-based methods. Three categories of molecules as potent, moderately potent and non-potent were identified based on target binding and parasite killing. The demonstrations validated two most potent stereoisomers of cladosporin so this information will allow their development for drug-like properties. The significance of chirality in modern drug discovery has also been highlighted through these efforts.

Introduction:

Malaria is a common and life-threatening disease in many tropical and subtropical areas. There are currently over 100 countries and territories where there is a risk of malaria transmission, and these are visited by more than 125 million international travellers every year. Each year many international travellers fall ill with malaria while visiting countries/territories where malaria is endemic, and well over 10 000 are reported to become ill with malaria after returning home; however, underreporting means that the real figure may be considerably higher. International travellers to countries/territories with ongoing local malaria transmission arriving from countries with no transmission are at high risk of malaria infection and its consequences because they lack immunity. Migrants from countries/territories with malaria transmission living in malaria-free countries and returning to their home countries to visit friends and relatives are similarly at risk because of waning or absent immunity. Travellers who fall ill during travel may find it difficult to access reliable medical care. Travellers who develop malaria upon returning to a country that is malaria-free face particular problems: medical personnel may be unfamiliar with malaria, the diagnosis may be delayed, and effective antimalarial medicines may not be registered and/or available, resulting in progression to fatality rates.–severe and complicated malaria and, consequently, high case Fever occurring in a traveller

within 3 months of leaving a country in which there is risk of malaria is a potential medical emergency and should be investigated urgently to exclude malaria. In the absence of rapid access to reliable diagnostic facilities, stand-by emergency treatment (SBET) is indicated.

In Present study we have assessed the fungal metabolite-inspired molecules (Cladosporin stereoisomers) as potential lead antimalarials. Novel synthetic routes were developed in the laboratory to access this natural product. In addition, the team has synthesized all the possible stereoisomers of Cladosporin using novel synthetic organic chemistry protocols. After the successful synthesis of all eight compounds (called Cladologs), the teams tested it against malaria parasites to address their potency. Enzyme and structure-based studies were done to address mechanistic details of the drug interactions. The important cladologs were co-crystallized with the target enzyme lysyl-tRNA synthetase of malaria parasite in order to provide atomic details. The bases for wide differences in antimalarial potency between various sterioisomeric forms of cladosporin using an elegant chemistry, strong biochemisty and modern structure-based methods. Three categories of molecules as potent, moderately potent and non-potent were identified based on target binding and parasite killing. The demonstrations validated two most potent stereoisomers of cladosporin so this information will allow their development for drug-like properties. The significance of chirality in modern drug discovery has also been highlighted through these efforts.

Materials and Methodology:

Protein Sequence retrieval and Primary analysis:

Protein sequence of protein minor nucleoprotein Cladosporin (ribosomal protein S5 [*Aspergillus flavus*]) was retrieved from Gene bank database. The physicochemical analysis were calculated by ProtParam tool (http://web.expasy.org/protparam/), including *pI*, total number of negatively and positively charged residues, the instability index (II), aliphatic index, and grand average of hydrophilic (GRAVY).

Structural Charecterization:

Similarity search was carried out by using BLAST software (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins). SOPMA (Geourjon and Deléage, 1995) server (https://npsaprabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html). SOPMA is using homologue method of Levin *et al*. According to this method; short homologous sequence of amino acids will tend to form similar secondary structure. As well it also done by using Phyre2 (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index) software and visualized by using Chimera (https://www.cgl.ucsf.edu/chimera/) software.

Homology modeling and Model evaluation:

Homology modeling was used for determining 3D structure of protein. Then, BLASTP was performed against PDB (Protein Databank, Bernstein *et al*., 1977) to retrieve the best suitable templates for homology modeling. Preferred hit contains maximum identity and lowest e-value that it was used as a template. The modeling of the 3D structure of the protein was performed by using Swiss-Modeler (http://swissmodel. expasy.org/) program (Arnold *et al*., 2006; Bordoli *et al*., 2009).

Molecular Docking:

Molecular docking is an attractive scaffold to understand drugbiomolecular interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule (ligand) into the preferred binding site of the target specific region of the DNA/protein (receptor) mainly in a non-covalent fashion to form a stable complex of potential efficacy and more specificity. The information obtained from the docking technique can be used to suggest the binding energy, free energy and stability of complexes. At present, docking technique is utilized to predict the tentative binding parameters of ligand-receptor complex beforehand.in this project docking was carried out between Cladosporin and ribosomal proteins5 to find proper drug structure for future applications.

Binding site Prediction:

The binding site of Cladosporin protein was predicted by RaptorX server (http://raptorx.uchicago.edu/BindingSite/). The binding site shows the small pockets of the tertiary structure where ligands bind to using the weak forces.

1] Organism : *Aspergillus flavus*

2] Protein : ribosomal protein S5

3] Accession id: Gen Bank: RAQ63165.1

4] Sequence:

>RAQ63165.1 ribosomal protein S5 [*Aspergillus flavus*]

MADAAPRGRGGFGSRGDRGGDRGRGRGRRGRRGGK
QEEKEWQPVTKLGRLVKAGKITSMEQIYLHSLPIKEY
QIVDFFLPKLKDEVMKIKPVQKQTRAGQRTRFKAVVI
IGDSEGHIGLGIKTSKEVATAIRAAITIAKLAVLPVRRG
YWGSNLGEPHSLPVKQSAKCGSVSVRLIPAPRGTGLV
ASPAVKRLLQLAGVQDAYTSSSGSTKTLENTLKATFL
AVVNTYGFLTPNLWKETKLIRSPLEEFGDVLRQGKKY

5) **Drug used:**

I) **Source name :** *Aspergillus flavus*

II) **Chemical compound : Cladosporin**

III) **Pubchem id:** 13990016

IV) **Molecular Formula:** $C_{16}H_{20}O_5$

V) **Chemical Names:** Cladosporin
Asperentin
UNII-81PR0D5FI4
81PR0D5FI4
35818-31-6

VI) **Related compounds with annotation:**

1. Taleranol
2. Mellein
3. (4S)-8,16,18-Trihydroxy-4-methyl-3-oxabicyclo[12.4.0]octadeca-1(14),15,17-trien-2-one
4. Altenuene
5. Isocoumarin, 3,4-dihydro-6,8-dihydroxy-3-(6-methyl-tetrahydro-2H-pyran-2-yl)

Results and discussion:

Protein Sequence retrieval and Primary analysis:

The physicochemical analysis of Ribosomal Protein S5 protein was performed using Protparam and results were shown in Table 1. Protein contains 259 amino acids with molecular weight 28274.85 Dalton and Theoretical pI 10.54

Sr.No.	***Parameters***	***Values***
1	Molecular weight	28274.85 D
2	Theoretical pi	10.54
3	Instability index	37.17
4	Extinction coefficients	25440
5	Total number of negatively charged residues (Asp + Glu):	21
6	Total number of positively charged residues (Arg + Lys):	47
7	Aliphatic index:	86.22
8	GRAVY -	-0.396

Table 3. Physico-chemical properties of *Ribosomal Protein*

Protparam tool computed that the protein is basic in nature and stable on the basis of parameters Theoretical pi and instability index. According to the GRAVY index protein is hydrophilic. The aliphatic index of a protein is 86.22 which defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). The total number of positively charged residues (Arg+Lys 47) was found higher than the total number of negatively charged residues (Asp+Glu 21).

Structural Characterization:

The secondary structure of the protein was predicted using SOPMA server. It was observed that predominant with alpha helix (33.98%) followed by random coil (41.31%), and extended strand (15.83%). Random coils have important functions in proteins for flexibility and conformational changes such as enzymatic turnover (Buxbaum, 2007).

Table 4. Secondary structure of protein using SOPMA

Sr.No.	***Parameters***	***Values***
1	Alpha Helix	80
2	Bita Sheets	23
3	Random coils	107
4	Extended strand	41

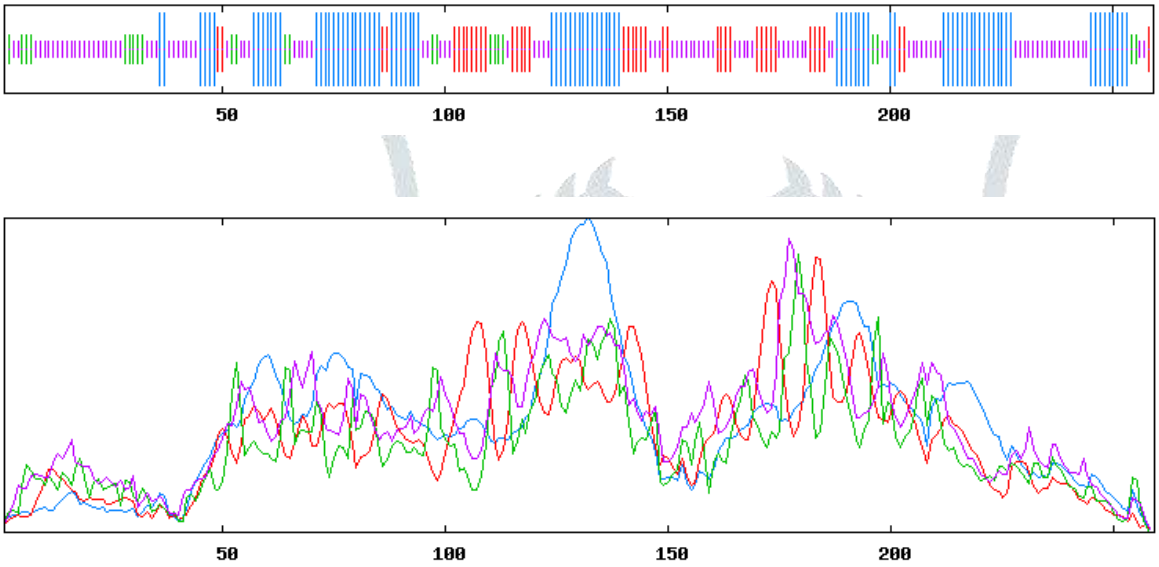
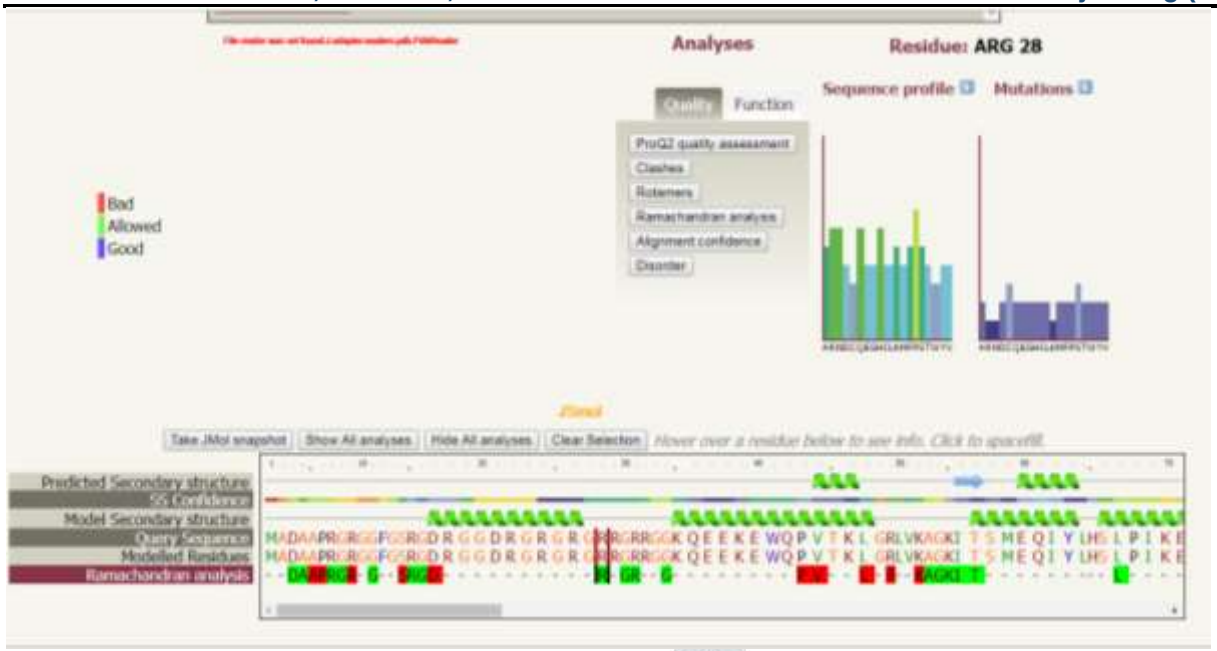
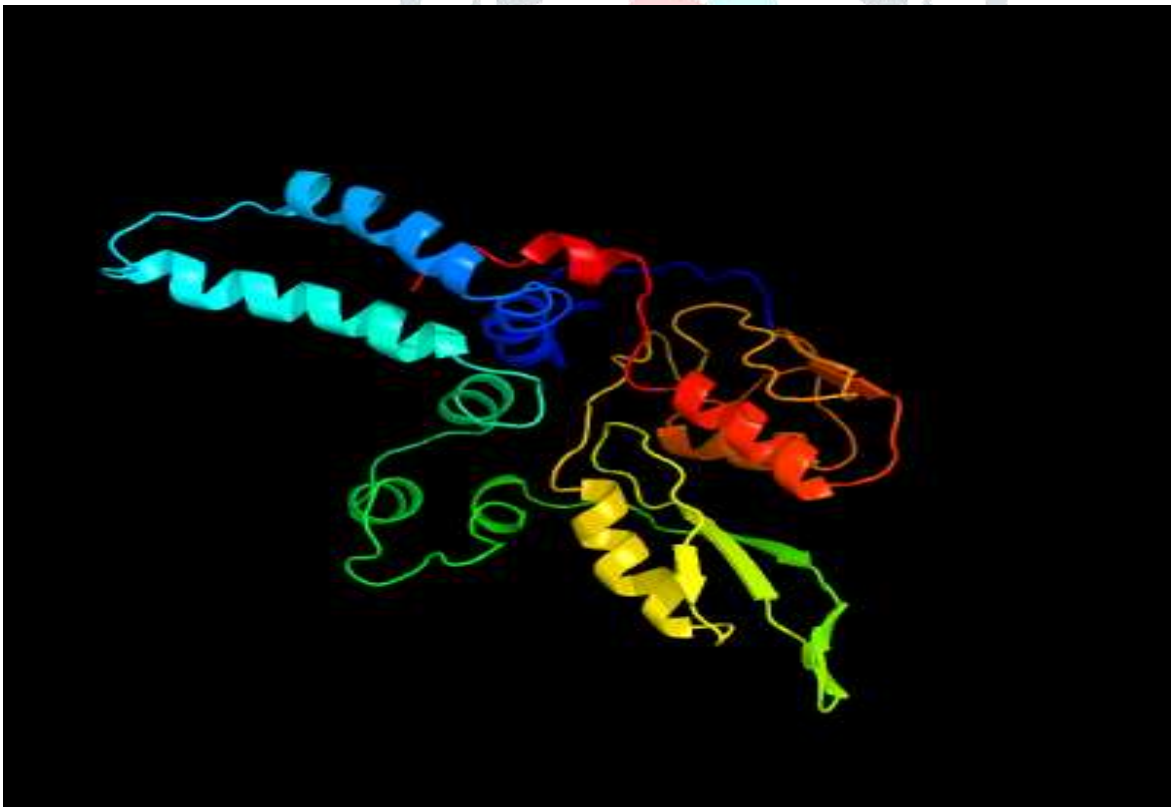


Figure 5. Secondary structure of protein using SOPMA

Phyre2 Secondary structure prediction:

According to structure prediction by Phyre2 ribosomal s5 protein is known to antagonize interferon signaling by binding host karyopherin a proteins, The crystal structures and accompanying biochemical analysis map differences between pathogenic and nonpathogenic viruses, offer templates for drug design, and provide the three dimensional framework necessary for biological dissection of the many functions of ribosomal S5 protein.





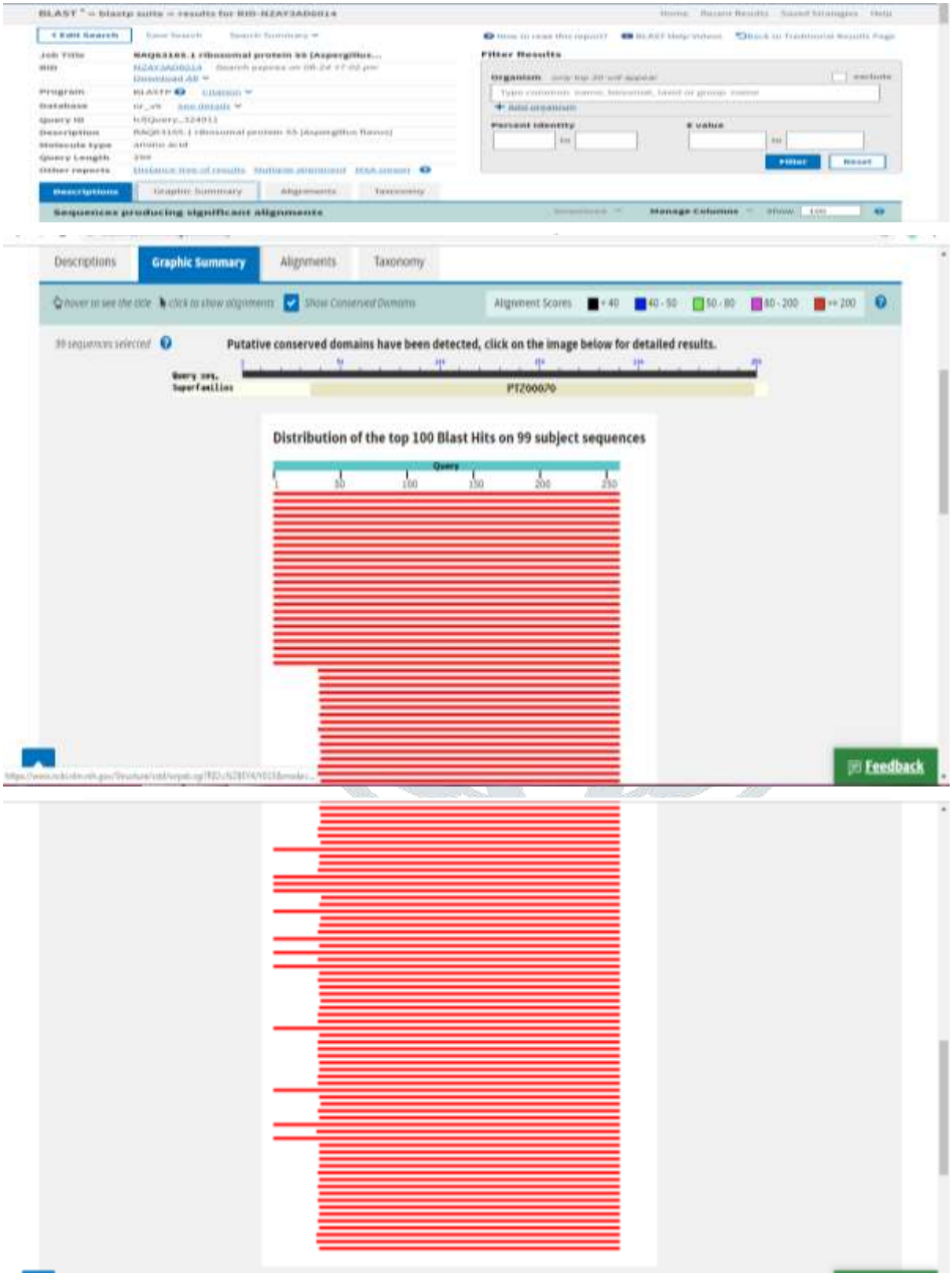
Secondary structure with Helix.

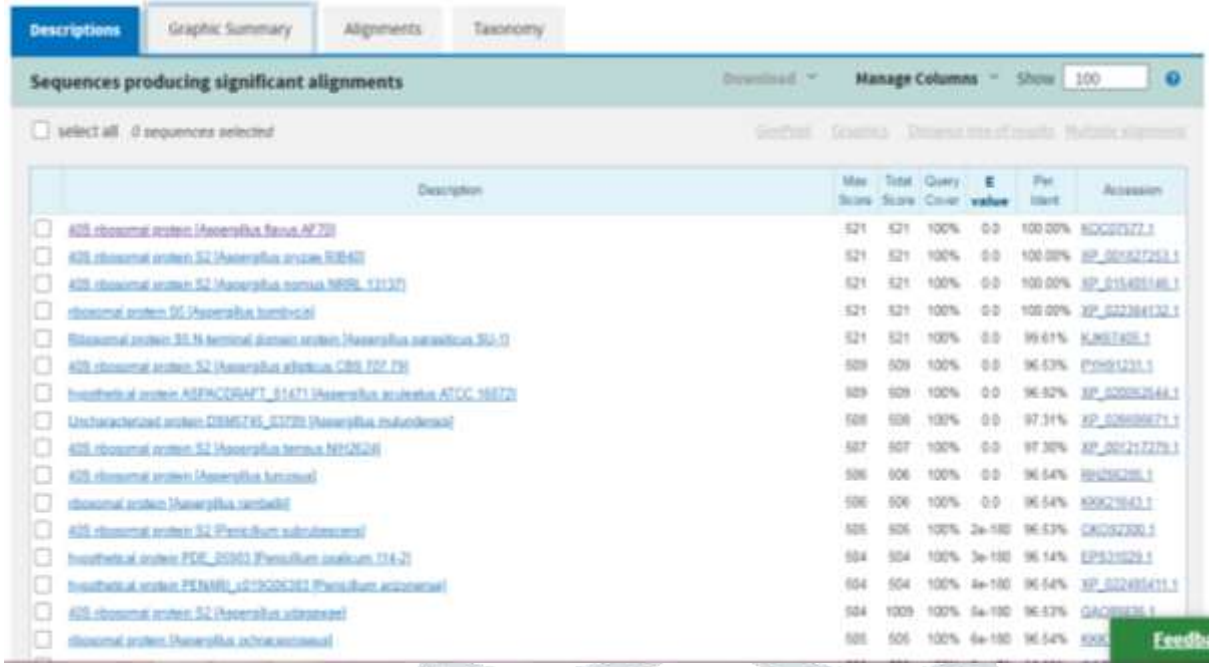
Homology modeling and Model evaluation:

The SWISS-MODEL homology modeling program was used for the predicting of three dimensional structures of the Ribosomal s5 proteins (Figure 5). BLASTP was performed against PDB (Protein Databank, Bernstein *et al*., 1977) to retrieve the best suitable templates for homology modeling. Preferred hit contains maximum identity and lowest e-value that it was used as a template PDBe 3iZb. A (small subunit protein 40s) was selected as template with 100% sequence identity to query sequence. The quality and validation of the model was evaluated by Ramachandran plot analysis using PDBsumserver (Figure 5&6). Based on Ramachandran plot

analysis **118** structures of resolution of at least **2.0** Angstroms and *R*-factor no greater than **20.0** a good quality model would be expected to have over **90%** in the most favored regions (A,B,L) it also showed that only 4.2% residues in outlier region, 16.9% allowed region indicating that the models were of reliable and good quality.

BLASTRP Results:




Descriptions
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Descriptions
405 ribosomal protein, partial [Aspergillus flavus AF70]
Sequence ID: KOC07577.1 Length: 295 Number of Matches: 1
Range 1: 37 to 295 GenPept Graphics
Score Expect Method Identities Positives Gaps
521 bits(1343) 0.0 Compositional matrix adjust. 259/259(100%) 259/259(100%) 0/259(0%)
Query 1 MADAAPRGRGGFGSRGDRGGDRGRGRGRRGRRGGKQEEKEWQPVTKLGRLVKAGKITSME 60
Sbjct 37 MADAAPRGRGGFGSRGDRGGDRGRGRGRRGRRGGKQEEKEWQPVTKLGRLVKAGKITSME 96
Query 61 QIYLHSLPIKEYQIVDFFLPKLKDEVMKIKPVQKQTRAGQRTRFKAVVIIGDSEGHIGLG 120
Sbjct 97 QIYLHSLPIKEYQIVDFFLPKLKDEVMKIKPVQKQTRAGQRTRFKAVVIIGDSEGHIGLG 156
Query 121 IKTSKEVATAIRAAITIAKLAVLPVRRGYWGSNLGEPHSLPVKQSAKCGSVSVRLIPAPR 180
Sbjct 157 IKTSKEVATAIRAAITIAKLAVLPVRRGYWGSNLGEPHSLPVKQSAKCGSVSVRLIPAPR 216
Query 181 GTGLVASPAVKRLLQLAGVQDAYTSSSGSTKTLENTLKATFLAVVNTYGFLTPNLWKETK 240
Sbjct 217 GTGLVASPAVKRLLQLAGVQDAYTSSSGSTKTLENTLKATFLAVVNTYGFLTPNLWKETK 276
Query 241 LIRSPLEEFGDVLRQGKKY 259
Sbjct 277 LIRSPLEEFGDVLRQGKKY 295
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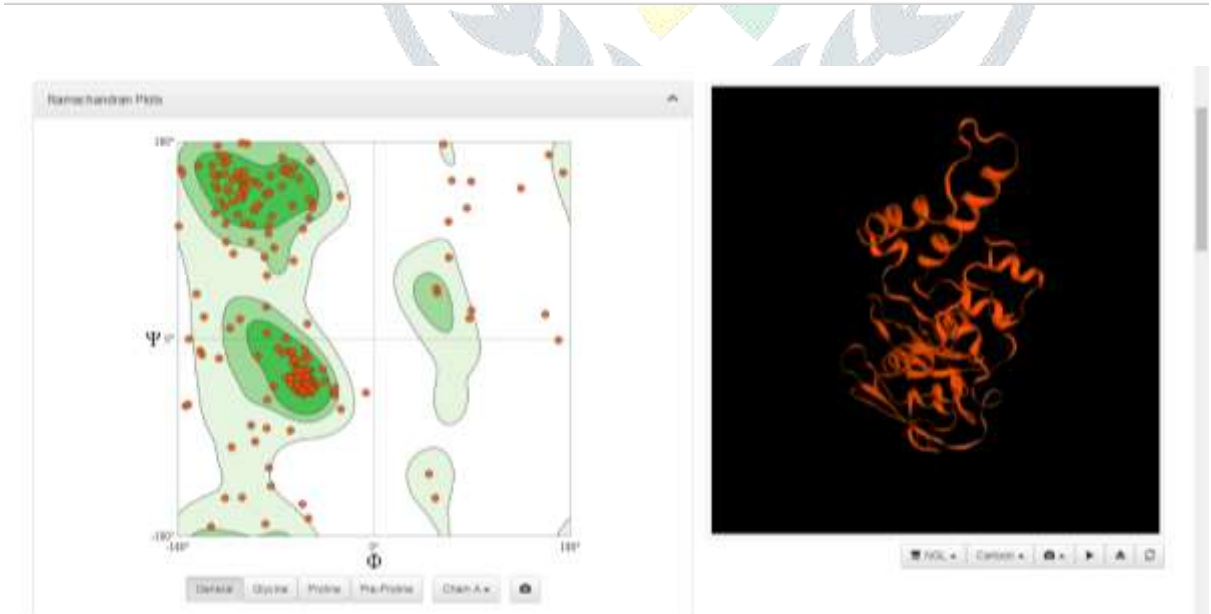
SWISS modeling results



The SWISS-MODEL template library (SMTL version 2019-08-14, PDB release 2019-08-09) was searched with BLAST (Camacho et al.) and HHBlits (Remmert et al.) for evolutionary related structures matching the target sequence in Table T1. For details on the template search, see Materials and Methods. Overall 664 templates were found (Table T2).

Models:

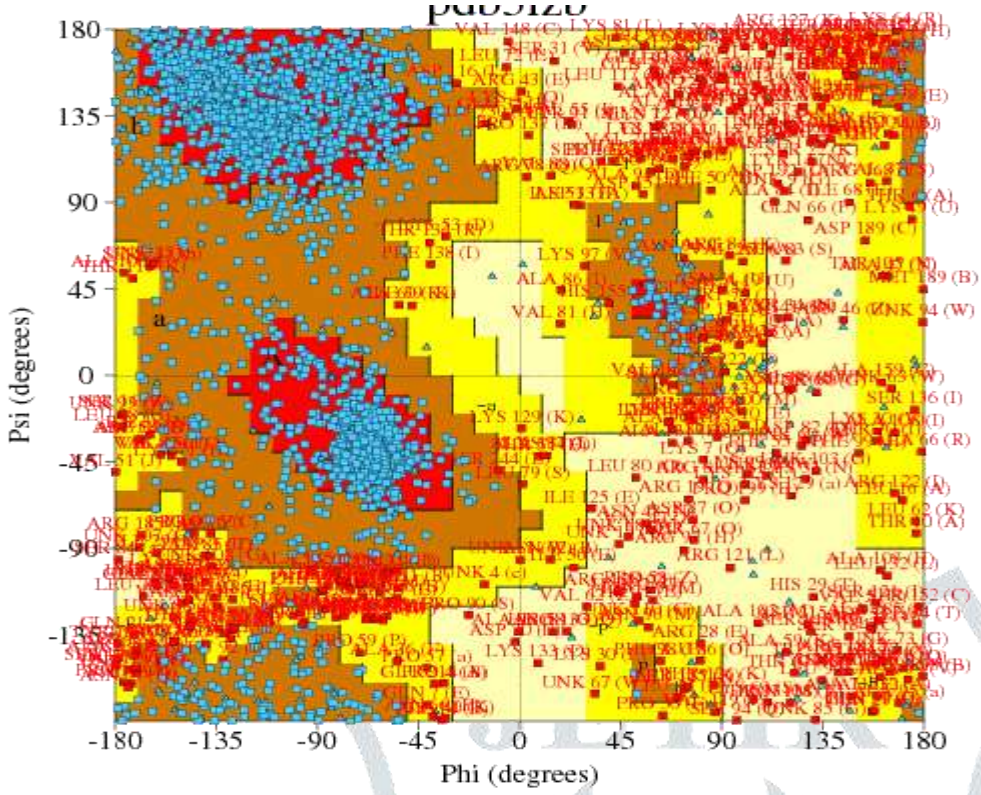
The following models were built (see Materials and Methods "Model Building"):

Model #01	File	Built with	Oligo-State	Ligands	GMQE	QMEAN
	PDB	ProMod3 Version 2.0.0.	monomer	None	0.75	-9.78

QMEAN	-9.78
Cβ	-5.13
All Atom	-6.21
solvation	-5.04
torsion	-7.45

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Range	Coverage	Description
4v3p.5.A	59.53	monomer	HHblits	EM	34.00Å	0.48	1 - 258	0.99	40S ribosomal protein S2

Structure validation by Ramachandran plot:



PROCHECK statistics

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	2394	72.7%**
Additional allowed regions	[a,b,l,p]	530	16.1%
Generously allowed regions	[~a,~b,~l,~p]	228	6.9%
Disallowed regions	[XX]	142	4.3%*
Non-glycine and non-proline residues		3294	100.0%
End-residues (excl. Gly and Pro)		64	
Glycine residues		259	
Proline residues		140	
Total number of residues		3757	

Based on an analysis of **118** structures of resolution of at least **2.0** Angstroms and *R*-factor no greater than **20.0** a good quality model would be expected to have over **90%** in the most favoured regions [A,B,L].

2. G-Factors

Parameter	Average Score
Dihedral angles:-	
Phi-psi distribution	-0.81*
Chi1-chi2 distribution	-0.66*
Chi1 only	-0.37
Chi3 & chi4	0.39
Omega	**-1.30****
	-0.75*
Main-chain covalent forces:-	
Main-chain bond lengths	**-2.64****
Main-chain bond angles	**-3.88****
	-3.36**
OVERALL AVERAGE	**-1.78****

G-factors provide a measure of how **unusual**, or out-of-the-ordinary, a property is.

Values below -0.5* - unusual

Values below **-1.0**** - highly unusual

Important note: The main-chain bond-lengths and bond angles are compared with the Engh & Huber (1991) ideal values derived from small-molecule data. Therefore, structures refined using different restraints may show apparently large deviations from normality.

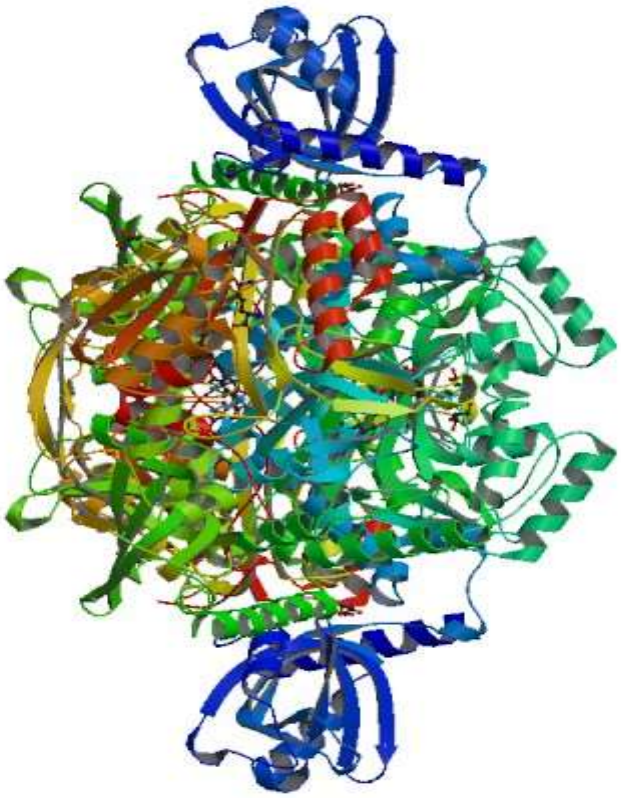
Binding site prediction:

	PDB code	Model	Length	% Iden identity	a.a. overlap	z-score	Ligands	Protein name
1.	1jj6(E)		254	71.5%	242	1418.2		Localization of the small subunit ribosomal proteins into a cryo-em map of saccharomyces cerevisiae translating 80s rib
2.	1jj6(E)		283	61.5%	262	1356.9		Localization of the small subunit ribosomal proteins into a cryo-em map of triticum aestivum translating 80s ribosome
3.	5l6(R)		226	73.0%	222	1345.8	SF4, ANP	Structure of the 40s abce1 post-splitting complex in ribosome recycling and translation initiation
4.	3jam(C)		217	75.4%	211	1339.7		Cryoem structure of 40s-eif1a-eif1 complex from yeast
5.	3j00(C)		217	75.4%	211	1339.7		Cryoem structure of 40s-eif1-eif1a preinitation complex
6.	5t5h(C)		217	75.4%	211	1339.7		Structure of the yeast kluyveromyces lactis small ribosomal subunit in complex with the cricket paralysis virus ires
7.	3j51(C)		217	75.4%	211	1339.7	MET	Cryoem structure of a partial yeast 48s preinitation complex
8.	6emr(R)		220	72.7%	220	1331.1		Cryo-em structure of a late pre-40s ribosomal subunit from saccharomyces cerevisiae
9.	3o2z(B)	X-ray 4.00Å	219	72.9%	216	1327.5	CHX	Yeast 80s ribosome. This entry consists of the 40s subunit o first 80s in the asymmetric unit.
10.	3o30(B)	X-ray 4.00Å	219	72.9%	216	1327.5	CHX	Yeast 80s ribosome. This entry consists of the 40s subunit o second 80s in the asymmetric unit.
11.	4u3d(D)	X-ray 2.80Å	217	74.4%	211	1323.9	CHX	Crystal structure of lactimidomycin bound to the yeast 80s r
12.	4u4g(g)	X-ray 2.80Å	217	74.4%	211	1323.9	CHX	Crystal structure of lactimidomycin bound to the yeast 80s r
13.	4u4i(D)	X-ray 2.90Å	217	74.4%	211	1323.9	CHX	Crystal structure of cycloheximide bound to the yeast 80s ri
14.	4u6d(g)	X-ray 2.90Å	217	74.4%	211	1323.9	CHX	Crystal structure of cycloheximide bound to the yeast 80s ri
15.	3u5c(C)	X-ray 3.00Å	217	74.4%	211	1323.9		The structure of the eukaryotic ribosome at 3.0 a resolution entry contains proteins of the 40s subunit, ribosome a
16.	3u5h(C)	X-ray 3.00Å	217	74.4%	211	1323.9		The structure of the eukaryotic ribosome at 3.0 a resolution entry contains proteins of the 40s subunit, ribosome b
17.	4u6i(D)	X-ray 3.00Å	217	74.4%	211	1323.9	CHX	Crystal structure of lycorine bound to the yeast 80s ribosom
18.	4u6m(g)	X-ray 3.00Å	217	74.4%	211	1323.9	CHX	Crystal structure of lycorine bound to the yeast 80s ribosom
19.	4u4z(D)	X-ray 3.00Å	217	74.4%	211	1323.9	CHX	Crystal structure of anisomycin bound to the yeast 80s ribos
20.	4u51(g)	X-ray 3.00Å	217	74.4%	211	1323.9	CHX	Crystal structure of anisomycin bound to the yeast 80s ribos
21.	4u3f(D)	X-ray 3.00Å	217	74.4%	211	1323.9	CHX	Crystal structure of homoharringtonine bound to the yeast 80
22.	4u5l(g)	X-ray 3.00Å	217	74.4%	211	1323.9	CHX	Crystal structure of homoharringtonine bound to the yeast 80
23.	4u3j(D)	X-ray 3.00Å	217	74.4%	211	1323.9	CHX	Crystal structure of naglactonE C bound to the yeast 80s ri

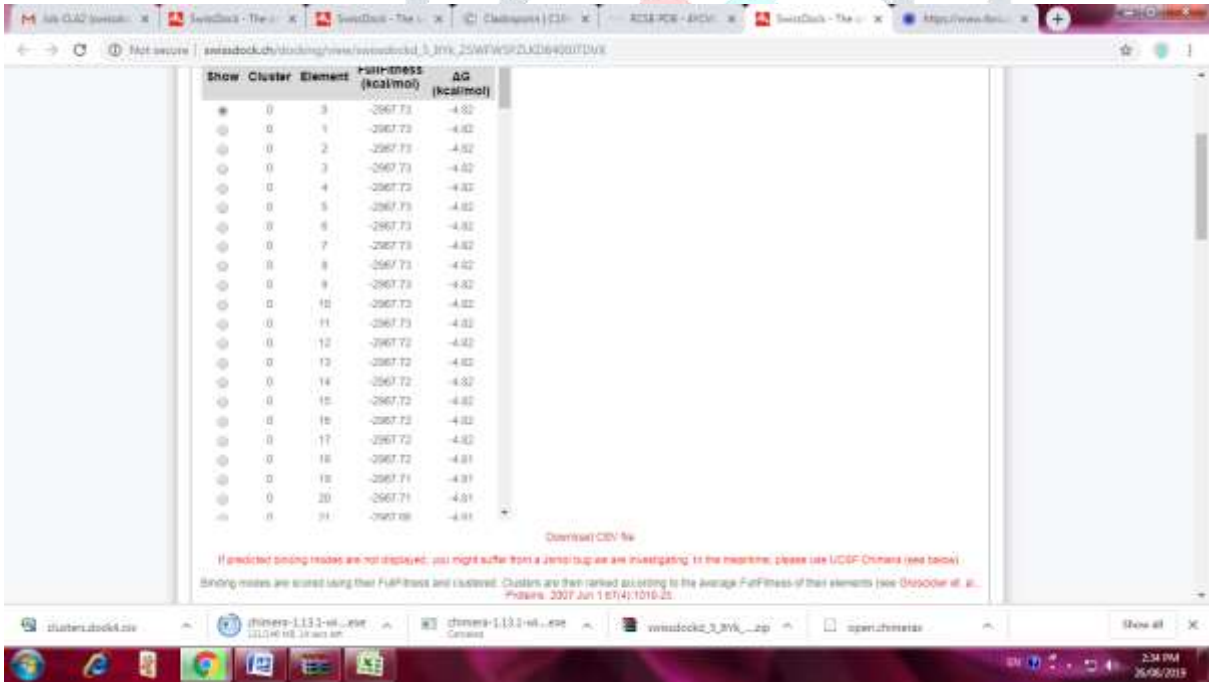
Molecular Docking:

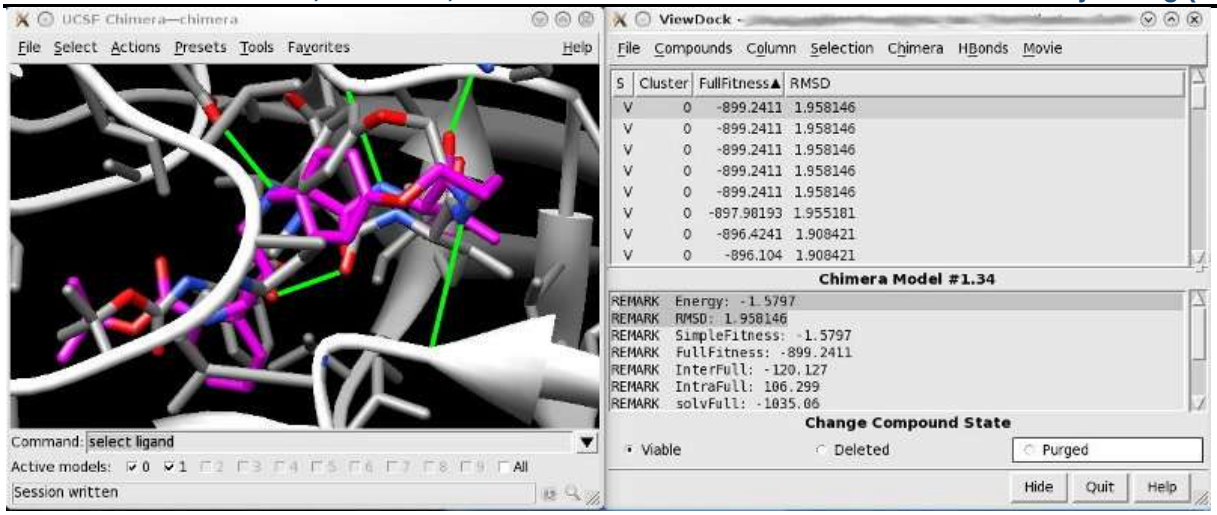
Molecular docking was done by using online free software for docking Swiss Dock. This website provides an access to **SwissDock**, a web service to predict the molecular interactions that may occur between a target protein and a small molecule. **S3DB**, a database of manually curated target and ligand structures, inspired by the Ligand-Protein Database. SwissDock is based on the docking software EADock DSS, whose algorithm consists of the following steps:

1. Many binding modes are generated either in a box (local docking) or in the vicinity of all target cavities (blind docking).
2. Simultaneously, their CHARMM energies are estimated on a grid.
3. The binding modes with the most favorable energies are evaluated with FACTS, and clustered.
4. The most favorable clusters can be visualized online and downloaded on your computer.



Target for Docking Crystal structure of Cladosporin.-4YCU





http://www.swissdock.ch/img/material/viewdock.jpg Image address.

Conclusion

The Cladosporin (Rps5)protein is involve in the transcription of virus. The present study we analyzed the physicochemical properties of protein by using Protparam tool. The 3D structure of protein was predicted using SWISS MODEL server. The final model was further evaluated by using Procheck and Ramachandran plot analysis. Binding site of the protein was studied using PDBsum database. From the present study it has been concluded that ribosomal protein s5 protein can be used as target for the inhibition of virus. The molecular structural insight encompasses to the development of new drug for inhibition of protein by using Cladosporin.

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