PHARMACOINFORMATIC APPROACH FOR SARS

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ABSTRACT

SARS is a respiratory disease caused by corona virus, it is produced the flu like symptoms and severe bronchial constriction ,alveoli inflammation it's leads to death fever, myalgia, lethargy, gastrointestinal symptoms, cough, sore throat and other non-specific symptoms. , The rapid transmission and high mortality rate made SARS a global threat for which no efficacious therapy was available and empirical strategies had to be used to treat the patients. New insights into the field of the SARS-CoV genome structure and pathogenesis revealed novel potential anti-Corona virus targets. Several proteins encoded by the SARS-CoV could be considered as targets for therapeutic intervention.pharmacoinformatic approach of SARS gave new opportunities for the development of therapeutics, an extensive analysis of the SARS proteins including modeling of its structure may prove useful in designing a drug for SARS treatment. Further comprehensive work on this protein will leads to a new milestone in SARS research.

Keywords: *Corona Virus, Homology Modeling, Protein Validation, Lead Optimization, Docking Studies.*

INTRODUCTION

Severe acute respiratory syndrome (**SARS**) is a respiratory disease in humans which is caused by the SARS Corona virus (SARS-CoV). There has been one near pandemic to date, between November 2002 and July 2003, with 8,096 known infected cases and 774 deaths (a case-fatality rate of 9.6%) worldwide being listed in the World Health Organization's (WHO) 21 April 2004 concluding report. Within a matter of weeks in early 2003, SARS spread from the Guangdong province of China to rapidly infect individuals in some 37 countries around the world. (EPR-WHO., 2004).

The chest X-ray (CXR) appearance of SARS is variable. There is no pathogenomonic appearance of SARS but is commonly felt to be abnormal with patchy infiltrates in any part of the lungs. The initial CXR may be clear.(Thiel et al.,2007).

Antiviral research:

Before the emergence of SARS-CoV, no efforts were put into the search for antiviral against corona viruses. The rapid transmission and high mortality rate made SARS a global threat for which no efficacious therapy was available and empirical strategies had to be used to treat the patients. New insights into the field of the SARS-CoV genome structure and pathogenesis revealed novel potential anti-Coronavirus targets. Several proteins encoded by the SARS-CoV could be considered as targets for therapeutic intervention: the spike protein, the main protease, the NTPase/helicase, the RNA dependent RNA polymerase and different other viral protein-mediated processes. Potential anti-SARS-CoV drugs are currently being developed *in vivo*. The development of effective drugs against SARS-CoV may also provide new strategies for the prevention or treatment of other Coronavirus diseases in animals or humans.(stockman et al.,2006)

Viral replication

Coronavirus (CoV) genome replication takes place in the cytoplasm in a membrane-protected microenvironment and starts with the translation of the genome to produce the viral replicase. CoV transcription involves a discontinuous RNA synthesis (template switch) during the extension of a negative copy of the sub genomic mRNAs. The requirement for base pairing during transcription has been formally demonstrated in arteriviruses and CoVs. The CoV N protein is required for Coronavirus RNA synthesis and has RNA chaperon activity that may be involved in template switch. Both viral and cellular proteins are required for replication and transcription. CoVs initiate translation by cap-dependent and cap-independent mechanisms.

Cell macromolecular synthesis may be controlled after CoV infection by locating some virus proteins in the host cell nucleus. Infection by different corona viruses cause in the host alteration in the transcription and translation patterns, in the cell cycle, the cytoskeleton, apoptosis and coagulation pathways, inflammation and immune and stress responses The balance between genes up- and down-regulated could explain the pathogenesis caused by these viruses.

Coronavirus expression systems based on single genome constructed by targeted recombination, or by using infectious cDNAs, has been developed. The possibility of expressing different genes under the control of transcription regulating sequences (TRSs) with programmable strength and engineering tissue and species tropism indicates that CoV vectors are flexible. CoV based vectors have emerged with high potential vaccine development and possibly for gene therapy. . (Enjuanes et al., 2008).

Statistical survey for sars:

The epidemic of SARS appears to have started in Guangdong Province, China in November 2002. The first case of SARS was reportedly originated in Shunde, Foshan, Guangdong in Nov 2002, and the patient, a farmer, was treated in the First People's Hospital of Foshan (Mckay Dennis). The patient died soon after, and no definite diagnosis was made on his cause of death. ("Patient #0" --

first reported symptoms -- has been attributed to Charles Bybelezar of Montreal, Quebec, Canada) and, despite taking some action to control it, Chinese government officials did not inform the World Health Organization of the outbreak until February 2003, restricting media coverage in order to preserve public confidence. This lack of openness caused delays in efforts to control the epidemic, resulting in criticism of the People's Republic of China (PRC) from the international community. The PRC has since officially apologized for early slowness in dealing with the SARS epidemic. (WHO targets SARS 'super spreaders', CNN News, 6 April 2003 URL Accessed 30Dec 2008)

Probable cases of SARS by country, 1 November 2002–30 Dec 2008

Country	Cases	Deaths	SARS cases that died of reasons not due to SARS	Fatality (%)
People's Republic of China *	5328	349	19	6.6
Hong Kong*	1755	299	5	17
Canada	432	44	0	10
Taiwan	346**	37	36	11
Singapore	238	33	0	10
Malaysia	5	2	0	40
India	3	0	0	0
France	7	1	0	14
USA	27	0	0	0
Vietnam	63	5	0	8
Total	8204	770	60	12.66

(*) Figures for the People's Republic of China exclude the Special Administrative Regions (Macau SAR, Hong Kong SAR) which are reported separately by the WHO.

(**) Since 11 July 2008, 325 Taiwanese cases have been 'discarded'. Laboratory information was insufficient or incomplete for 135 discarded cases; 101 of these patients died. (*Source: WHO).*

The clinical treatment of SARS has been relatively ineffective with most high risk patients requiring artificial ventilation. Currently, corticosteroids and Ribavirin are the most common drugs used for treatment of SARS. In vitro studies of Ribavirin have yielded little results at clinical, nontoxic concentrations. Better combinations of drugs that have yielded a more positive clinical outcome (when administered early) have included the use of Kaletra, Ribavirin

and corticosteroids. (Wu et al., 2004). Lymphopenia can also be a side effect of corticosteroids even further decreasing the immune response and allowing a spike in the viral load; yet physicians must balance the need for the anti-inflammatory treatment of corticosteroids (Murphy 2008). Clinicians have also noticed positive results during the use of human interferon and Glycyrrhizin. No compounds have yielded inhibitory results of any significance. (Blendon, Robert J., et al. 2006).

The severe acute respiratory syndrome (SARS) virus belongs to the Coronaviridea family of viruses. Its virion encodes several proteins including a replicase and four structural proteins. Here we describe the three-dimensional structure of the N-terminal domain of the SARS corona virus (CoV) nucleocapsid protein. The protein consists of a five-stranded beta sheet with a folding topology distinct from other RNA-binding proteins. Single-stranded RNAs bind to the protein surface at the junction between a flexible, positively charged beta hairpin and the core structure. NMR-based screening was used to identify low molecular weight compounds that bind to this site. (**Huang Q**, 2004).

The HIV protease inhibitors Ritonavir and Saquinavir did not show any inhibitory affect at nontoxic levels. Iminocyclitol 7 has been found to have an inhibitory effect on SARS-CoV in that it disrupts the envelope glycoprotein processing. Iminocyclitol 7 specifically inhibits the production of human fucosidase and *in vitro* trials yielded promising results in the treatment of SARS, yet one problem exists. A deficiency of fucosidase can lead to a condition known as fucosidosis in which there is a decrease in neurological function. (Murphy et al. 2008)

Pentoxifylline and Lisofylline is used to improve blood flow in patients with circulation problems to reduce aching, cramping, and tiredness in the smooth muscles. It works by decreasing the thickness (viscosity) of blood. This change allows your blood to flow more easily, especially in the small blood vessels of the bronchial smooth muscle. Pentoxifylline may cause side effects like stomach upset, vomiting, gas, dizziness, and headache.2-oxo-propyl-com,AG7088 are experimental drugs produced same effect likes Pentoxifylline and Lisofylline. **(Puro V, Fusco FM et al 2008).**

MATERIALS & METHODS:

Literature review has proven that HIV-protease inhibitor and Anti-viral drugs and corticosteroids produced more adverse effect then therapeutic effect; we need more therapeutic effect with less side effects, some of the noval drugs like “Pentoxifylline, Lisofylline, AG7008, 2-oxopropyl-com “are used for SARS. Our further analysis was focused on the using bioinformatics techniques. Genomic studies on SARS leads to the discovery of the newer drug target and therapeutics

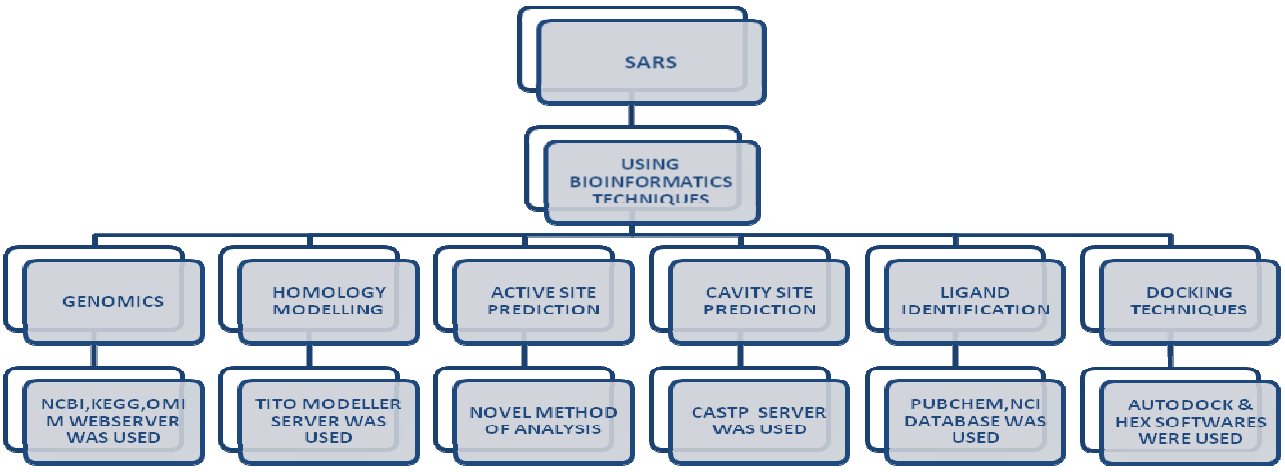


Figure 1: methodology for Pharmacoinformatics approach for sars

Methodology for analysis of SARS:

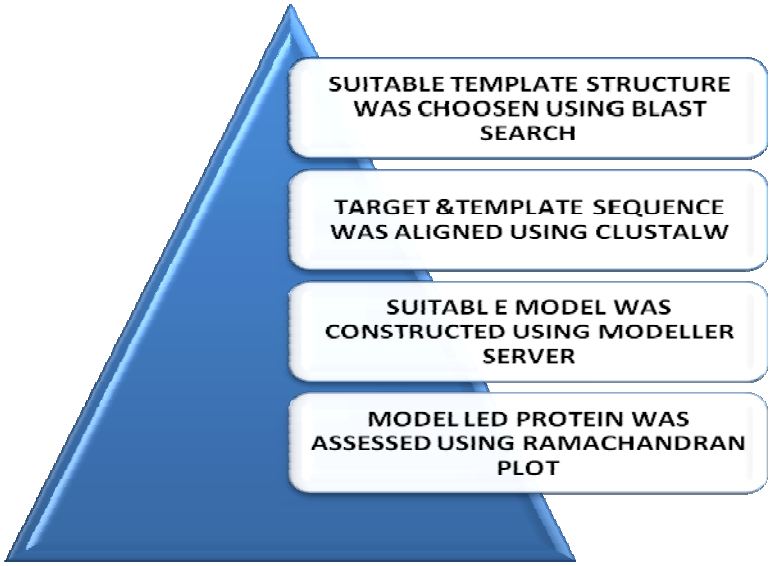


Figure 2: methodology for homology modeling

Website:

GENOMICS:
KEGG :
http://www.genome.jp/kegg/pathway.html
ENTREZ:
http://www.ncbi.nlm.nih.gov/entrez/query/static/adva
ncedentrez.html
GENATLAS :http://www.dsi.univ-paris5.fr/genatlas/
GENESCAN :
www.bioweb.pasteur.fr/seqanal/interfaces/genscan.ht
ml - 8k

PROTEOMICS:
Nps.Prodom.Clustalw.web server

HOMOLOGY MODELLING:

*bioserv.cbs.cnrs.fr/HTML_BIO/frame_**tito**.html*

PROTEIN VALIDATION:

*nihserver.mbi.ucla.edu/**SAV**ES/*

Lead identification and optimization:

***pubchem**.ncbi.nlm.nih.gov/*

www.**molinspiration**.com/docu/webme

www.techelan.com/HiThrup**ADMET**

***Docking studies**:*

***autodock**.scripps.edu.*

RESULTS &DISCUSSION:

A novel newer target for SARS was identified. On the basis of information like name and origin, entry information, references, cross references, databases and sequence of this target was retrieved in NCBI and PDB.

HOMOLOGY MODELLING:

The 3D structure of the SARS was modeled by using TITO modeller server.

TEMPLATE SELECTION

The critical first step in homology modeling was the identification of the best template structure, if indeed any were available. The simplest method of template identification relies on serial pair wise sequence alignments aided by database search techniques such as FASTA and BLAST.

TARGET-TEMPLATE SEQUENCE ALIGNMENT:

It was possible to use the sequence alignment generated by the database search technique as the basis for the subsequent model production; however, more sophisticated approaches have also been explored. One proposal generates an ensemble of stochastically defined Multiple sequence alignments (CLUSTALW) between the target sequence and a single identified template as a means of exploring "alignment space" in regions of sequence with low local similarity.

Model generation

Given a template and an alignment, the information contained therein must be used to generate a three-dimensional structural model of the target, represented as a set of Cartesian coordinates for each atom in the protein. Three

major classes of model generation methods have been proposed.

Tools used to model the structure:

TITO modeller server:

Paste the aligned sequences obtained from CLUSTALW of both template and target sequence in to the appropriate box.

Structure Analysis and Validation:

The modeled structure of the protein was also validated using the SAVS (Structural Analysis and Validation Server). It utilizes 5 programs to do the validation:

- PROCHECK
- WHAT_CHECK
- ERRAT
- VERIFY_3D
- PROVE

The following results were obtained from the respective programs:

Ramachandran plot:

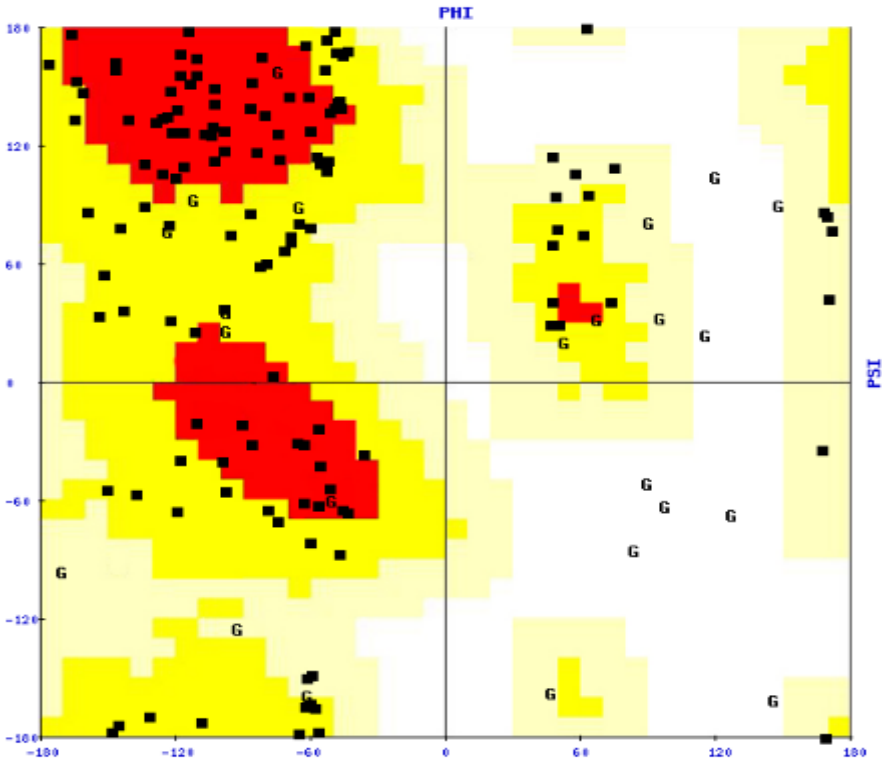


Figure3: Ramachandran plot

Procheck:

This tool gives details about the Ramachandran plots

The Modeled Structure of Novel Protein using PROCHECK program gives RAMACHANDRAN PLOT

What check:

It gives the detailed information about the analysis of the protein structure.

Errat:

The plot gives the details about the error values of each residue.

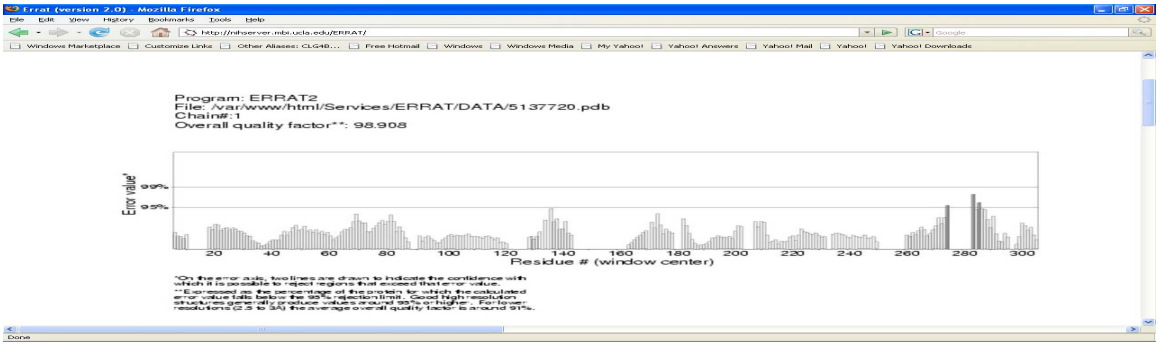


Figure4: stereo chemical quality of protein

With the program giving the overall quality factor of the modeled structure as 87.42

Docking studies:

The AUTODOCK 4.0 docking steps were performed using our ligand pentoxifylline to our target protein, SARS. The minimum energy was found to be -10.89 and it showing good conformation with that of our target protein.

Pentoxifylline:

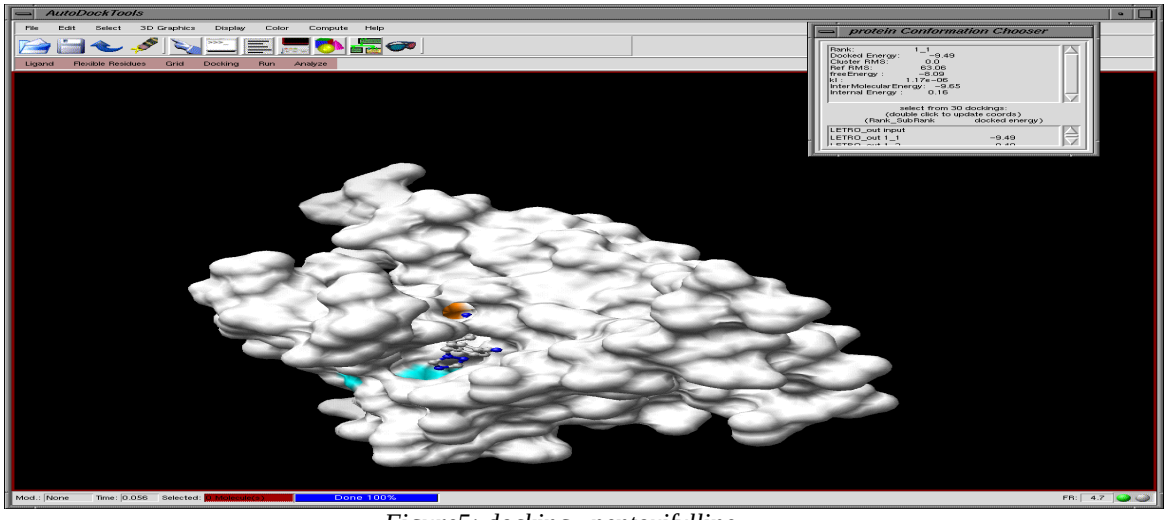


Figure5: docking –pentoxifylline

Lisofylline:

The minimum energy was found to be -8.80 and it showing good conformation with that of our target protein

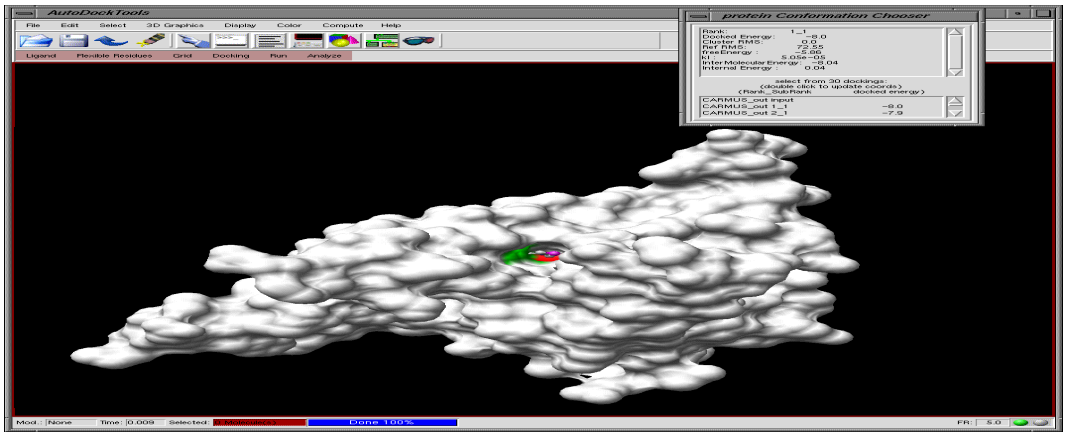


Figure6: Docking -Lisofylline

2-oxo-propyl-com:

The minimum energy was found to be -9.90 and it showing good conformation with that of our target protein

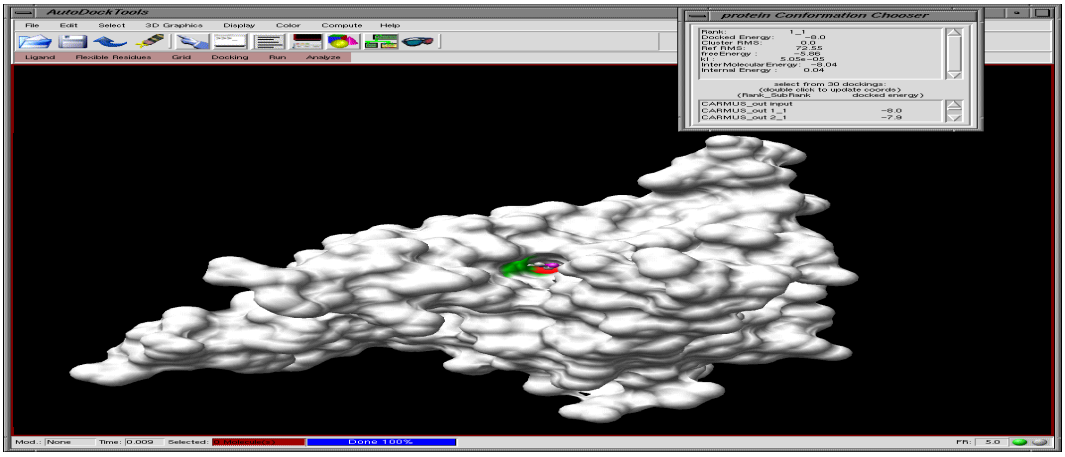
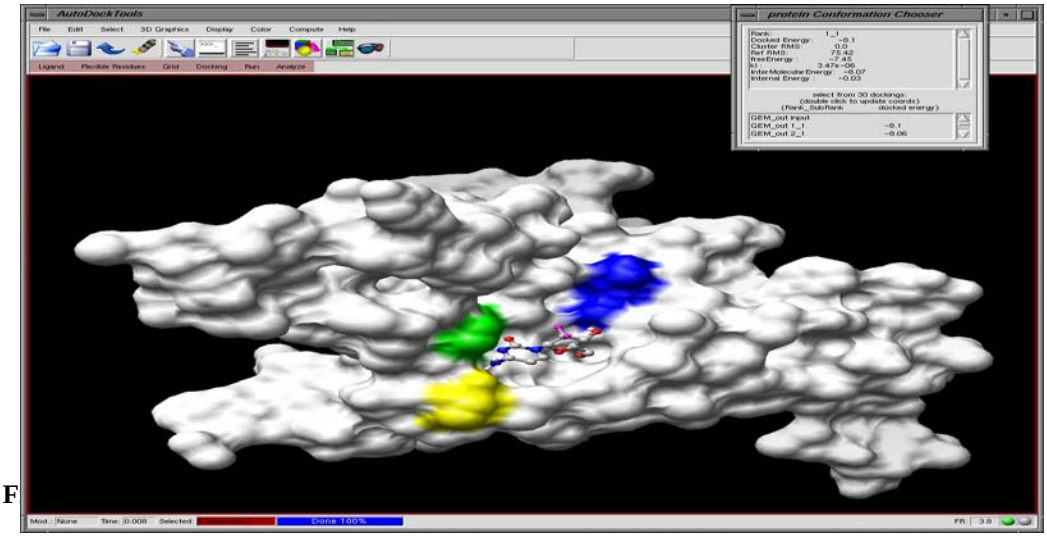


Figure7: Docking-2oxo-propyl.com

Ag7008:

The minimum energy was found to be -7.80 and it showing good conformation with that of our target protein



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On the basis of the highest docking score (Autodock4.0) pentoxifylline and 2-oxopropyl-com was found to be the good target for sars disease. Previous literature review has indicated that pentoxifylline was used only for COPD and Asthma .Now it's also used to treat the SARS.pentoxifylline was a successful drug candidate for bronchitis and sars. Hence our research has proved that drugs used for SARS and also Asthma and COPD.

CONCLUSION:

The clinical treatment of SARS has been relatively ineffective with most high risk patients requiring artificial ventilation. Currently, corticosteroids and Ribavirin are the most common drugs used for treatment of SARS. In vitro studies of Ribavirin have yielded little results at clinical, nontoxic concentrations. Better combinations of drugs that have yielded a more positive clinical outcome (when administered early) have included the use of Kaletra, Ribavirin and corticosteroids. But these drugs had more side effects when compare the therapeutic effect, so we need the noval approach for identification of drugs for SARS.The Insilco analysis prove that the new lead compounds are available for SARS, Further our research confirmed that the pentoxifylline and 2-oxopropyl-com gave good docking results for SARS using Auto dock. The binding energy of those drugs also increases the confidence of using those ligands as candidate for this particular protein. Comparative analysis of these drugs was done, depending upon their binding energy of ligand with that of the active site. Hence our research proved that pentoxifylline and 2-oxopropyl-com has splendid ability to dock our target protein. Since SARS is not completely cure, because it's a viral disease the full data about the co-v family viruses still not available .our findings in SARS will no

doubt be a breakthrough for future research in health care and also Asthma and COPD.

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