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Computational Modeling and Drug Interaction Studies of Neuraminidase from influenza a virus (strain a/swine/new jersey/11/1976 h1n1) using discovery studio 2.5"

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ABSTRACT

Swine flu (swine influenza) is a respiratory disease caused by viruses (influenza viruses) that infect the respiratory tract of pigs and result in nasal secretions. Swine flu produces most of the same symptoms in pigs as human flu produces in people. The 2009 swine flu strain, first seen in Mexico, is termed novel H1N1 flu since it is mainly found infecting people and exhibits two main surface antigens, H1 (hemagglutinin type 1) and N1 (neuraminidase type1).This study deals with the structure prediction and computer aided drug interaction studies for the neuraminidase protein from H1N1 virus. Selection of neuraminidase was done since its function is to move the virus out of host cell and hence spread more disease. Targeting this protein with potential drug compounds will stop further spread. Accelrys Discovery studio 2.5 was the software employed in this work. Target sequence selection was done from PIR database and homology modeling was carried out using in-build modeler in DS. Quality of the model has been analyzed and binding sites were predicted. Drugs suitable for swine flu were retrieved from drug bank and through a 3D database search in DS, different conformations has been developed. Molecular docking was performed using Ligandfit in DS. Selection of best drug was done based on highest dock score which was further studied for ADMET (Absorption, Distribution, Metabolism, Elimination and Toxicity) properties. TOPKAT predictions, which give results for carcinogenicity, ocular irritation, skin irritation was also carried out. The results of this study can be useful further for QSAR studies and various in-vitro analysis later.

Introduction

Swine influenza (also called pig influenza, swine flu, hog flu and pig flu) is an infection by any one of several types of swine influenza virus. Swine influenza virus (SIV) or S-OIV (swine-origin influenza virus) is any strain of the influenza family of viruses that is endemic in pigs.[1]As of 2009, the known SIV strains include influenza C and the subtypes of influenza A known as H1N1, H1N2, H3N1, H3N2, and H2N3.It has been classified in to three genera which causes human flu, two also cause influenza in pigs, with influenza A being common in pigs and influenza C being rare.[3] Influenza B has not been reported in pigs. Within influenza A and influenza C, the strains found in pigs and humans are largely distinct, although because of reassortment there have been transfers of genes among strains crossing swine, avian, and human species boundaries. Influenza C viruses infect both humans and pigs, but do not infect birds. Transmission between pigs and humans has occurred in the past. For example, influenza C caused small outbreaks of a mild form of influenza amongst children in Japan and California. Because of its limited host range and the lack of genetic diversity in influenza C, this form of influenza does not cause pandemics in humans. Swine influenza is known to be caused by influenza A subtypes H1N1, H1N2, H2N3, H3N1, and H3N2. In pigs, three influenza A virus subtypes (H1N1, H1N2, and H3N2) are the most common strains worldwide. In the United States, the H1N1 subtype was exclusively prevalent among swine populations before 1998; however, since late August 1998, H3N2 subtypes have been isolated from pigs. As of 2004, H3N2 virus isolates in US swine and turkey stocks were triple reassortants, containing genes from human (HA, NA, and PB1), swine (NS, NP, and M), and avian (PB2 and PA) lineages. The symptoms of H1N1 in people are similar to the symptoms of common seasonal flu. The common symptoms of H1N1 Flu include: Fever – particularly a fever of over 100 degrees, Sore throat, Cough,Chills and fatigue, Body aches, Headache Occasionally, vomiting and diarrhea. H1N1 in 2009 (sometimes called "swine flu") is a new influenza virus causing illness in people. This new virus was first detected in people in the United States in April 2009. This virus is spreading from person-to-person worldwide, probably in much the same way that regular seasonal influenza viruses spread. On June 11, 2009, the World Health Organization (WHO) declared that a pandemic of 2009 H1N1 flu was underway. People at High Risk for Developing Flu-Related Complications like Children younger than 5, but especially children younger than 2 years old, Adults women. People who have medical conditions like Asthma ,Neurological and neurodevelopment conditions [including disorders of the brain, spinal cord, peripheral nerve, and muscle such as cerebral palsy, epilepsy (seizure disorders), stroke, intellectual disability (mental retardation), moderate to severe developmental delay, muscular dystrophy, or spinal cord injury, Chronic lung disease (such as chronic obstructive pulmonary disease [COPD] and cystic

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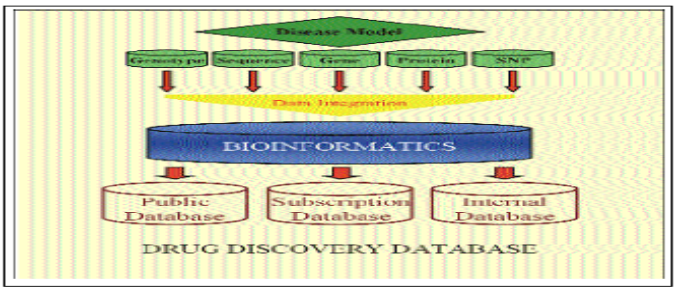
fibrosis),Heart disease (such as congenital heart disease, congestive heart failure and coronary artery disease),Blood disorders (such as sickle cell disease),Endocrine disorders (such as diabetes mellitus),Kidney disorders, Liver disorders, Metabolic disorders (such as inherited metabolic disorders and mitochondrial disorders),Weakened immune system due to disease or medication (such as people with HIV or AIDS, or cancer, or those on chronic steroids),People younger than 19 years of age who are receiving long-term aspirin therapy In addition, some studies have shown that obese persons (body mass index ≥30) and particularly morbidly obese persons (body mass index ≥40) are at higher risk, perhaps because they have one of the higher risk conditions above but do not realize it.The following picture depicts the various steps followed in drug discovery and development and various drug discovery databases.



Material And Method

Swiss-Prot strives to provide reliable protein sequences associated with a high level of annotation (such as the description of the function of a protein, its domains structure, post-translational modifications, variants, etc.), a minimal level of redundancy and high level of integration with other databases.

Protein Information Resource (PIR) is an integrated public bioinformatics resource to support genomic, proteomic and systems biology research and scientific studies (Wu *et al.*, 2003).

Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches.

Swiss-PdbViewer is an application that provides a user friendly interface allowing analyzing several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts.

SAVS: **Structural Analysis and Verification Server.** For docking studies, drugs that are related to the diseases are obtained from DRUG BANK databases. PubChem is a database of chemical . The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States, National Institutes of Health (NIH).

PubChem can be accessed for free through a web user interface. PubChem contains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds. More than 80 database vendors contribute to the growing PubChem database These drugs are obtained from PUBCHEM and it provides the name, structure, smiles, pharmacological effects, and mechanism of action about the drug. Structures of the relevant drugs smiles were saved and opened in the **DS.**

Discovery Studio is an interactive modeling & simulation environment for life sciences researchers. Built on Accelrys' open pipelining platform, SciTegic™ Pipeline Pilot 6.0, Discovery Studio provides seamless integration of multiple premium software application modules. Accelrys provides software for chemical research, especially in the areas of drug discovery and materials science.

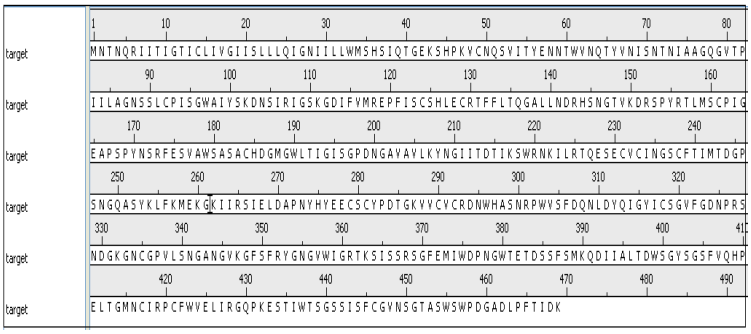
Results and discussion

Sequence retrieval

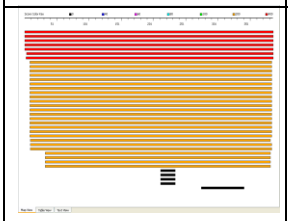
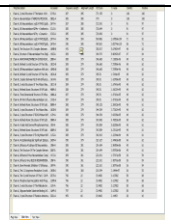
The sequence retrieval is done from PIR database. The target sequence did not have a PDB id. This shows there is no experimentally resolved structure available for this Protein. Length of the sequence is 469 amino acid.

Protein AC/ID	Protein Name	Length	Organism Name	PIRSF ID	Related Seq.	PDB ID
Q9IGQ0/NRAM_I76AI /ProClass UniProtKB/Swiss-Prot	Neuraminidase BioThesaurus	469	Influenza A virus (strain A/Swine/New Jersey/11/1976 H1N1)	PIRSF001075	300	

Sequence Opened With DS 2.5



BLAST RESULTS:The query protein was subjected to a BLAST search against PDB database and it was completely aligned with highly similar template proteins.

MAP VIEW	TABLE VIEW	TEXT VIEW
		

Selection of template

The following two protein structure hits, which show high similarities, were selected as templates for the project. They are 3CYE A chain and 2HTY A chain with 100% and 91% identity respectively.

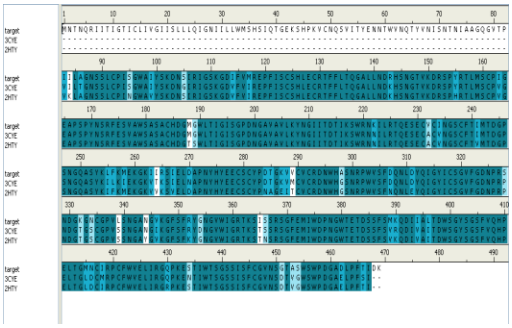
Template	Crystal with Lattice-Translocation Defects >gi|195927504|pdb|3CYE|	N1 Neuraminidase from N1 subunit of influenza virus|2HTY|A chain
Sequence length	387	387
Alignment Length	385	385
Bit score	771.155	723.391
E value	0	0
Identity	100	91
Positive	100	97

Alignment of input model sequence with template sequence:

The Input Model Sequence is aligned to a set of protein structures based on their sequence similarity. If there are more than one template structures, the sequences of the structures will be first aligned based on their structure similarity. The following steps are performed:

1. Align Template Structures
2. Align Model Sequence with Templates

The Structure for the above mentioned species where uploaded and alignment of the same was done based on similarity. The alignment was done based on 3D structural similarity. The 3DMA program align by finding the matching segments of protein structure pairs by comparing the C-α torsion angle and optimizes by discriminative extension of matching segment.



The output file is Clustal -1- bsml is opened and viewed for further steps.

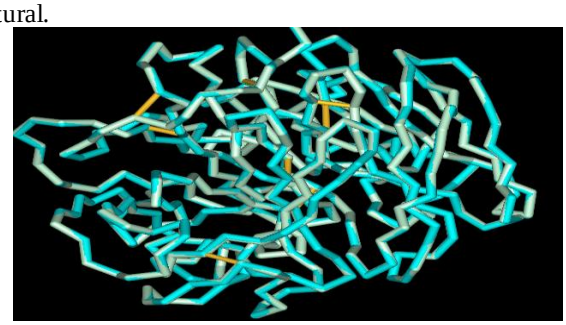
Discussion:

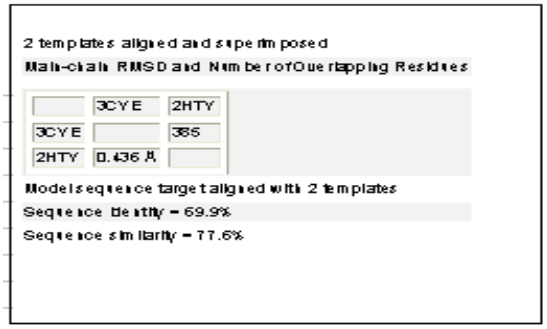
The shades of blue colors shows the extend of similarity between the sequences. Dark shaded regions are fully identical and the density of colour will decrease with low similarity and dissimilar regions will be white in colour.

Superimposed Template Structure

The templates are superimposed after aligning the protein.RMSD value was found to be 0.436 A^{o}.

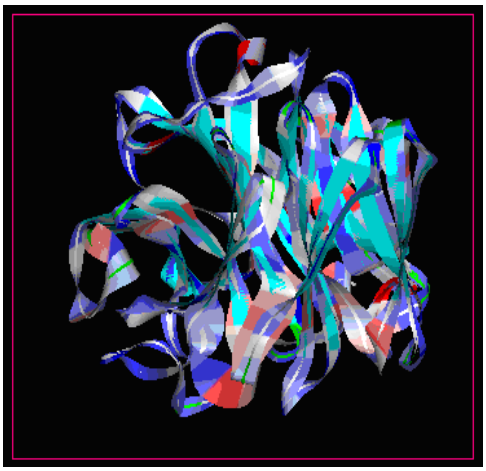
Discussion The structural similarity between proteins is usually verified using the Root- Mean-Square Distance (RMSD) between equivalent atoms. After optimal superposition of the template structures, a low RMSD value shows high degree of structural.

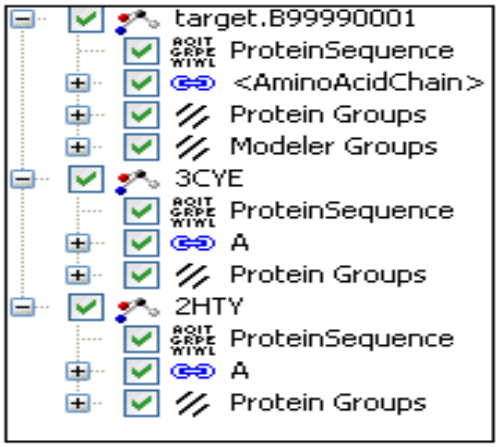




The templates are superimposed after aligning the protein. RMSD value was found to be 0.436 A^{o}

Build Homology Model Using One Or More Template Structure



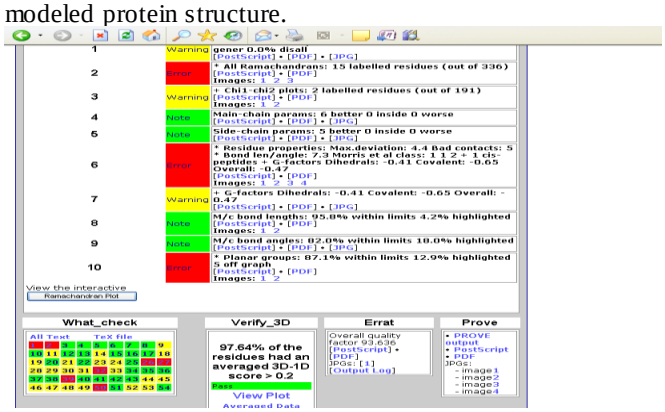


Uses "MODELER" to build homology models. An accurate sequence alignment between the model and the template proteins are essential to achieve high quality models. After alignment between sequence and the template the protocol "BUILD HOMOLOGY" was run .The figure shows the structure of the target protein obtained by homology modeling

Discussion: The Molecule with lowest DOPE score was selected. The blue region with thick representation is completely resolved without any problem. The red region is problematic, that needs to be refined to obtain a good model for target protein.

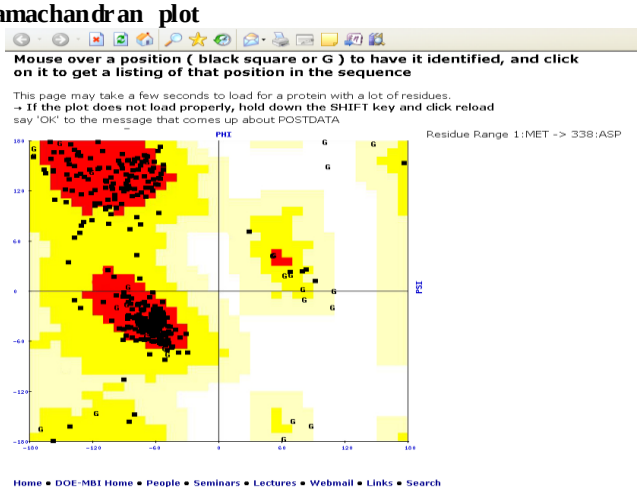
SAVS: STRUCTRAL ANALYSIS VERIFICATION SERVER

The analysis was performed to verify the quality of the modeled protein structure.



Discussion: Using this tool 3D structure of protein was verified. The structure analysis gave the following results. A pass certificate was obtained from verify_3D as 97.64% of residues had an aveaged 3D-1D score greater than 0.2. Overall quality factor from Errat was found to be as 93.636.

Ramachandran plot



it's to visualize dihedral angles φ against ψ of amino acid residues in protein structure. it shows the possible conformations of φ and ψ angles for a polypeptide.all the residues were found with in allowed region

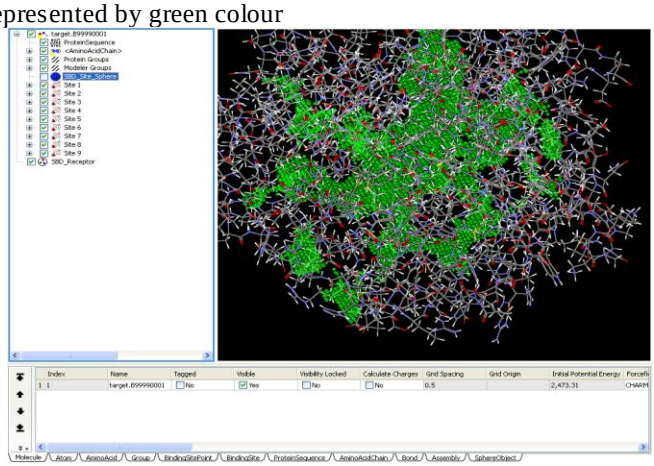
3D structure was also verified using Accerlys software Verify (Profiles-3D) protocol: The result shows "verify score" of 158.19 which is higher than the expected high score of 153.95. Hence it can be concluded that protein is having good quality and is suitable for further structure based studies.

Applying force field for the target molecule

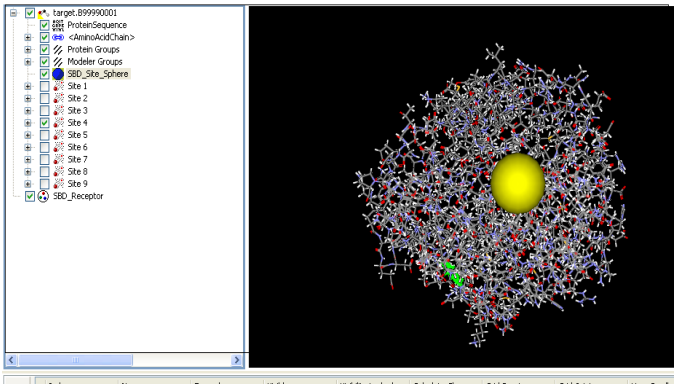
The CHARM m force field was applied: The Initial potential energy was found as **-17815.4451kcal/mol** in the initial stage, and then the force field was applied for sequential steps until the energy value reaches the minimal value of **-22676.15952kcal/mol.**

Prediction of binding site: In this tool the number of the binding sites is found in the modeled protein. There are about nine site. Ligand binding sites present in the protein represented by green colour.

Below picture shows ligand binding sites present in the protein represented by green colour



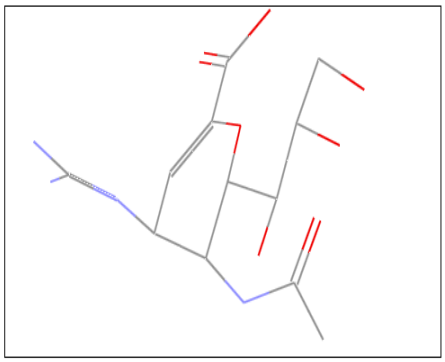
The below picture shows an example for "Site Sphere" view for site 4



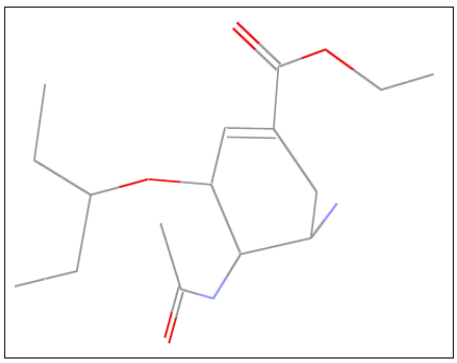
Drug selected for docking: The following drug were chosen from Drug bank for Neuraminidase

Structure of Drugs

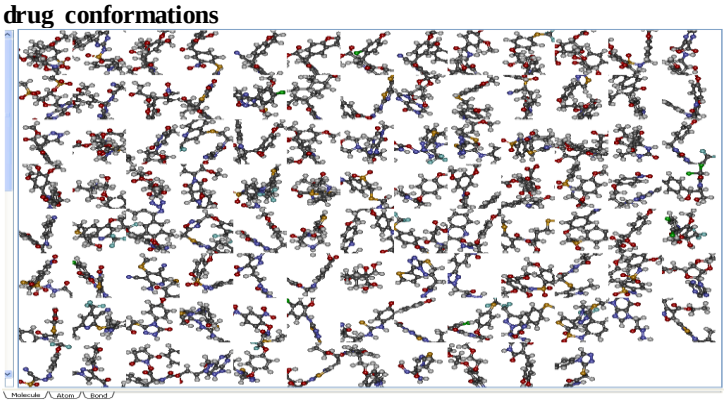
Zanamivir



Oseltamivir



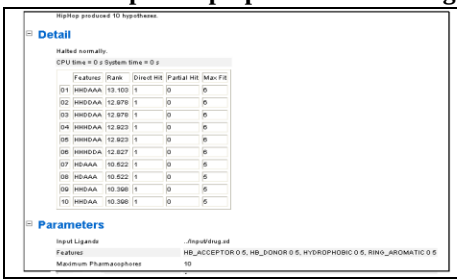
3D Search database from Accelrys DS gave the following drug conformations



Discussion:

From 3D Search database around 205 drug conformations were identified based on similarity with above mentioned drugs which were considered for docking with target protein.

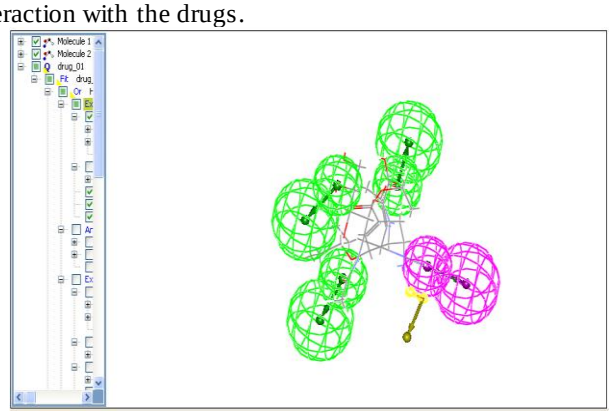
Pharmacophore properties of the drug



10 pharmacophore models were generated out of which the first model was selected for further studies.

Pharmacophore View in Discovery studio

Molecular Docking: The different drug conformations were subjected to molecular docking with the 9 binding sites of the target protein. Out of the 9 sites the following sites showed interaction with the drugs.



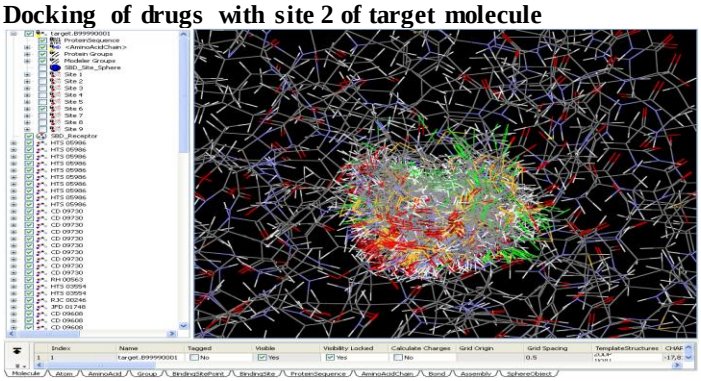
Pharmacophore properties were found in this drug.

a. Hydrogen Bond acceptor denoted by green colour.

b. Hydrogen Bond donor denoted by pink colour

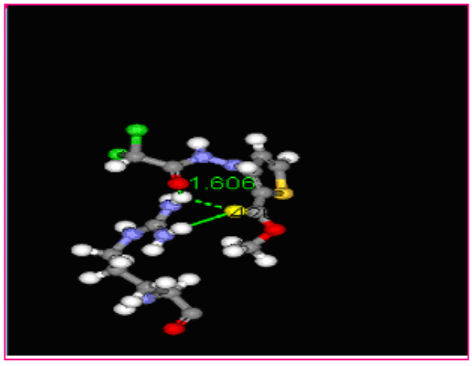
Site2 Docking Studies: Site2 of target was subjected for docking which has resulted in 432 poses.

Docking of drugs with site 2 of target molecule

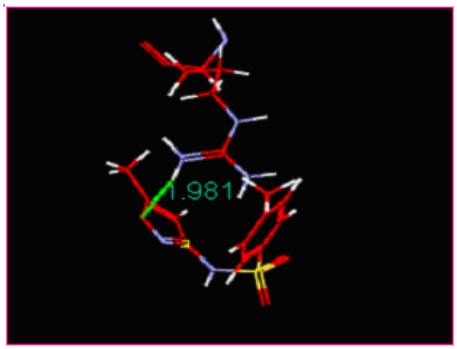


The following figure shows hydrogen bond between target site and ligand. It also displays hydrogen bond distance.DS represents dock score.

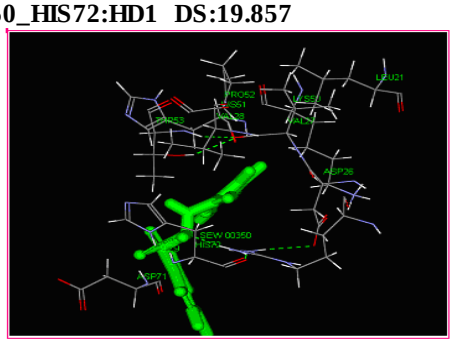
GK 02408_ARG2HH11 DS: 32.009



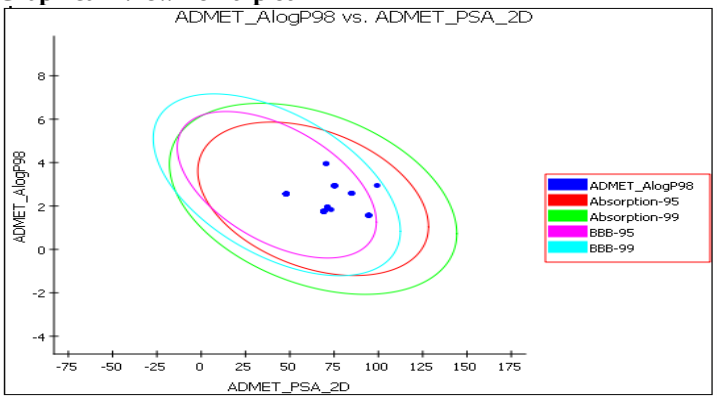
BTB 05702_ARG2HH12 DS:21.76



SEW 00350_HIS72:HD1 DS:19.857



Graphical View-Point plot

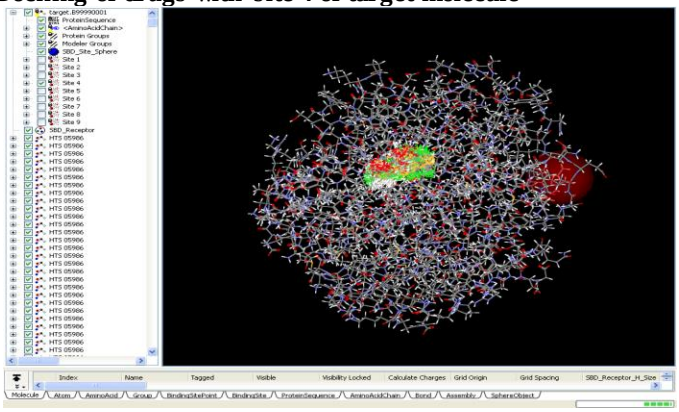


Discussion

From this graph, we can conclude that which drugs have shown to be absorbed 95% and 99% in the body. The blue color dots indicate the drugs and the rings that are specified for absorption and Blood Brain Barrier confidence ellipses. Drugs are analyzed based on their ADMET_AlogP98 and ADMET_PSA_2D values.

From the above table, it is clear that all these drugs shows good absorption and are not inhibiting cytochrome P450,but some are causing hepatotoxicity.

Docking of drugs with site 4 of target molecule

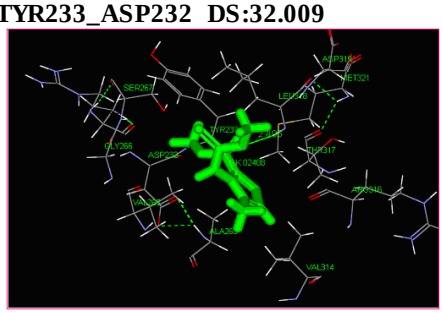


Drug Name	BBB Level	Absorbtion level	Solubility level	Hepatotoxicity	PBB Level	CYP2D6 binding
GK 02408	3(Low penetration)	0(Good Absorption)	3(Yes good)	1(toxic)	0(Non-inhibitor)	2(>=95% binding)
SEW00350	2(Medium penetration)	0(Good Absorption)	3(Yes good)	1(toxic)	0(Non-inhibitor)	2(>=95% binding)
BTB05702	3(Low penetration)	0(Good Absorption)	3(Yes good)	1(toxic)	0(Non-inhibitor)	2(>=95% binding

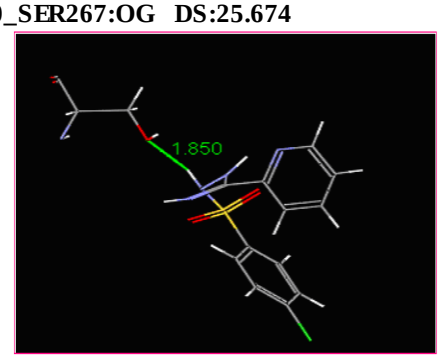
Drug Name	BBB Level	Absorbtion level	Solubility level	Hepato toxicity	CYP2D6	PBB Level
HTS05986	2(Medium penetration)	0(Good Absorption)	3(Yes, good)	1(toxic)	0(Non-inhibitor)	2(>=95% binding)
GK 02408	2(Medium penetration)	0(Good Absorption)	3(Yes, good)	1(toxic)	0(Non-inhibitor)	2(>=95% Binding)
SEW 00350	3(Low penetration)	0(Good Absorption)	3(Yes, good)	1(toxic)	0(Non-inhibitor)	2(>=95% binding)

The following shows hydrogen bond between target site and ligand. It also displays hydrogen bond distance. DS represents dock score.

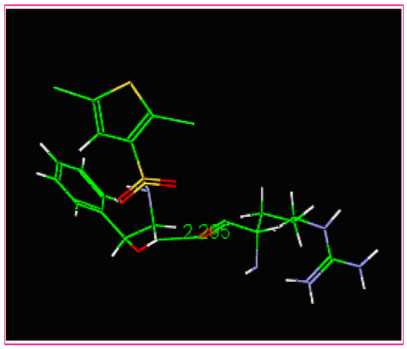
Gk02408_TYR233_ASP232 DS:32.009



SEW00350_SER267:OG DS:25.674



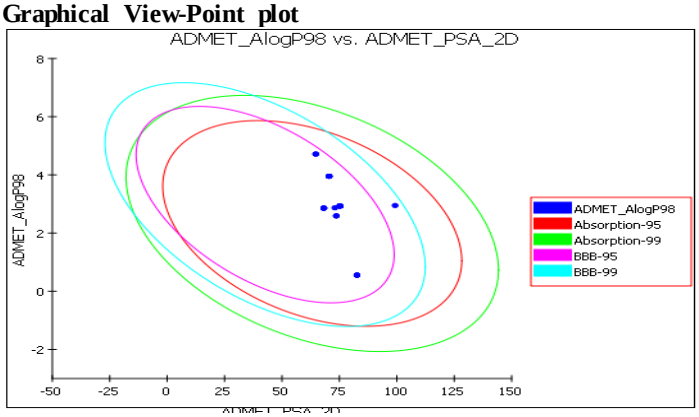
HTS 05986_ARG316: O DS:23.896



Discussion

From this graph, we can conclude that which drugs have shown to be absorbed 95% and 99% in the body. The blue color dots indicate the drugs and the rings that are specified for absorption and Blood Brain Barrier confidence ellipses. Drugs are analyzed based on their ADMET_AlogP98 and ADMET_PSA_2D values.

Graphical View-Point plot



ADMET prediction levels:

Discussion:

From the above table, it is clear that all these drugs shows good absorption and are not inhibiting cytochrome P450,but some are causing hepatotoxicity.

Conclusion:

Swine influenza virus (SIV) or S-OIV (swine-origin influenza virus) is a strain of the influenza family of viruses that is endemic in pigs. In this study the neuraminidase protein from this virus has been selected as the target protein. By using Accelrys Discovery studio 2.5 software, homology modeling of the target protein has been carried out. Blast search has been performed against PDB database to identify the suitable template structures. Selected templates were further superimposed for their identities. Homology modeling resulted in 3 possible models, out of which the one with lowest DOPE score was selected as the best model. 3D-Structure verification was done using SAVS server and "verify (profiles 3D)" of Discovery Studio 2.5. Energy optimization was performed to produce stable structure for docking, and the final potential energy was found to be -22676.16kcal/mol. The drugs (Zanamivir and Oseltamivir) for target protein has been retrieved from drug bank and by means of a 3D search database of Discovery studio, 205 conformations were generated and selected for docking studies. 9 binding sites were predicted on target receptor out of which only site 2 and 4 gave interactions with drugs and resulted in 432 and 1448 docked poses respectively. Selection of best drug structures was done based on highest dock score which were further carried out for ADMET property analysis for Absorption, Distribution, Metabolism, Elimination and Toxicity. TOPKAT prediction which gives results for carcinogenicity, ocular irritation, skin

irritation etc. was also carried out for the promising compounds. Hence the chemical ligands with best properties and interactions can be further selected and used for in-vitro studies and drug development.

References

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