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AUTHORIZATIONS OF EFFECTIVE NEURAMINIDASE INHIBITORS THROUGH THE LIGAND AND STRUCTURE-BASED VIRTUAL SCREENING

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ABSTRACT

Various inhibitors have been developed for neuraminidase but resistance against these drugs in many viral strains makes it an advantageous and interesting task to discover compounds which can be more promising in preventing viral infection through neuraminidase. Virtual screening methods have been proved as an efficient in silico approach for drug discovery processes. In the present study, we used ligand based virtual screening process for identifying potent inhibitors against viral neuraminidase enzyme. The approach utilized in this study has been successful in identifying docking results indicate that out of 33 Marine compounds, there were three inhibitory compounds for Neuraminidase as target for swine flu. Thus, our study confirms ZINC2043006, ZINC5884077 and ZINC13802909 are potential inhibitors for Neuraminidase as target for tuberculosis. The results of the present study are reported here in so that researchers, who are having required laboratory facilities for synthesizing drugs, can utilize findings of this study for developing new drugs against Influenza with better efficacy.

Keywords: neuraminidase, swine flu, ZINC2043006, ZINC5884077 and ZINC13802909

1. INTRODUCTION

Computational methods have been developed and widely applied to pharmacology hypothesis development and testing. In silico methods includes databases, quantitative structure-activity relationships, similarity searching, pharmacophores, homology models and other molecular modeling, machine learning, data mining, network analysis tools and data analysis tools that use a computer. Such methods have seen frequent use in the discovery and optimization of novel molecules with affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization **[1]**.

1.1 Computer Aided Drug Designing (CADD)

It is generally recognized that drug discovery and development are very time and resources consuming processes **[2]**. There is an ever-growing effort to apply computational power to the combined chemical and biological space in order to streamline drug discovery, design, development and optimization. In biomedical arena, computer-aided or in silico design is being utilized to expedite and facilitate hit identification, hit-to-lead selection, optimize the absorption, distribution, metabolism, excretion and toxicity profile and avoid safety issues. Commonly used computational approaches include ligand-based drug design (pharmacophore, a 3-D spatial arrangement of chemical features essential for biological activity), structure-based drug design (drug-target docking), and quantitative structure-activity and quantitative structure-property relationships. Regulatory agencies as well as pharmaceutical industry are actively involved in development of computational tools that will improve effectiveness and efficiency of drug discovery and development process, decrease use of animals, and increase predictability. It is expected that the power of CADD will grow as the technology continues to evolve.

1.2 Ligand Based Drug Designing

Flexible ligand docking is a computational screening technique, where the candidate ligands are fitted to the 3D structure of the target receptor with allowance for the conformational flexibility of the ligands (http://www.simbiosys.ca/).

1.3 Structure Based Drug Designing

Drug discovery has evolved through various stages into more rational and evidence-based drug designing. Compared to conventional methods which were time consuming and less logical, new drug designing based on structure is rational, evidence based, faster and more scientific in nature. In the era of modern medicine, where newer insights into molecular level of disease processes are available, it is very essential that drug designing be based on molecular mechanism of pathologic processes. Structure-based drug designing has made tremendous contributions in the field of cancer

chemotherapy, drug resistant infections, neurological diseases, to mention a few. New drug discovery methods are furthered by developments in the technology especially computers, bioassay techniques and calibrated instruments. Computational structure-based drug designing opens the door to novel treatments in modern medicine **[3]**.

1.4 Swine Flu

Swine flu is an illness caused by viruses from Orthomyxoviridae family made from genome of seven-eight ssRNA segments. It's named for a virus that pigs can get. People do not normally get swine flu, but human infections can and do happen. The virus is contagious and can spread from human to human. Symptoms of swine flu in people are similar to the symptoms of regular human flu and include fever, cough, sore throat, body aches, headache, chills and fatigue **[4]**.

1.5 Description about H1N1 Flu [6]

H1N1 flu is contagious. H1N1 flu is an influenza virus causing illness in people. H1N1 flu is NOT caused by eating pork or pork products. Illness with the H1N1 flu virus ranges from mild to severe. Senior prioritized for antiviral treatment to limit risk of complication if they got flu.

1.6 Virion Structure

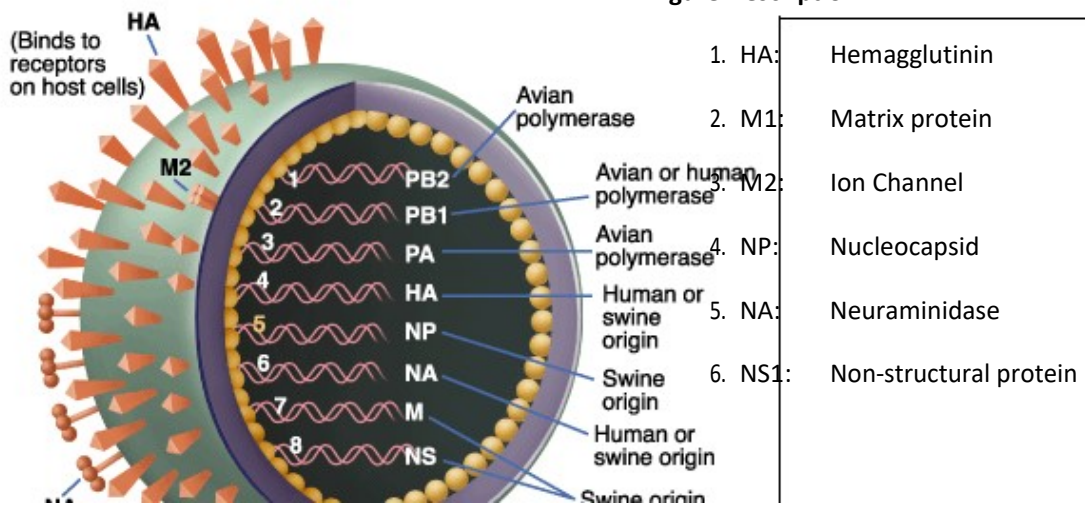


Figure Description:

1. HA: Hemagglutinin
2. M1: Matrix protein
3. M2: Ion Channel
4. NP: Nucleocapsid
5. NA: Neuraminidase
6. NS1: Non-structural protein

Figure 1: Swine Flu [5]

1.7 3D view of H1N1

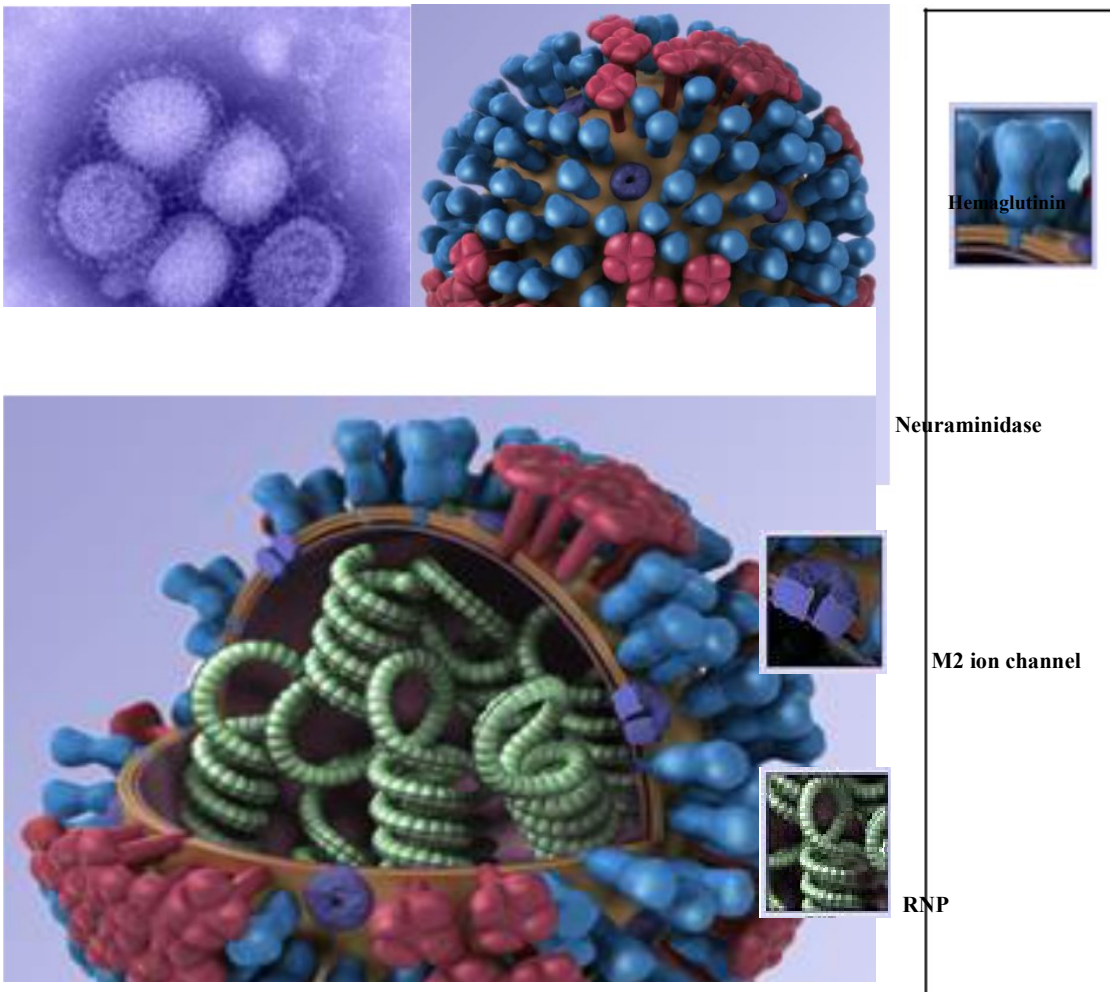


Figure 2: 3D Structure of the H1N1 virion [4]

H1N1 flu is an influenza virus causing illness in people. It has two genes from flu viruses that normally circulate in pigs in Europe and Asia, plus avian genes and human genes. Scientists call this a "quadruple reasserting" virus. H1N1 flu is not a food borne disease; it is a respiratory disease. About 70 percent of people who were hospitalized with H1N1 flu had one or more medical conditions that placed them in the "high risk" category for serious seasonal flu-related complications. These include pregnancy, diabetes, heart disease, asthma and kidney disease. Seniors (adults 65 years and older) were prioritized for antiviral treatment to limit risk of complication if they got flu. While your age means you have a lower risk of getting the flu, certain risk conditions (COPD, diabetes, etc.) mean if you get sick, you may have higher risk of complications from any influenza.

1.8 Starting of Disease [7]

The H1N1 is a descendant of the Spanish Flu which was a pandemic disease in the 2nd decade of the 20th century during 1918-1920. However, direct transmission from pigs to humans is quite rare, with only 12 cases have shown in the United States since 2005.

1.9 Death Statistics of Swine Flu Around the world [8]

Swine flu was the pandemic disease whole over the world and thousands of deaths occurred due to it. There is a statistic published by WHO till 21 May 2010. The Total Death Occurred according to WHO report was 18097 around the world.

1.10 Classification of swine flu

1.10.1 Influenza C

It is a virus belonging to the family Orthomyxoviridae, which includes the viruses causing the flu. The only species of this genus is called "influenza C". It has been confirmed that influenza C viruses infect humans and pigs, causing flu. However, influenza type C is not very common in comparison with influenza A virus and influenza B virus, but can become severe and cause epidemics premises.

1.10.2 Influenza A

It is known that the swine flu caused by viruses of influenza A (H1N1), H1N2, H3N1, H3N2 and H2N3. In the town there are three subtypes of swine influenza A (H1N1, H3N2 and H1N2) circulating throughout the world. In the United States, the H1N1 subtype has been a frequent cause of infection among the population before swine until 1998, but since late August of that year, the H3N2 subtype was isolated from pigs. Since 2004, the H3N2 virus strains isolated in Turkey and United States, but came to find genetic traces of human (HA, NA and PB1), swine (NS, NP, and M) and poultry (PB2 and PA).

1.11 Update 2011 of swine flu [9]

1.11.1 Swine is still active

By August 2010, the WHO declared H1N1 swine flu was "post-pandemic", the last phase of any pandemic. However, H1N1 flu is still making people sick, even killing some patients in random areas of the world, and is expected to do so for years to come. According to the World Health Organization and various news reports, cases of H1N1 were reported as late as December 2010 and January 2011 in England, Ireland, Germany, Sri Lanka, Korea, New Zealand and India.

1.11.2 The 2011 Flu Vaccine [10]

Because new virus strains constantly evolve, and are therefore different from previous strains, we are not immune to the new strains. Once we are exposed to those new strains, our lack of immunity may mean we get that flu. In order to gain immunity, then, we must get flu vaccinations. The flu vaccine developed for the 2011 flu season seems to have been accurate, thereby rendering the vaccine very effective toward warding off the 2010-2011 flu viruses in those who have been vaccinated.

1.12 Included in the 2010-11 flu vaccine is protection from H1N1 swine flu [11]

The CDC recommends that even if you were vaccinated against H1N1 in 2009 or 2010, you should still plan to get the most current flu vaccination because you'll need the protection from the newer identified virus strains.

1.13 Molecular mechanism of Swine flu

HA (Hemagglutinin) is a glycoprotein on the viral coat that binds to sialic acid receptors in the membrane of cells that line the host's respiratory tract. After its binding the membranes of cell and viruses' fuse. The virus is taken into the cell. Once inside the cell, virus emerges from its covering. It migrates to the cell nucleus, crosses the nuclear membrane and hijacks the cell's machinery, and makes copies of viral components. The newly manufactured viral components migrate back to the cell membrane and bud out from the cell. This new virion still contains sialic acid receptors from the host cell. It is N or Na (neuraminidase) to enzymatically cleave the sialic receptors and thus allowing the virus to break free from the host cell. Now the virus able to bind to another respiratory cells and begin the process anew. The H protein allows the influenza virus to enter a cell while N protein allows to escape. An infected cell dies because virus triggers the suicide switch within the cell [12].

1.14 Protein of Interest: Neuraminidase [13]

Viral neuraminidase found on the surface of H1N1 viruses that enables the virus to be released from the host cell. Neuraminidases are enzymes that cleave

sialic acid groups from glycoprotein and are required for influenza virus replication. When influenza virus replicates, it attaches to the cell surface using hemagglutinin, a molecule found on the surface of the virus that binds to sialic acid groups. Sialic acids are found on various glycoproteins at the host cell surface, and the virus exploits these groups to bind the host cell. In order for the virus to be released from the cell, neuraminidase must enzymatically cleave the sialic acid groups from host glycoproteins. As an integral part of influenza replication, blocking the function of neuraminidase with neuraminidase inhibitors is an effective way to treat influenza.

3. MATERIALS & METHODS

3.1 Retrieval of the target protein sequence

The amino acid sequence of neuraminidase (NA) of influenza A virus subtype H1N1 of (A/Hyd/NIV51/2009(H1N1)) isolated from a patient of Hyderabad, India was retrieved from NCBI influenza virus resource with accession number ACZ97472 and is shown below in GenBank format.

```
LOCUS       ACZ97472                469 aa            linear   VRL 18-MAR-
2010
DEFINITION  neuraminidase [Influenza A virus (A/Hyd/NIV51/2009(H1N1))].
ACCESSION   ACZ97472
VERSION     ACZ97472.1  GI:273039070
DBLINK      Project: 37813
DBSOURCE    accession GU292383.1
KEYWORDS    .
SOURCE      Influenza A virus (A/Hyd/NIV51/2009(H1N1))
  ORGANISM  Influenza A virus (A/Hyd/NIV51/2009(H1N1))
            Viruses; ssRNA negative-strand viruses; Orthomyxoviridae;
            Influenzavirus A.
REFERENCE   1  (residues 1 to 469)
  AUTHORS   Potdar,V.A., Chadha,M.S., Jadhav,S.M., Mullick,J., Cherian,S.S.
and
            Mishra,A.C.
  TITLE     Genetic Characterization of the Influenza A Pandemic (H1N1) 2009
            Virus Isolates from India
  JOURNAL   PLoS ONE 5 (3), E9693 (2010)
   PUBMED   20300625
  REMARK    Publication Status: Online-Only
REFERENCE   2  (residues 1 to 469)
  AUTHORS   Mishra,A., Potdar,V., Chadha,M., Jadhav,S., Mullick,J. and
            Cherian,S.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-DEC-2009) Influenza, National Institute of
Virology,
            20-A, Dr Ambedkar Road, Pune, Maharashtra 411001, India
COMMENT     Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..469
                     /organism="Influenza A virus (A/Hyd/NIV51/2009(H1N1))"
                     /strain="A/Hyd/NIV51/2009"
                     /serotype="H1N1"
                     /host="Homo sapiens"
                     /db_xref="taxon:698887"
```

```
                     /segment="6"
                     /country="India"
                     /collection_date="May-2009"
                     /note="lineage: swl"
     Protein         1..>469
                     /product="neuraminidase"
     CDS             1..469
                     /gene="NA"
                     /coded_by="GU292383.1: 1.>1407"
ORIGIN
        1 mnpnqkiiti gsvcmtigma nlilqigniv siwishsiql gnqnqietcn qsvityennt
       61 wvnqtyvnis ntnfaagqsv vsvklagnss lcpvsgwaiy skdnsirigs kgdvfvirep
      121 fiscsplecr tffltqgall ndkhsngtik drspyrtlms cpigevpspy nsrfesvaws
      181 asachdginw ltigisgpdn gavavlkyng iitdtikswr nnilrtqese cacvngscft
      241 vmtdgpsdgq asykifriek gkivksvemn apnyhyeecs cypdsseitc vcrdnwhgsn
      301 rpwvsfnqnl eyqigyicsg ifgdnprpnd ktgscgpvss ngangvkgfs fkygngvwig
      361 rtksissrng femiwdpngw tgtdnnfsik qdivginews gysgsfvqhp eltgldcirp
      421 cfwvelirgr pkentiwtsg ssisfcgvns dtvgwswpdg aelpftidk
```

3.2 Blast of the protein from swine flu strain: (A/Hyd/NIV51/2009(H1N1))

Table 1: Blast results of the protein from H1N1 virus

Accession	Description	Max score	Total score	Query coverage	E value
3NSS_A	**Chain A, 2009 H1n1 Neuraminidase**	**795**	**795**	**82%**	**0.0**
2HTY_A	**Chain A, N1 Neuraminidase**	**749**	**749**	**82%**	**0.0**
3CL2_A	**Chain A, N1 Neuraminidase**	**743**	**743**	**82%**	**0.0**
3CKZ_A	**Chain A, N1 Neuraminidase**	**742**	**742**	**82%**	**0.0**
3CYE_A	**Chain A, Crystal Structure**	**736**	**736**	**82%**	**0.0**
3BEQ_A	**Chain A, Neuraminidase**	**732**	**732**	**82%**	**0.0**
2HTV_A	**Chain A, N4 Neuraminidase**	**560**	**560**	**82%**	**4e-160**
2HT5_A	**Chain A, N8 Neuraminidase**	**466**	**466**	**81%**	**7e-132**
3O9J_A	**Chain A, Influenza Na**	**464**	**464**	**80%**	**4e-131**
1NMB_N	***Chain N, influenza***	***382***	***382***	***99%***	***2e-106***
1NNA_A	**Chain A**	**362**	**362**	**80%**	**2e-100**
1XOE_A	**Chain A**	**362**	**362**	**80%**	**2e-100**
1NCA_N	**Chain N, Refined Crystal Structure**	**362**	**362**	**80%**	**2e-100**
1A14_N	**Chain N, Complex Between Nc10**	**362**	**362**	**80%**	**2e-100**
5NN9_A	**Chain A, Refined Atomic Structures**	**361**	**361**	**80%**	**3e-100**

3.3 Computational Approach for Protein 3-D Structure Prediction

There are three computational approaches to protein three-dimensional structural modeling and prediction. They are H1N1 flu is an influenza virus causing illness in people, Homology modeling, Threading, & Ab-initio prediction. The first two are knowledge-based methods; they predict protein structures based on knowledge of existing protein structural information in databases. Homology modeling builds an atomic model based on an experimentally determined structure that is closely related at the sequence level. Threading identifies proteins that are structurally similar, with or without detectable sequence similarities. The ab-initio approach is simulation based and predicts structures based on physicochemical principles governing protein folding without the use of structural templates.

3.3.1 Homology Modeling

As the name suggests, homology modeling predicts protein structures based on sequence homology with known structures. It is also known as comparative modeling. The principle behind it is that if two proteins share a high enough sequence similarity, they are likely to have very similar three-dimensional structures. If one of the protein sequences has a known structure, then the structure can be copied to the unknown protein with a high degree of confidence. Homology modeling produces an all-atom model based on alignment with template proteins. The overall homology modeling procedure consists of seven steps like Template Selection, Sequence Alignment, Backbone Model Building, Loop Modeling, Side Chain Refinement, Model Refinement Using Energy Function and Model Evaluation.

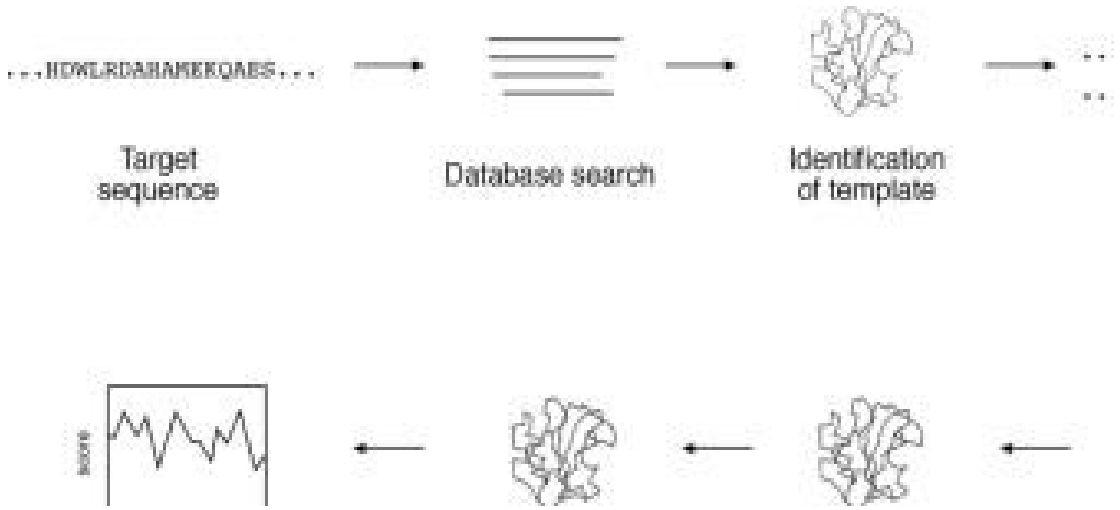


Figure 3: Flowchart showing steps involved in homology modeling

3.4 Comprehensive Modeling Programs

A number of comprehensive modeling programs are able to perform the complete procedure of homology modeling in an automated fashion. The automation requires assembling a pipeline that includes target selection, alignment, model generation, and model evaluation.

3.4.1 Modeller

Modeller (http://bioserv.cbs.cnrs.fr/HTML BIO/frame mod.html) is a web server for homology modeling. The user provides a predetermined sequence alignment of a template(s) and a target to allow the program to calculate a model containing all of the heavy atoms (nonhydrogen atoms). The program models the backbone using a homology-derived restraint method, which relies on multiple sequence alignment between target and template proteins to distinguish highly conserved residues from less conserved ones. Conserved residues are given high restraints in copying from the template structures. Less conserved residues, including loop residues, are given less or no restraints, so that their conformations can be built in a more or less ab-initio fashion. The entire model is optimized by energy minimization and molecular dynamics procedures. Most important method used for structure determination of proteins utilizes NOE experiments to measure distances between pairs of atoms within the molecule.

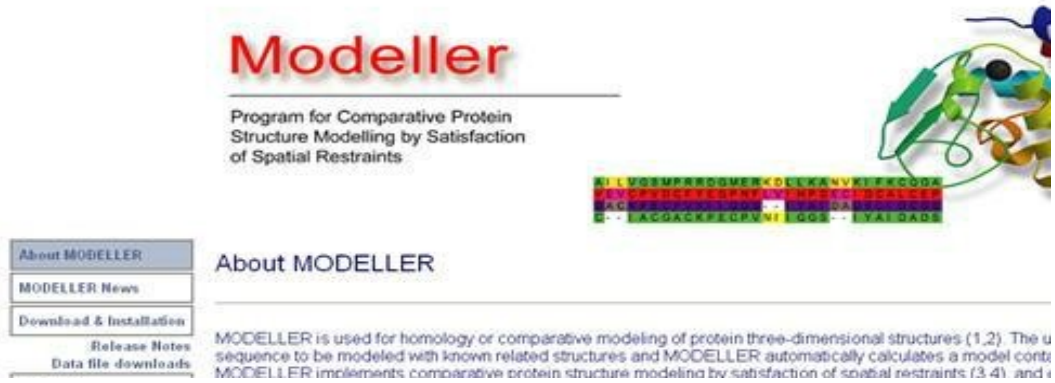


Figure 4: Graphical representation of modeler home page

Subsequently, the obtained distances are used to generate a 3D structure of the molecule using a computer program.

3.5 Evaluation of Model

Procheck (*http://www.biochem.ucl.ac.uk/*) **is** the program to check the stereochemical quality of the given protein structure, as compared with well refined structures at the same resolution and to give indication of its local, residue by residue reliability. Procheck generates a number of output files which have the same name as the original PDB file, but with different extensions .ps, out,. nb,. new,. pln etc. The

seven executable files of procheck are: clean.exe, tplot.exe, pplot.exe, bplot.exe, anglen.exe, nb.exe, secstr.exe. 10 plots were generated that helps us to determine the stereochemical quality of our protein structure.These are: Ramachandran plot, Glycine & Prolein Ramachandran plot, Chi1-Chi2 plot, Main chain parameter, Side chain parameter, Residue properties, Main chain bond length distribution, Main chain bond angle distribution, RMS distances from planarity, Distorted geometry plots. The plots were generated with .ps extension which can be viewed using Ghostscript viewer (*http://pages.cs.wisc.edu/~ghost/*). Ghostscript is an interpreter for the PostScript (TM) language. A PostScript interpreter usually takes as input a set of graphics commands. GSview is a graphical interface for Ghostscript under MS-Windows or OS/2.

3.6 Software used for the visualization of 3-D structure of Protien

Pymol is an open-source, user-sponsored, molecular visualization system created by Warren Lyford DeLano and commercialized by DeLano Scientific LLC, which is a private software company dedicated to creating useful tools that become universally accessible to scientific and educational communities. It is well suited to producing high quality 3D images of small molecules and biological macromolecules such as proteins.PyMOL is one of few open source visualization tools available for use in structural biology. The Py portion of the software's name refers to the fact that it extends, and is extensible by, the Python programming language

3.7 Retrieval of 3D structure of known Inhibitors

3.7.1 Drug Bank

The DrugBank database is a unique bioinformatics and cheminformatics resource that combines detailed drug (i.e., chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e., sequence, structure, and pathway) information. The database contains 6827 drug entries including 1437 FDA-approved small molecule drugs, 134 FDA-approved biotech (protein/peptide) drugs, 83 nutraceuticals and 5206 experimental drugs. Additionally, 4436 non-redundant protein (i.e., drug target/enzyme/transporter/carrier) sequences are linked to these drug entries. Each DrugCard entry contains more than 150 data fields with half of the information being devoted to drug/chemical data and the other half devoted to drug target or protein data.

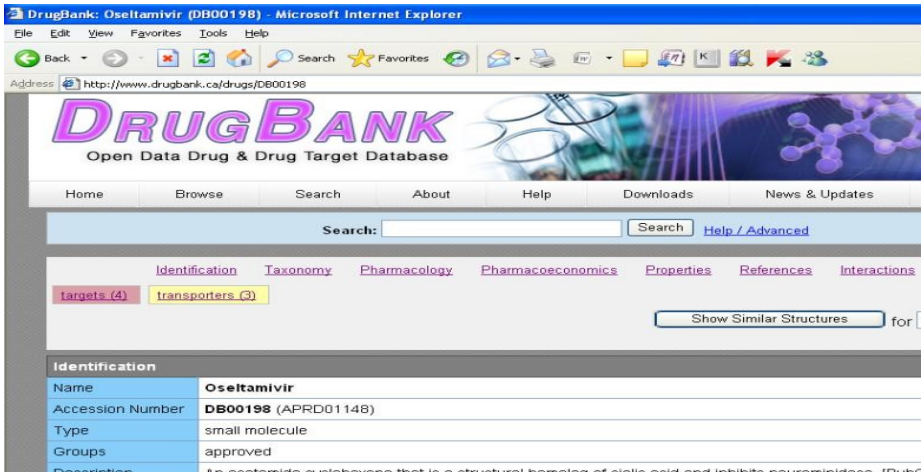


Figure 5: Snapshot of Drug Bank database

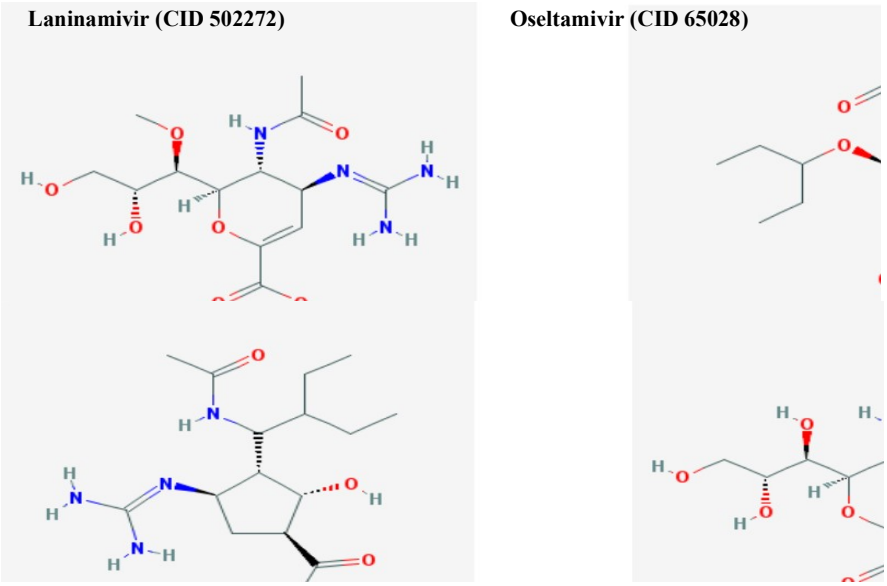


Figure 6: Known inhibitors of H1N1 Neuraminidase

3.7.2 ZINC database

ZINC database contains over 13 million commercially available compounds in ready-to-dock, 3D formats for structure based virtual screening.

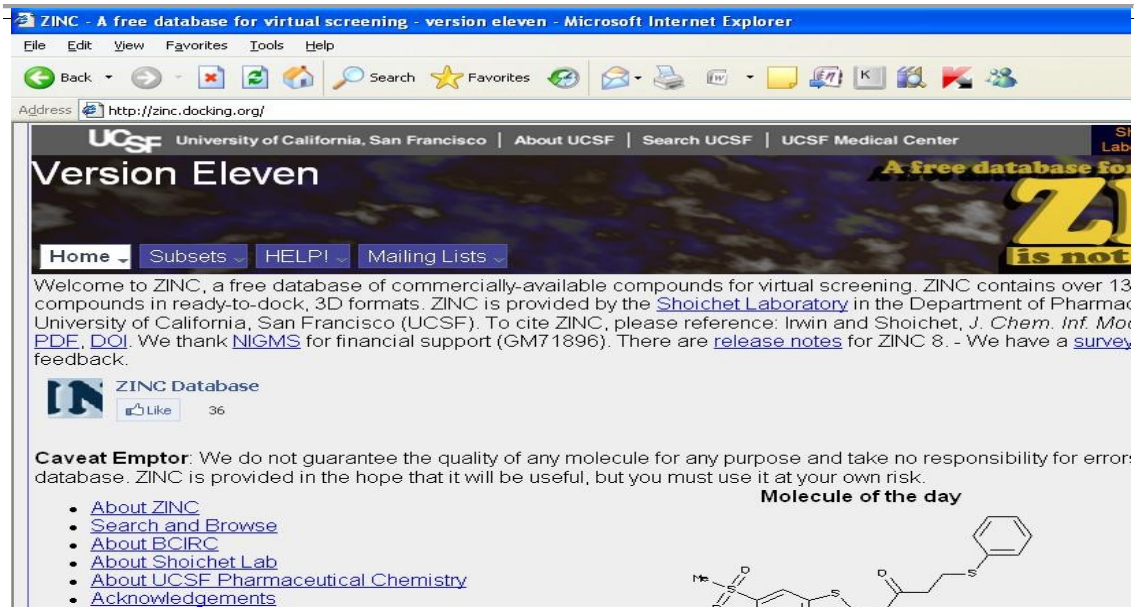


Figure 7: Snapshot of ZINC database

3.7.3 NCBI Pubchem Compound database

PubChem Structure Search allows PubChem Compound Database to be queried using a chemical structure. Chemical structure queries may be sketched using the PubChem Sketcher. You may also specify the structural query input by PubChem Compound Identifier (CID), SMILES, SMARTS, InChI, Molecular Formula, or by upload of a supported structure file format. This standardizing allows NCBI to compute chemical parameters and similarity relationships between compounds. The compounds are grouped into levels of chemical similarity from most general to most specific: same bonding connectivity and any tautomer; same bonding connectivity; same stereochemistry; same isotopes; and same stereochemistry and isotopes. PubChem Compound also indexes these chemicals using 34 fields, many of which represent computed chemical properties such as the number of chiral centres, the number of hydrogen bond donors/acceptors, molecular formula and weight, total formal charge, and octanol-water partition coefficients (XlogP). These groups are provided as Entrez links that allow similar compounds to be retrieved quickly.

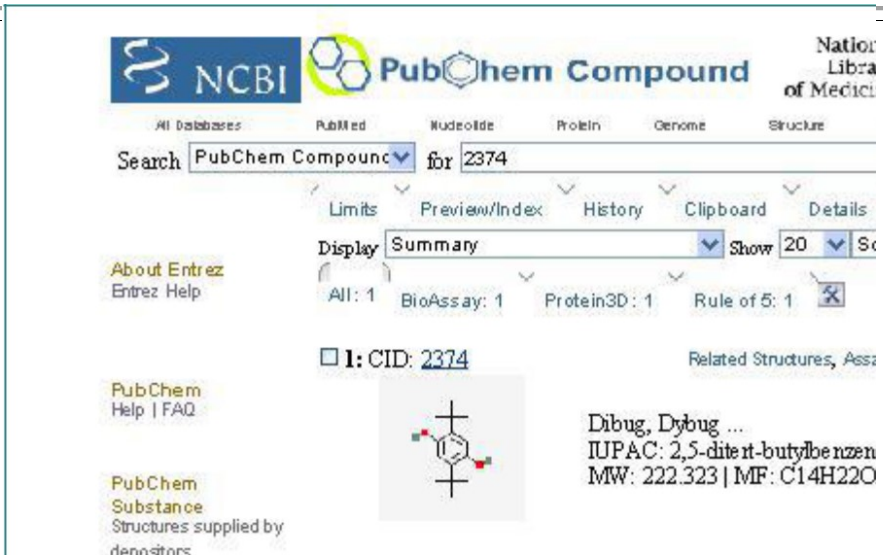


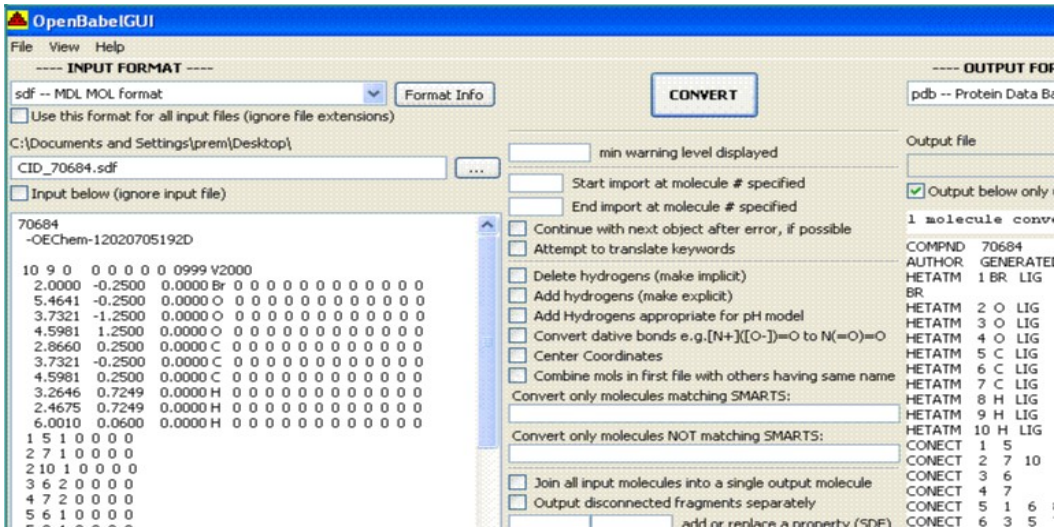
Figure 8: Representation of NCBI PubChem Compound database

Structure of potent inhibitors are obtained by submitting the name of inhibitor to NCBI's Pubchem compound and save it in SDF format then later it is converted into PDB format through Babel Molecule Format Convertor software, which is freely available.

3.8 Conversion of .sdf/. mol format to .pdb format

3.8.1 Babel Molecule Format Converter:

Babel is a cross-platform program designed to interconvert between many file formats and is used in molecular modeling and computational chemistry and related areas. Babel is a chemical toolbox designed to allow anyone to convert, analyze, or store data from molecular modeling, chemistry, solid-state materials, biochemistry, or related areas Interface of Babel Molecular format converter for converting SDF format to PDB format is shown below:



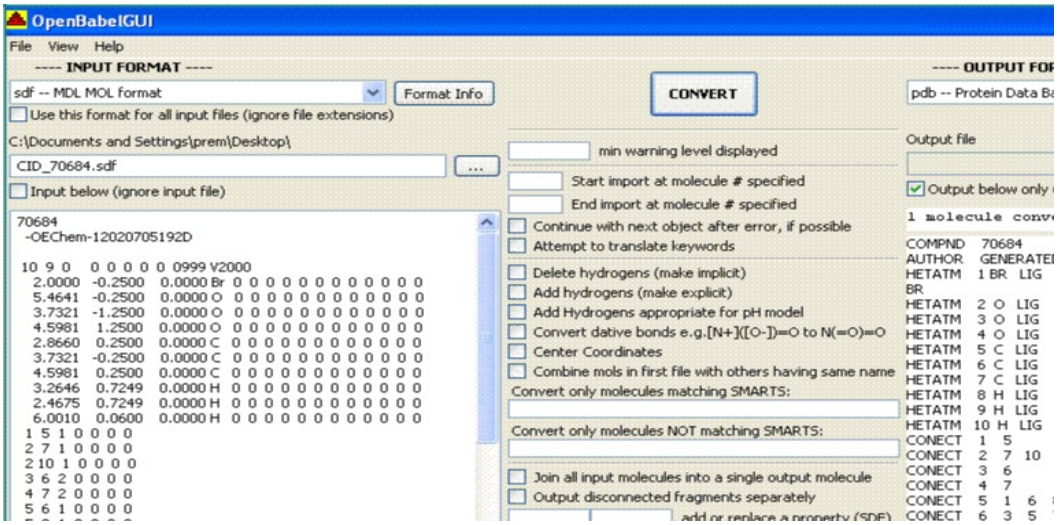


Figure 9: Input format of Babel Molecule Format Converter

Procedure to convert the SDF files in to PDB file

First of all, open the Babel molecular format converter page. Set the parameter for input and output file i.e., SDF for input file & PDB for output file. Upload the SDF file through browse command. Set the Path of output file. Click on the convert Button.

3.9 Optimization of Inhibitors

Optimization means, trying to find maxima and minima of a function after a number of inhibitor compounds have been found, SBDD techniques are especially effective in refining their 3D structures to improve binding to protein active sites, a process known as lead optimization. In lead optimization researchers systematically modify the structure of the lead compound, by some software docking each specific configuration of a drug compound in a protein's active site, and then testing how well each configuration binds to the site. In a common lead optimization method known as bioisosteric replacement, specific functional groups in a ligand are substituted for other groups to improve the binding characteristics of the ligand. With SBDD researchers can examine the various bioisosteres and their docking configurations, choosing only those that bind well in the active site we have optimize inhibitors with help of Argus Lab.

3.9.1 Argus Lab

Argus Lab is a molecular modeling program that runs on Windows 98, NT, and 2000. Argus Lab consists of a user interface that supports OpenGL graphics display of molecule structures and runs quantum mechanical calculations using the Argus compute server. The Argus compute server is constructed using the Microsoft Component Object Model (COM).

3.9.2 Procedure of using Argus lab to optimize the inhibitors

Open the Argus lab. Click on file menu & click open to open the inhibitor's PDB file. Go to Calculation then Optimize Geometry and click OK. When calculation finished it automatically show calculation Finished. After some time, calculation finish message will appear then save the file with (.PDB) extension.

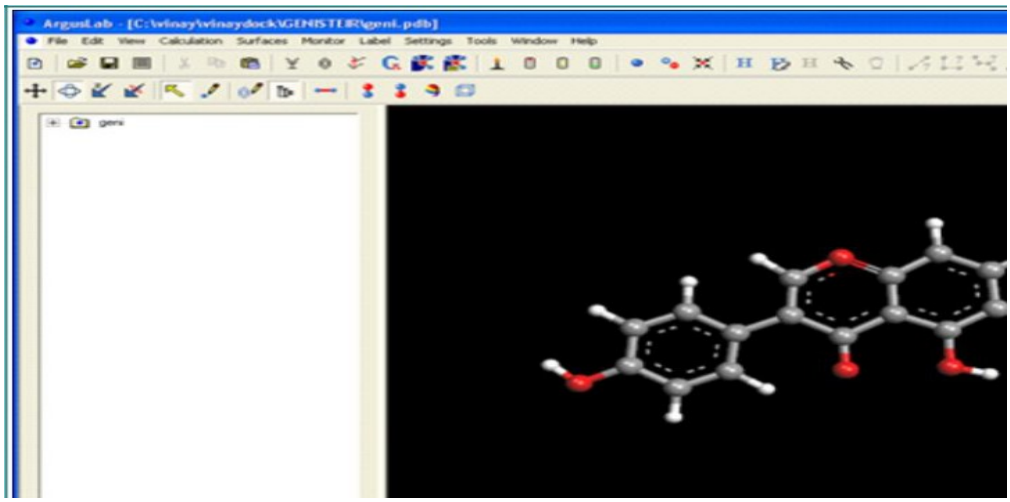


Figure 10: Window showing optimization of protein by Argus lab

3.10 Docking of Flexible Ligands to the Receptors Theory of Docking

Three-dimensional molecular structure is one of the foundations of structure-based drug design. Often, data are available for the shape of a protein and a drug separately, but not for the two together. Docking is the process by which two molecules fit together in 3D space. There are an estimated 15,700 known protein-protein interactions in humans only, therefore, understanding such interactions is important for insights into molecular recognition and networks such as signal transduction pathways in cells. To assist in studying protein interactions, we can use DOCK programs for protein-protein docking as well as complementary tools, Evolutionary trace, Profiles-3D etc. Overall, we find protein-protein docking and complementary tools are useful to study protein-protein interactions of unknown complex assemblies. The original procedure developed for AutoDock used a Monte Carlo (MC)simulated annealing (SA) technique for configurational exploration with a rapid energy evaluation using grid-based molecular affinity potentials. It thus combined the advantages of exploring a large search space and a robust energy evaluation. This has proven to be a powerful approach to the problem of docking a flexible substrate into the binding site of a static protein.

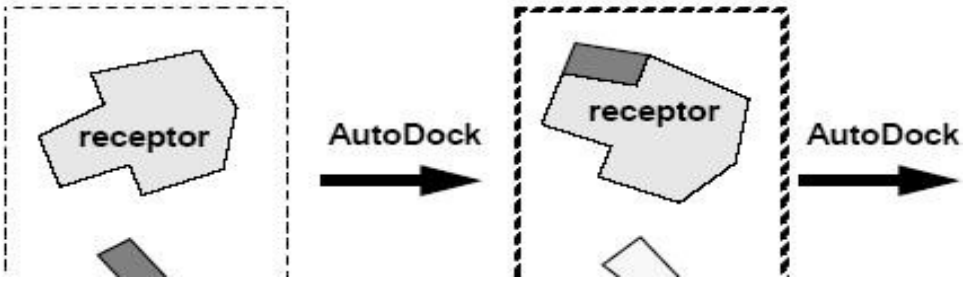


Figure 11: Binding of Receptor to Ligand through Autodock

Docking Software's Programme

Table 2: Shows a list of various docking software

S. N.	Name	License Term	Platform	Keyword
1.	Autodock	Commercial	UNIX, LINUX, SGI	GA/LGA, MC
2.	Affinity	Commercial	SGI	Monte Carlo method
3.	Dock Vision	Commercial	LINUX.IRIS	MC, GA
4.	DOT (Daughter of Turnip)	Free	Supercomputers, UNIX	
5.	Flex X	Commercial	UNIX	Fragment Based
6.	Shape	E-mail request	UNIX	Structure and chemistry of molecular surface
7.	LEAPFROG	Commercial	SGI	ligand design
8.	Q site	Commercial	UNIX, LINUX, SGI	Mixed quantum and molecular mechanics
9.	HINT	Commercial	Windows 2000, SGI, LINUX	Hydropathic interaction
10.	GOLD	Free evaluation	UNIX	GA

Autodock 3.0.5

Autodock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. AutoDock actually consists of two main programs: AutoDock performs the docking of the ligand to a set of grids describing the target protein; Auto Grid pre-calculates these grids. In additions to using them for docking, the atomic affinity grids can be visualized. This can help, for example, to guide organic synthetic chemists design better binders.

AutoDock Tools

AutoDockTools, or ADT, is the free GUI for AutoDock developed by the same laboratory that develops AutoDock. We can use it to set up, run and analyze AutoDock dockings and isocontour AutoGrid affinity maps, as well as compute molecular surfaces, display secondary structure ribbons, compute hydrogen-bonds, and do many more useful things.

Figure12: Interface of Autodock Tool

Cygwin

Cygwin is a collection of free software tools originally developed by Cygnus Solutions to allow various versions of Microsoft Windows to act similar to a Unix system. It aims mainly at porting software that runs on POSIX systems (such as Linux, BSD, and Unix systems) to run on Windows with little more than a recompilation.

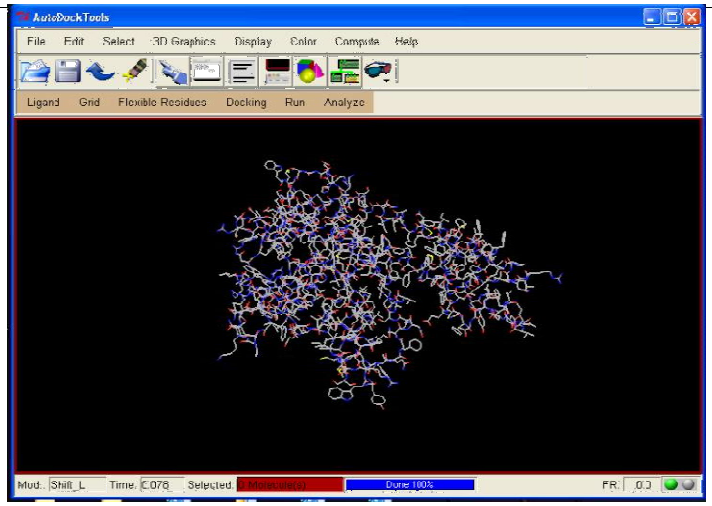


Figure 13: Interface of Cygwin

Running Autodock 3.0.5

Preparing a ligand file for Autodock

For preparing the ligand file, first open the **ligand** menu button. Goto **input** and open the PDBQ files: (*. pdbq) in **Open…AD3.**

Then Goto **Ligand** and open the **torsion tree** and press **Detect Root**.

Goto **Ligand, Opentorsion tree** and **choose Torsions**.

Goto **Ligand**, Open **torsion tree** and **set no. of torsions**

Goto **Ligand Openoutput** and save the file as **(*. pdbq) …AD3**

Preparing a macromolecule file for Autodock

For preparing the macromolecule file, first open the **Grid** menu button. Goto **Macromolecule** and open the **Choose…AD3 file** and save as *. PDBQS

Open **Grid** and Goto **Set map types**.

Preparing the grid parameter file

Open **Grid** and Goto **Set map types**.

Open **Grid** and Goto **Grid Box** and cover the active site of the receptor and save as **Close Saving Current.**

Open **Grid** andGoto **Output** and **Save GPF … (AG3).**

Starting autogrid

Open **Run** andGoto **Run Auto Grid**

Goto **Program Pathname** and set up the location to **AutoGrid3.exe**

Goto **Parameter Filename** and set up the receptor files with gpf file

*Goto **cmd*** and copy the command

Open **Cgywin** window and paste the copied command

Goto **Run Auto Grid …** and **launch** the cmd command.

Preparing a docking parameter file for Autodock

Open **Docking** and Goto **Macromolecule** and **Choose …(AD3)**

Open **Docking** and Goto **Ligand** then Choose**…(AD3)** and Select Ligand

Open Docking and Goto Search **Parameter File** and select Genetic

Algorithm …

Open **Docking** Goto **Output** and Select **Lamarckian GA …(AD3)**

Open **Docking** and Goto **Edit DPF…**

Starting Autodock

Open **Run** andGoto **Run AutoDock**

Goto **Program Pathname** and set up the location to **AutoDock 3.exe**

Goto **Parameter Filename** and set up the receptor file with dpf exe.

*Goto **cmd*** and copy the command

Open **Cgywin** window and paste the copied command

Goto **Run AutoDock …** and **launch** the cmd command

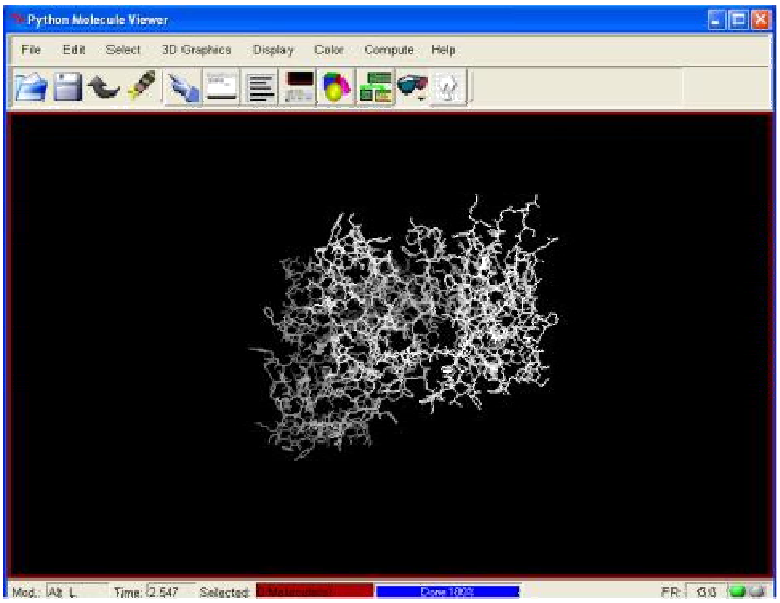


Figure 14: Interface of PMV

Analysing Autodock results reading docking logs

Goto **Analyse** and Open **Docking** then **open…** the ligand dlg exe. File

Goto **Analyse** and Open **Conformations** then **Load …** the Receptor.

Analysing autodock results visualizing docking conformations

Goto to **Analyse** and Open **Conformation** and press the **Play**…

Check the **Conformation** and **Analyse** the result**.**

Visualization of Autodock Result

PMV (Python Molecular Viewer)

Python Molecular Viewer is a tool to view the binding of hydrogen bonds in the target molecule. It helps to visualize and analyse the hydrogen bonds. The process of operation of PMV is enlisted below:

Procedure for Operation of PMV:

First Open 'PMV'. Then Go to 'File', then 'Browse commands. Choose 'PMV' then 'Load molecule'. After that 'trace commands', again 'load molecule'. At last, choose 'hydrogen bond commands' then 'load molecule' and 'Dismiss'. Go to 'file', choose 'Read molecule' and then 'Open protein.pdbqs'. Go to 'Compute', click on 'Trace' and then 'Compute extrude trace'. Go to 'Colour' then 'Choose colour. Click on 'All geometries' and 'OK'. Select colour from table and then click on 'Dismiss'. Go to 'Display', choose 'Lines', now click on 'undisplay' and then 'ok'. Now go to 'Select'. Choose 'select from string'. Type all the 'residues' which came while analysing Auto dock results in Residue box & '*' in Atom box. Then 'Add' and 'Dismiss'. In the next step go to 'Display'. Choose 'Sticks & balls. Now set 'sticks radius':0.1 & 'ball radius':2.0 and 'Sticks& balls quality': 5.0. Click on 'OK'. Go to 'Colour', choose 'By atom type' then select 'sticks &balls', click on OK. Go to 'display', choose 'Label' now 'by properties. Click on 'Residue' then 'name'. After that 'choose label color', select black colour and then OK. Go to 'File', then 'read molecule'. Now open "inhibitor.docked.pdbq" file. Now go to 'Select'. Choose 'Direct select', click on 'molecule list'. Now 'select protein'. Again, click on 'molecule list'. Now select 'inhibitor file'. Then 'Dismiss'. Go to 'Display', choose lines then 'select display'. Click 'OK'. Go to 'Color', then 'choose color'. Click on 'all Geometries 'then 'ok'. Now select 'black colour' then 'Dismiss'. Go to 'Display', then 'label'. Choose 'by properties', click on 'molecule' then 'name' and 'ok'. In next step go to 'Select' then 'Direct select'. Choose 'Molecule list' then 'inhibitor file'. Again, go to 'molecule list' then 'model' and 'Dismiss'. Go to 'Display' then choose 'trace'. Now select 'Undisplay' then 'OK'. Go to 'Select' then 'direct select'. Now go to 'molecule list' then select 'protein' and 'Dismiss'. Go to 'Hydrogen bonds' then choose 'build'. Now 'Set Params+build' then 'specify two sets. choose 'molecule list (1)' then 'select protein' and 'molecule list (2). Now 'select inhibitor'. Click on 'OK'. Go to 'Hydrogen bonds' then 'Display as lines. Click on 'Déjà Vu GUI. Now click on 'camera 'of property box. Then click 'Scene antialising. now select '15' from no. table. Click on background color. Then scroll down and click on 'SW'. At the end go to 'File', then 'save. After that 'save image as '.tif' file then click on 'OK'

4. RESULTS AND DISCUSSION

4.1. Homology modeling of Neuraminidase (NA) protein

The sequence alignment of the query NA sequence (ACZ97472) of (A/Hyd/NIV51/2009(H1N1) virus and

template (1NMB) shows sequence identity and similarity were 45% and 59% respectively. The result of alignment was employed to build new homology model. MODELLER generates five models of H1N1-Neuraminidase. Modeller Function and DOPE Score for five models were listed in the next Table 3. Reliability of new homology model for NA was identified by Ramachandran plot (Table 4). After the optimization and energy minimization process, the best model was selected among five 3D models generated for NA protein on the basis of modeller scores. Energy minimization of 3D structure is vital for providing the maximum stability to the protein. Ramachandran plot drawn through PROCHECK program validated the model with 76% of the total residues in most favoured region and residues in additional allowed regions was 3.1% and 19.1 % in the generously allowed region. This stipulates that protein backbone dihedral angles phi(φ) and psi(ψ) occupied reasonably accurate positions in the selected 3D model.

Table 3: Modeller Objective Function and DOPE Score of five models predicted by MODELLER 9V8

MODEL	MODELLER OBJECTIVE FUNCTION (kcal/mol)	DOPE SCORE (kcal/mol)
Neuraminidase.B99990001	5720.22	-36164.96
Neuraminidase. B99990002	5990.88	-26402.80
Neuraminidase. B99990003	5759.83	-35051.16
Neuraminidase. B99990004	5920.65	-36052.41
Neuraminidase. B99990005	5946.36	-35958.81

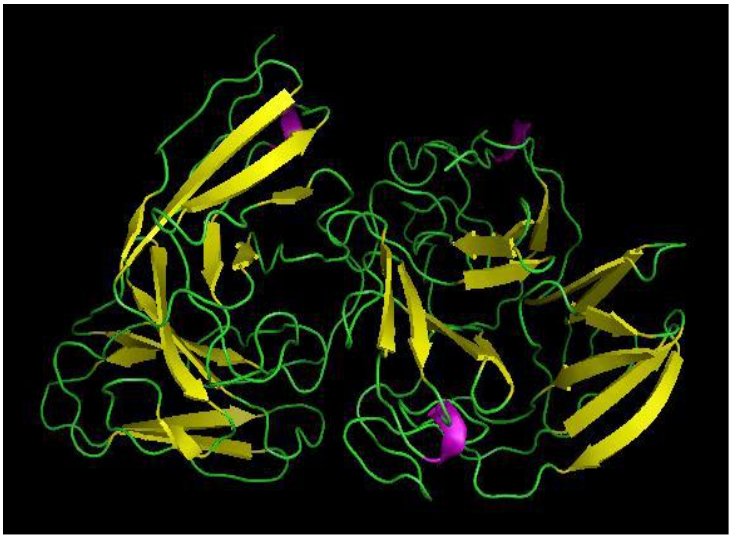


Figure 15: Computationally modelled 3D structure of Neuraminidase obtained from Modeller

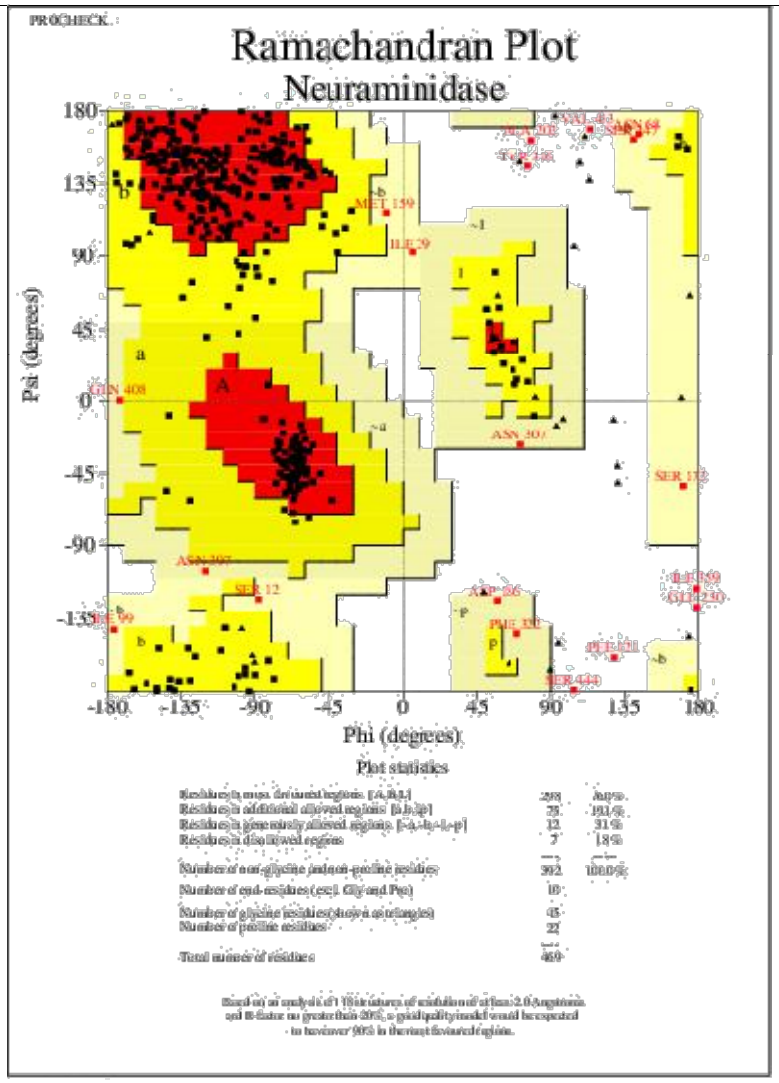


Figure 16: Ramachandran plot of model Neuraminidase (Neuraminidase. B99990001)

4.2 ZINC Screening

Pharmacophore of known drugs Peramivir, Laninamivir, Zanamivir and Oseltamivir was developed using PharmaGist. Snapshot of PharmaGist is shown in figure 17. ZINC database was screened using the pharmacophore (shown in figure 18) based on antiflu drugs Laninamivir, Zanamivir and Oseltamivir 3D structures. A total of 33 compounds were screened.

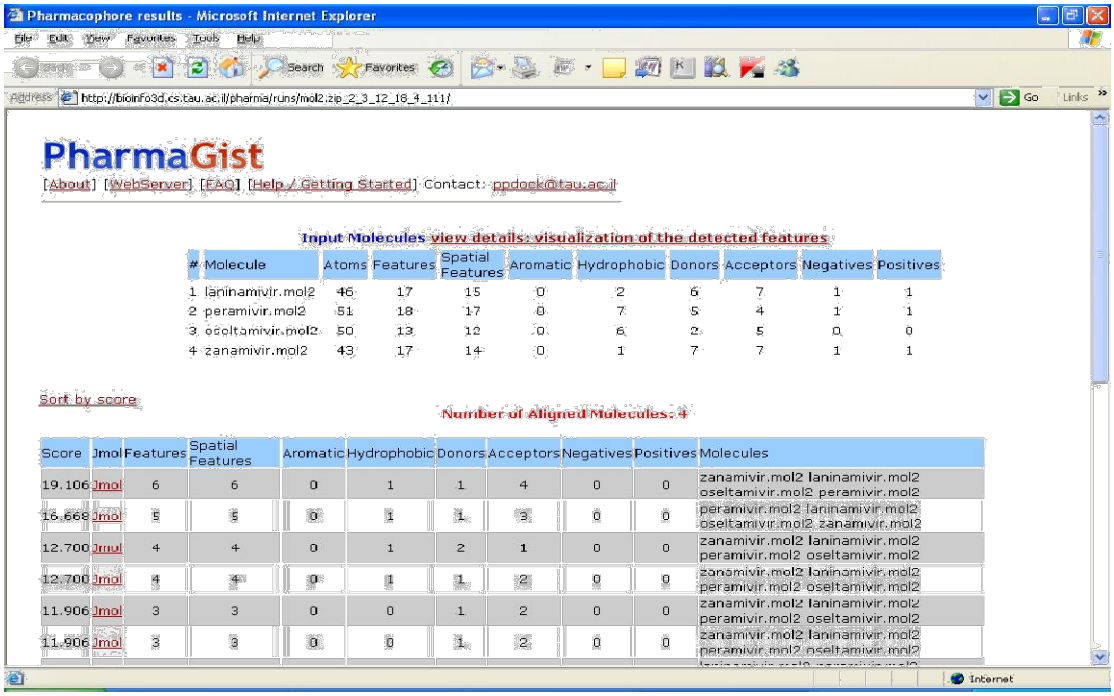


Figure 17: Snapshot of PharmaGist

Table 4: Ramachandran plot statistics of Neuraminidase

Model	Residues in most favoured regions	Residues in additional allowed regions (%)	Residues in generously allowed regions (%)	Residues in disallowed regions (%)
Neuraminidase. B99990001	**76 %**	**19.1 %**	**3.1 %**	**1.8 %**
Neuraminidase. B99990002	**74 %**	**19.4 %**	**3.6 %**	**2.6 %**
Neuraminidase. B99990003	**74 %**	**18.9 %**	**4.3 %**	**2.0 %**
Neuraminidase. B99990004	**75 %**	**18.4 %**	**4.3 %**	**1.8 %**
Neuraminidase. B99990005	**74.5%**	**19.6 %**	**3.3 %**	**2.6 %**

Table 5: The docking results of the thirty-three compounds with NA model structure.

Sl. No.	ZINC ID	MWT	xLogP	HBD	HBA	psa	charge	rb	Docked Energy (kcal/mol)	Ref RMS
1	ZINC2043006	290.296	-1.22	9	9	174	1	5	-14.85	97.61
2	ZINC5434455	290.248	-3.01	5	9	159	-1	5	-1.01	92.45
3	ZINC4096466	290.248	-3.01	5	9	159	-1	5	-3.65	93.11
4	ZINC5884079	290.272	-3.57	7	9	166	0	5	-10.45	92.69
5	ZINC5884077	290.296	-1.22	9	9	174	1	5	-15.26	96.27
6	ZINC29559740	346.34	-3.24	8	11	194	0	7	-7.75	94.05
7	ZINC13443807	316.314	-2.94	8	10	185	0	6	-12.6	91.77
8	ZINC13443833	328.369	-0.71	6	9	154	0	7	-6.88	96.14
9	ZINC26739500	299.287	-2.83	7	10	174	0	4	-7.4	95.71
10	ZINC6778874	341.368	-1.71	6	10	164	0	6	-9.19	94.63
11	ZINC28006289	290.272	-3.5	7	9	167	0	5	-8.85	99.85
12	ZINC29551048	332.313	-3.64	9	11	205	0	6	-6.76	96.78
13	ZINC3952211	346.34	-3.27	8	11	191	0	7	-7.62	93.0
14	ZINC27706508	300.355	-0.31	5	7	126	0	6	-8.88	97.92
15	ZINC33360168	341.368	-1.71	6	10	165	0	6	-6.86	94.68
16	ZINC4134500	332.313	-3.64	9	11	205	0	6	--7.79	98.07
17	ZINC22047269	290.248	-3.01	5	9	159	-1	5	-0.93	93.49
18	ZINC16051908	330.297	-4.04	8	11	202	0	6	--7.0	97.74
19	ZINC34817410	327.341	-1.95	7	10	174	0	6	-8.91	96.81
20	ZINC34817412	313.314	-3.03	6	10	165	0	4	-9.03	99.96
21	ZINC14768526	332.313	-3.51	9	11	203	0	7	--7.13	98.59
22	ZINC59537700	332.313	-3.35	8	11	194	0	7	-2.92	93.91
23	ZINC59149365	314.338	-1.81	6	8	147	0	4	-9.68	97.56
24	ZINC59149553	304.299	-3.45	7	9	167	0	6	-10.2	97.64
25	ZINC37033736	332.313	-3.64	9	11	205	0	6	--7.38	98.16
26	ZINC22047259	290.248	-3.01	5	9	159	-1	5	-2.41	93.81
27	ZINC22047264	290.248	-3.01	5	9	159	-1	5	3.34	92.07
28	ZINC29561586	291.284	-3.91	11	10	204	1	5	-15.82	96.49
29	ZINC13828169	332.313	-3.64	9	11	205	0	6	-8.2	95.82
30	ZINC13802909	392.288	-4.03	8	9	165	0	5	-14.08	97.52
31	ZINC37033733	332.313	-3.64	9	11	205	0	6	-10.77	95.0
32	ZINC40747113	290.272	-3.5	7	9	167	0	5	-8.17	97.41
33	ZINC40527504	290.248	-3.01	5	9	159	-1	5	-2.54	97.11

MWT: Molecular weight; HBD: number of hydrogen bond donors; HBA: Number of hydrogen bond acceptors; psa: polar surface area; rb: rotatable bonds; NA: neuraminidase; RefRMS: ref root means square deviation

Each compound was docked with Neuraminidase one by one and validated in two parts: (i) Hydrogen bond details of the best-ranked docked pose and (ii) prediction of docking energy between the docked inhibitor with Neuraminidase using AutoDock 3.0.5.

4.2.1 Docking Energy and RefRMS Values

We docked all 33 optimized compounds screened from ZINC database with modeled Neuraminidase, using AutoDock 3.0.5 and evaluated binding compatibility with receptor based on docked energy (in kcal/mol). The docking tool generated 30 conformations for each docked inhibitor in approximately 25 minute of CPU time. Based on docking energy it was predicted that the compounds ZINC2043006(-14.85kcal/mol), ZINC5884077(-15.26kcal/mol) and ZINC13802909(-14.08kcal/mol) have good binding affinities towards the protein and their Root mean square deviation from a reference structure is shown in table 6.

4.2.2 Hydrogen Bond Details

A close view of the binding interactions of Neuraminidase with the ZINC2043006, ZINC5884077 and ZINC13802909 were analysed through Python Molecular viewer. Ligand is coloured in Magenta (in PMV Results) whereas amino acids involved in hydrogen bonding were labelled in black colour. Details of hydrogen bonds formation between each compound and Neuraminidase is shown in table 7. The compound ZINC5884077formed one hydrogen bond with ASP151 active site residue of Neuraminidase. The inhibitors form two hydrogen bonds with active site residue ASP151 (shown as green lines in PMV Results). The details of atoms in formation of hydrogen bond with bond length were also given in table 7 for each compound, which may provide useful information for in-depth under-standing binding mechanism of the compound to the active site of the protein.

Table 6: Hydrogen bond interaction between Neuraminidase with compounds

Sl. No.	Molecule	Amino acid with position	Atom in Amino Acid	Atom in Inhibitor	H-Bond Length (Å)
1	ZINC2043006	ASP151	OD1	H	2.223
2	ZINC5434455	ARG152	N	H	2.187
3	ZINC4096466	-	-	-	-
4	ZINC5884079	-	-	-	-
5	ZINC5884077	ASP151	OD1	H	1.991
6	ZINC29559740	ASP151	OD2	H	1.907
7	ZINC13443807	-	-	-	-
8	ZINC13443833	ASP151	OD2	H	2.1
9	ZINC26739500	ASP151	OD2	H	1.924
10	ZINC6778874	-	-	-	-
11	ZINC28006289	-	-	-	
12	ZINC29551048	ASP151	OD2	H	1.821
13	ZINC3952211	ASP151	OD2	H	2.168
14	ZINC27706508	-	-	-	-
15	ZINC33360168	ASP151	OD2	H	2.213
16	ZINC4134500	ASP151	OD2	H	2.009
17	ZINC22047269	ASP151	OD1	H	1.845
18	ZINC16051908	ASP151	OD2	H	1.693
19	ZINC34817410	ASP151	OD1	H	2.149
		ASP151	OD1	H	2.113
20	ZINC34817412	-	-	-	-
21	ZINC14768526	ASP151	OD1	H	2.235
22	ZINC59537700	-	-	-	-
23	ZINC59149365	-	-	-	-
24	ZINC59149553	-	-	-	-
25	ZINC37033736	ASP151	OD1	H	1.867
26	ZINC22047259	-	-	-	-
27	ZINC22047264	-	-	-	-
28	ZINC29561586	-	-	-	-
29	ZINC13828169	ASP151	OD2	H	1.967
30	ZINC13802909	ASP151	OD2	H	2.199
		ASP151	OD1	H	2.056
31	ZINC37033733	-	-	-	-
32	ZINC40747113	ASP151	OD1	H	1.773
33	ZINC40527504	-	-	-	-

4.3 Python Molecular Viewer (PMV) Results:

Figure19: Hydrogen Bond Formed Between Model Neuraminidase (ASP151) and ZINC2043006 compound

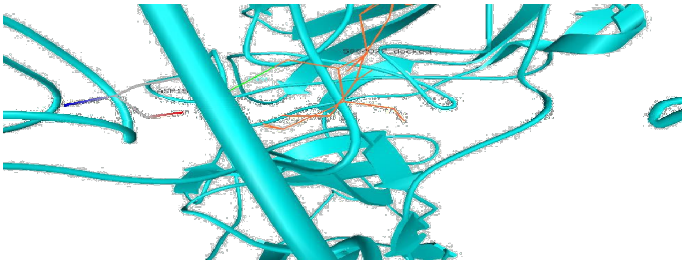


Figure 20: Hydrogen Bond Formed Between Model Neuraminidase (ASP151) and ZINC5884077 compound

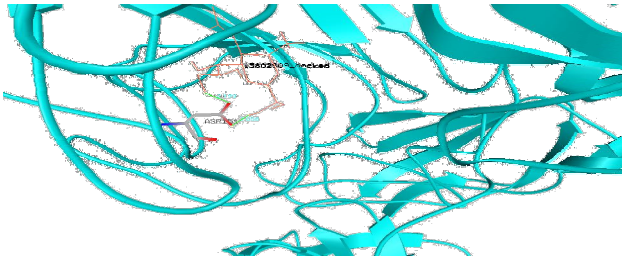


Figure 21: Hydrogen Bond Formed Between Model Neuraminidase (ASP151) and ZINC13802909 compound

5. CONCLUSION

Neuraminidase is one of the recent potent Drug targets for swine flu. In this work, we have constructed a 3D model of Neuraminidase, used the MODELLER software and obtained a refined model after energy minimization. The final refined model was further assessed by PROCHECK program, and the results show that the model was stable and reliable. The stable model was further used for Virtual Docking of ZINC database. Docking results indicate that out of 33 Marine compounds, there were three inhibitory compounds for Neuraminidase as target for swine flu. As it's well known, hydrogen bonding plays an important role for the structure and function of biological molecules, especially for inhibition in a complex. Thus, our study confirms ZINC2043006, ZINC5884077

and ZINC13802909 are potential inhibitors for Neuraminidase as target for tuberculosis forming a hydrogen bonding and with non-bonded interaction to act as a drug candidates yet Pharmacological study will yet confirm it to be promising.

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