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STRUCTURE BASED DRUG DESIGN FOR SWINE FLU: SURFACE ANTIGEN HEMAGGLUTININ AS A TARGET ON *IN SILICO* PLATFORM

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ABSTRACT

Swine flu or swine influenza is a highly contagious, air-borne viral respiratory disease, pandemic throughout the world. It is generally caused by subtypes H1N1/H1N2 and H3N2 of Type A Influenza virus. The pathogenesis of swine flu reflects, in part, the activity of lung cells. As it is an air-borne disease, it can easily spread from simple sneezing or coughing of an infected person. The virus can linger on tables and surface areas like door knobs, waiting to be picked up. The current work involves identification of the protein Hemagglutinin, main cause of swine flu and analysing its role in the disease by using bioinformatics tools. The sequence structure and functional analysis of the concerned protein has been studied through BLAST. Since the precise structure of Hemagglutinin protein is not yet available, theoretical model for the query protein was generated using Easy Modeller 4.0. The protein-ligand docking was done with use of Autodock software. The novel drugs designed through ChemSketch software showed an impressive binding energy of -10.3 kcal/mol, 3-(4-benzhydrylpiperazin-1-yl)-1-(4-fluorophenyl) pyrrolidine-2,5-dione) in comparison with the synthetic drug (binding energy: -8.8 kcal/mol, Flunarinzine) Chemsketch has been used to design new compounds that can serve as a novel compound for swine flu.

Keywords: Hemagglutinin, Swine Influenza, H1N1, H3N2, H1N2.

INTRODUCTION

Swine Influenza, SI (swine flu, hog flu, pig flu), a zoonotic, air-borne, highly contagious viral respiratory disease, which was first recognized in swine (pigs) in the Mid-Western US in summer/fall of 1918, with the infection Influenza A virus, from H1N1

lineage (1, 3, 7). Swine flu is also known as seasonal influenza because it tends to spread during temperate climates (in winters) (2). Besides pigs, seasonal influenza virus has been recognized to spread among humans across the globe. The subtypes of Influenza A virus that are found in the infected organisms (humans as well as swine) are H1N1/H1N2 and H3N2 (4, 6). Like regular flu, patients suffering from SI show various symptoms like sneezing, coughing, chills, sore throat, body aches, severe headaches, respiratory distress and sometimes cyanosis (3, 4, 5). In many cases, severe illness has been observed during SI infection like pneumonia, respiratory failure as well as death. SI has become a pandemic disease across the globe since 2009 currently.

SI virus has three subtypes or groups, labelled as A, B and C. Type A is the most common infectious virus which causes swine flu. The virus contain Hemagglutinin or HA and Neuraminidase or NA as a surface antigens which are able to undergo in two types of variations, called as antigenic drift and antigenic shift; former involve minor change(s) in the HA and NA, whereas antigenic shift involves major changes in these molecules resulting from replacement of the entire gene segment, respectively (1,2). Influenza virus replicates in the respiratory system in mammals and has high evolutionary rate in mammals. (1)

In the present study, hemagglutinin protein of type A influenza virus has been targeted for *in silico* studies. A suitable three dimensional computational structure has been modelled after retrieving FASTA sequence of the query protein with the help of bioinformatics tool. The modelled structure has been allowed to be docked to the active site of the query protein against various different ligands isolated from drug databases. These docking studies have shown the path for a de novo drug discovery against swine flu on in silico platform.

METHODOLOGY

Sequence Alignment

The FASTA sequence of hemagglutinin query protein has been obtained from online source of National Centre for Biotechnology Information, NCBI (http://www.ncbi.nlm.nih.gov) and aligned by using BLASTp sequence alignment tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Chain A of H1N1 influenza A virus (PDB ID: 4F15), has showed maximum identity with the query protein. Hence, it has been selected as a template protein for structure prediction of the targeted protein molecule.

Structure Prediction

The three dimensional secondary structure of hemagglutinin protein has been predicted with the help of online server 3DPSSM and Phyre 2. Nine protein templates have been selected showing maximum identity with

our query protein structure. The PDB file format of the selected templates have been obtained from Protein Data Bank (PDB), a computational database, having resolution <3.0, R-Value <5.0 and X-Ray crystallographic structures. With the help of the software EASY MODELLER (version 4.0), the 3-dimensional model of query protein has been created by submitting PDB file formats of these template protein to the software program.

Validation of Predicted Model (Loop Modelling):

Structural Analysis and Verification Server, SAVES (Prochek) is an online bioinformatics tool which has been used in our work for validation of the query protein model (Ramachandran Plot Analysis) of the query protein. The model query protein has been rebuilt into its secondary structure by further submitting it into the SAVES. The final secondary structure obtained after validation has been viewed using the Swiss PDB Viewer (SPDB 4.10).

Prediction of the Active Site

The query protein, having 566 amino acid residues, has been submitted to online Dog Site Scorer, an Active site prediction and Analysis server. Prediction of active site is necessary to identify the ligand binding site, present on the query protein.

Drug Library Search

Drug Bank database has been used to obtain the structure of drug molecules (in MOL format) already available for the treatment of swine influenza. The MOL files of these structures were then converted into PDB format with the help of software Open Bable. These molecules were used as ligand during molecular docking.

Molecular Docking

Molecular docking has been performed using Auto Dock software against the active site of the query protein with the available ligands. The ligand molecule that showed minimum free energy has been used as a base molecule for designing *in silico* structure of drug molecule against Swine Influenza.

Designing Computational Drug Molecule

With the help of ChemSketch software, the *in silico* drug molecules were designed. The molecule that showed minimum free energy when binding with the active site of the query protein has been selected as the final drug molecule.

Toxicity Prediction

The toxicity of the selected ligand has been checked with the help of an online server ACD Labs ILabs. This online server gives the information regarding the physical and chemical properties of any molecule by following the five rules of Lipinsky.

RESULTS AND DISCUSSION

The FASTA sequence of Hemaaglutinin obtained from NCBI has showed maximum identity of 99% with Chain A of H1N1 influenza A virus (PDB: 4F15) (Fig. 1). With the help of 3DPSSM and PHYRE 2 online servers, secondary structure of the query protein has been obtained showing the minimum dope score (Fig. 2). By using the secondary structure, 3- dimensional tertiary structure of the query protein has been created by using software Modeller. The obtained three dimensional computational model of query protein has been allowed for validation by following Ramachandran Plot analysis, using SAVES (Prochek), an online server. It has been viewed under Swiss PDB Viewer (Fig 3, 4, 5). The active site of the query protein obtained from Dog Site Scorer (Fig 6), has been allowed to molecular docking and a minimum free energy of -8.8 kcal/mol (Flunarizine; Drug ID: DB04841) (Fig 7) selected as a base molecule for further computational drug designing. The novel drug molecule obtained by us from ChemSketch software possess a free energy of -10.3 kcal/mol. IUPAC name of the novel drug stands 3-(4-benzhydrylpiperazin-1-yl)-1-(4-fluorophenyl) pyrrolidine-2,5-dione (Fig 8). The obtained molecule is the most suitable with consideration of the Lipinsky's rule for the toxicity of the chemical compounds in vivo (Table 1). Therefore, this molecule has been chosen for the best possible drug against Swine Flu.

CONCLUSION

In the present work we have found that Swine flu is caused by Hemagglutinin protein encoded in H1N1 virus. Through Homology modelling and loop modelling we have got the final structure of our protein and after its docking with the best result, Chemsketch made modifications has provided a suitable ligand having minimum free energy when docked with the active site of the query protein. In vivo toxicity of the novel drug has been checked. The de-novo structure based drug designing approach has led to a potential ligand (drug) molecule that blocks the active site of Hemagglutinin protein preventing the responsible gene of Swine flu.

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Description	Max score	Total score	Query cover	E value	Ident	Accession
Chain A, Molecular Basis Of Infectivity Of 2009 Pandemic H1n1 Influenza A Viruses [Influenza A virus (A/Korea/01/2	1046	1046	89%	0.0	99%	4F15_A
Chain A, Structure Of Influenza H2 Duck Ontario Hemagglutinin With Avian Receptor [unidentified influenza virus]	702	702	90%	0.0	63%	2WR3_A
Chain A, Structures Of Influenza H2 Hemagglutinins [unidentified influenza virus]	699	699	90%	0.0	63%	2WR0_A
Chain A, Structure Of H2 Japan Hemagglutinin [Influenza A virus (A/Japan/305+/1957(H2N2))]	696	696	89%	0.0	63%	2WRD_A
Chain A, Structure Of H2 Japan Hemagglutinin With Human Receptor [Influenza A virus (A/Japan/305+/1957(H2N2))]	696	696	89%	0.0	63%	2WRE_A
Chain A, The Structure Of Influenza H2 Human Singapore Hemagglutinin With Human Receptor [Influenza A virus (A/S	694	694	90%	0.0	62%	2WR7_A
Chain A, Structure Of H2 Avian Jena Hemagglutinin With Human Receptor [Influenza A virus (A/chicken/Potsdam/470	691	691	90%	0.0	62%	2WRF_A

Fig 1: Maximum Identity value of Hemagglutinin after running BLASTp

Filename	MOL pdf	DOPE score	GA341 score
query.B99990001.pdb	33402.42578	-43397.82031	1.00000
query.B99990002.pdb	31461.16797	-44809.94531	1.00000
query.B99990003.pdb	32744.52539	-43974.26953	0.99976
query.B99990004.pdb	28798.55273	-45995.11719	1.00000
query.B99990005.pdb	33979.85938	-43749.45703	1.00000
query.B99990006.pdb	32087.38281	-43740.45312	1.00000
query.B99990007.pdb	31686.76172	-44783.13672	0.99999
query.B99990008.pdb	36510.32812	-42603.83203	0.99998
query.B99990009.pdb	34452.33594	-42844.48047	0.99996

Fig. 2: Minimum Dope Score showed by the template protein molecules after Homology Modeling and Running Easy Modeller

SAVES results for output71.pdb

Procheck

Summary

1	Note	Ramachandran plot: 98.8% core 1.2% allow 0.0% gener 0.0% disall [PostScript] • [PDF] • [JPG]
2	Warning	+ All Ramachandrans: 1 labelled residues (out of 564) [PostScript] • [PDF] Images: 1 2 3
3	Error	* Chi1-chi2 plots: 13 labelled residues (out of 338) [PostScript] • [PDF] Images: 1 2
4	Warning	+ Main-chain params: 4 better 0 inside 2 worse [PostScript] • [PDF] • [JPG]
5	Note	Side-chain params: 5 better 0 inside 0 worse [PostScript] • [PDF] • [JPG]
6	Error	* Residue properties: Max.deviation: 10.5 Bad contacts: 126 * Bond len/angle: 19.4 Morris et al class: 1 2 4 + G-factors Dihedrals: -0.22 Covalent: -1.07 Overall: -0.51 [PostScript] • [PDF] Images: 1 2 3 4 5 6
7	Warning	+ G-factors Dihedrals: -0.22 Covalent: -1.07 Overall: -0.51 [PostScript] • [PDF] • [JPG]
8	Error	* M/c bond lengths: 95.2% within limits 4.8% highlighted 4 off graph [PostScript] • [PDF] Images: 1 2
9	Error	* M/c bond angles: 82.2% within limits 17.8% highlighted 20 off graph [PostScript] • [PDF] • [JPG]
10	Warning	+ Planar groups: 98.1% within limits 1.9% highlighted 2 off graph [PostScript] • [PDF] Images: 1 2 3

Fig. 3: Validation with SAVES (Prochek) Server

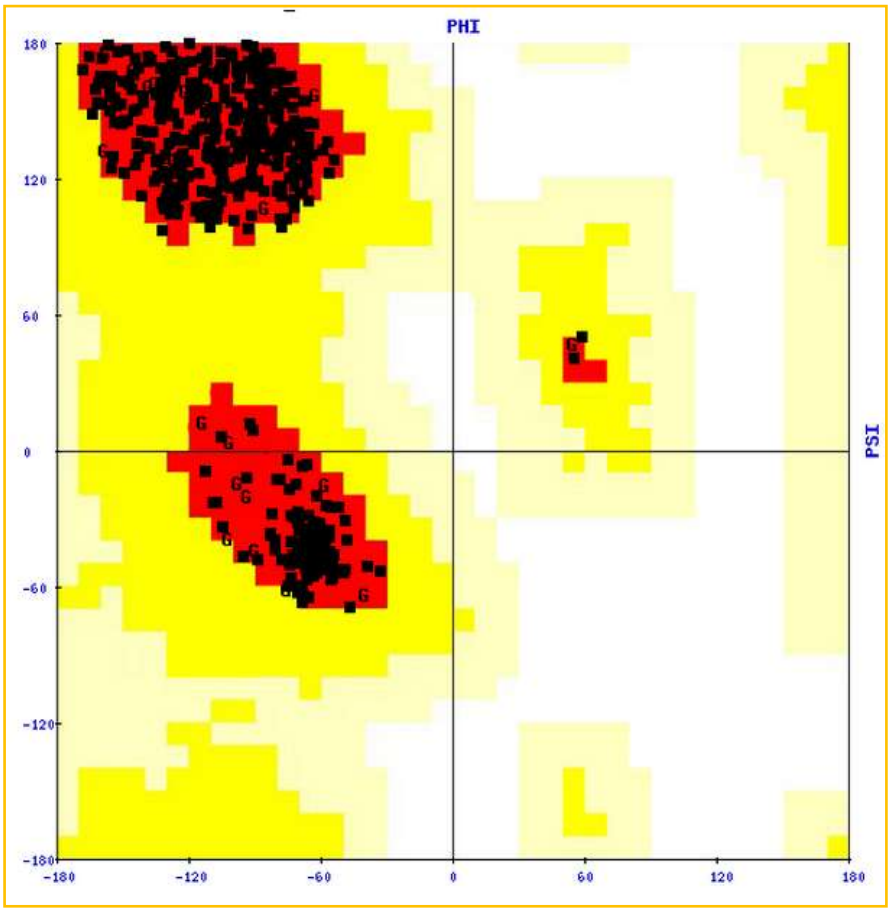


Fig 4: Ramachandran Plot Analysis with SAVES (Prochek)

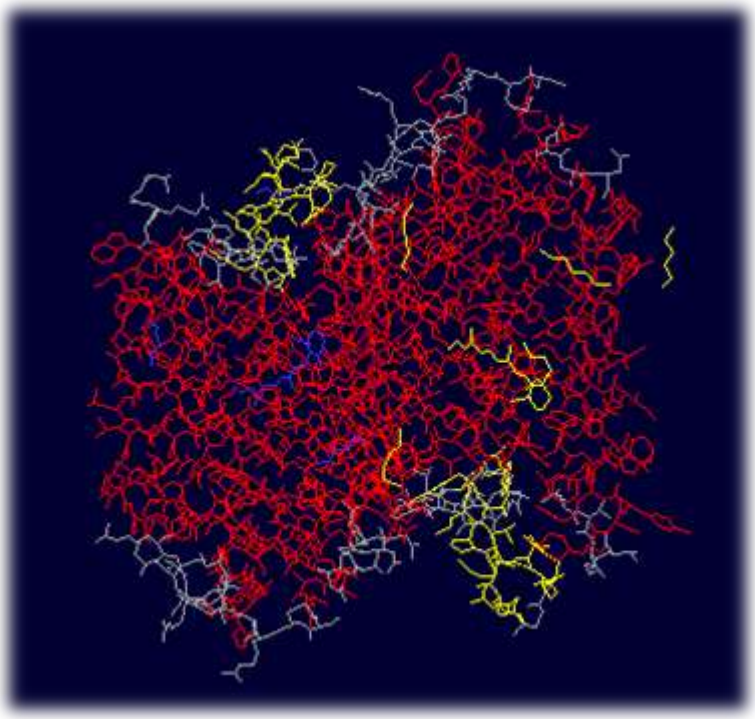
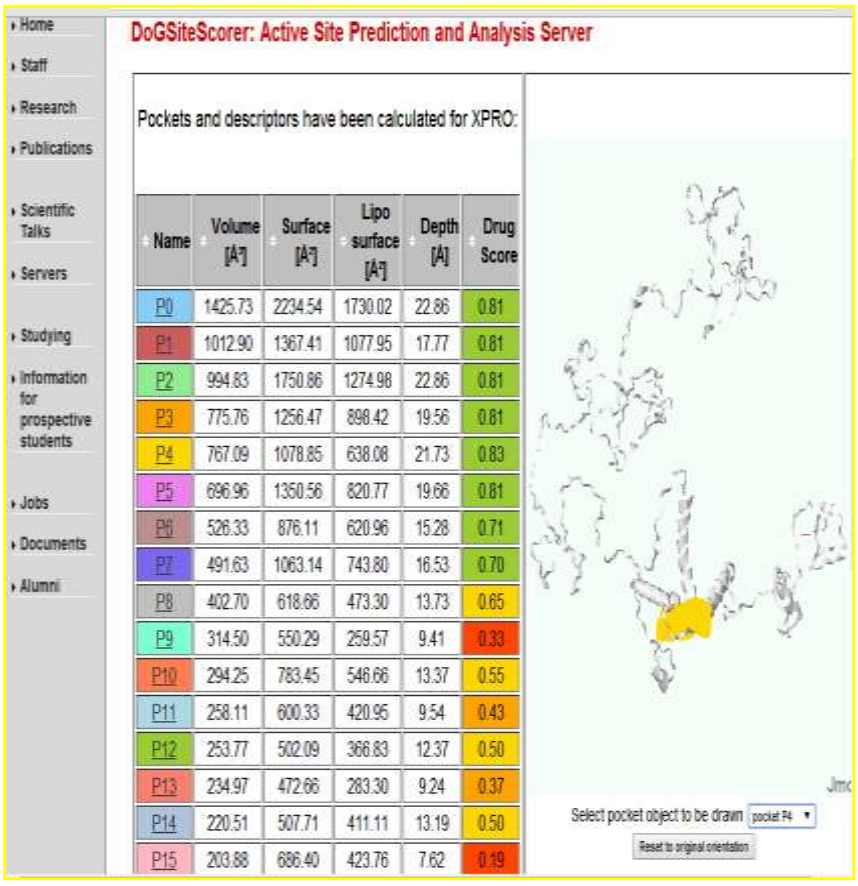


Fig. 5: Three dimensional model of Hemagglutinin protein viewed under Swiss PDB Viewer



Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0	1425.73	2234.54	1730.02	22.86	0.81
P1	1012.90	1367.41	1077.95	17.77	0.81
P2	994.83	1750.86	1274.98	22.86	0.81
P3	775.76	1256.47	898.42	19.56	0.81
P4	767.09	1078.85	638.08	21.73	0.83
P5	696.96	1350.56	820.77	19.66	0.81
P6	526.33	876.11	620.96	15.28	0.71
P7	491.63	1063.14	743.80	16.53	0.70
P8	402.70	618.66	473.30	13.73	0.65
P9	314.50	550.29	259.57	9.41	0.33
P10	294.25	783.45	546.66	13.37	0.55
P11	258.11	600.33	420.95	9.54	0.43
P12	253.77	502.09	366.83	12.37	0.50
P13	234.97	472.66	283.30	9.24	0.37
P14	220.51	507.71	411.11	13.19	0.50
P15	203.88	686.40	423.76	7.62	0.19

Fig. 6: Active Site of Query Protein obtained by Dog Site Scorer (in yellow colour)

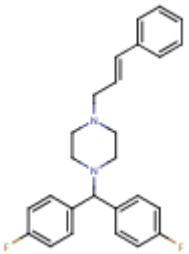


Fig. 7: Chemical Structure of Flunarizine

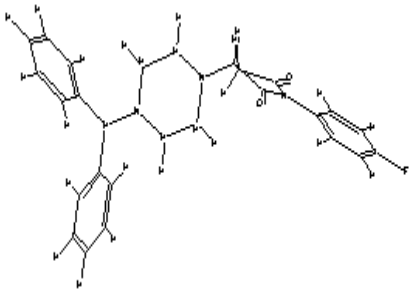


Fig. 8: Chemical Structure of 3-(4-benzhydrylpiperazin-1-yl)-1-(4-fluorophenyl) pyrrolidine-2,5-dione

Table 1: Toxicity Prediction of 3-(4-benzhydrylpiperazin-1-yl)-1-(4-fluorophenyl) pyrrolidine-2,5-dione

Molar Refractivity	**123.90 ± 0.3 cm3**
Molecular Weight	**443.51**
No. of Hydrogen Bond Donors	**0**
No. of Hydrogen Bond Acceptors	**5**