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In *silico* Analysis of Compounds from *Stemona tuberosa* as an Inhibitor for N1 Neuraminidase of H5N1 Avian Virus

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ABSTRACT

The worldwide spread of H5N1 avian influenza has raised concerns that this virus might acquire the ability to pass readily among humans and cause a pandemic.Two anti-influenza drugs currently being used to treat the infected patients are oseltamivir (Tamiflu) and zanamivir (Relenza), both of which target the neuraminidase enzyme of the virus. Reports of the emergence of drug resistance has made the development of new anti-influenza molecules a priority. Various compounds present in Stemona tuberosa a plant belonging to Stemonaceae family was tested for activity against H5N1 neuraminidase. Eight one molecules including stilbenoids, bibenzyls and various others were selected as probable compounds for lead molecules. These lead molecules were tested for toxicity tests and Lipinski rule in Pre ADMET server. Around 33 compounds cleared all these tests. Validated ligand molecules were docked against H5NI Neuraminidase active site residues using AUTODOCK 4 which showed better results in comparison with zanamivir and oseltamivir, anti- influenza drugs.

Key words: H5N1 Avian virus, Neuraminidase inhibiotors, *Stemona tuberosa*, Docking analysis

INTRODUCTION

Avian influenza or “bird flu” is a contagious disease that is hosted by the birds, which infects several species of mammals. H5N1 has evolved into a flu virus strain that infects more species than any previously known flu virus strain, is deadlier and more pathogenic. The worldwide spread of H5N1 avian influenza has raised concerns that this virus might acquire the ability to pass readily among the humans and cause a pandemic. Scientists are closely monitoring what looks like the birth of a super strain of one of humankind’s oldest and most persistent enemies, the influenza virus. This new strain has the potential to kill hundreds of millions given the right conditions. Avian influenza viruses are species specific but do occasionally cross the species barrier to infect humans and other mammals.

Avian influenza H5N1 is posing serious concerns to the medical community due to its stunning killing ability, a statistic known as the lethality of the disease. Influenza virus membranes contain two glycoproteins: haemagglutinin and neuraminidases. The influenza A virus particle or virion is 80–120 nm - It contains eight pieces of segmented negative-sense RNA (13.5 kilobases total), which encode 11 proteins (HA, NA, NP, M1, M2, NS1, NEP, PA, PB1, PB1-F2, PB2).Viral proteins are haemagglutinin and neuraminidase, two large glycoproteins found on the outside of the viral particles (Rupert et al. 2006).

Haemagglutinin mediates cell-surface sialic acid receptor binding to initiate virus infection. After virus replication, neuraminidase removes sialic acid from virus and cellular glycoproteins to facilitate virus release and the spread of infection to new cells1. The distinct antigenic properties of

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different haemagglutinin and neuraminidase molecules are used to classify influenza type A viruses into subtypes: 16 for haemagglutinin (H1–H16) and 9 for neuraminidase (N1–N9) (Von et al. 1993). Numerous combinations of haemagglutinin and neuraminidase subtypes are found in avian species. The N1 and N2 neuraminidases of viruses currently circulating in humans belong to two phylogenetically distinct groups. Group-1 contains N1, N4, N5 and N8 subtypes whereas group-2 contains N2, N3, N6, N7 and N9.

Neuraminidase has been targeted in structure-based enzyme inhibitor design programmes that have resulted in the production of two drugs, zanamivir (Relenza) (Von Itzstein et al. 1993) and oseltamivir (Tamiflu) (Kim C. U. et al. 1997). Reports of the emergence of drug resistance make the development of new anti-influenza molecules a priority(Aeron C et al. 2006, Jackson et al. 2000).

Structure of H5N1 avian influenza neuraminidase suggests new opportunities for drug design. Present investigation was undertaken to carry out the docking procedure using different ligands from *Stemona tuberosa* to find out potential inhibitor for N1 Neuraminidase of H5N1 avian virus.

Stemonaceae is a small family with four genera, namely, Croomia, Pentastemona, Stemona and Stichoneuron. *Stemona* is the largest genus with about 32 species, whereas the other genera each have one or two species. They are distributed in Asia, tropical Australia and North America.

Stemona tuberosa is a species registered in the Chinese Pharmacopoeia.The water extracts of the root tuber of this plant are used in Chinese, Japanese and Korean traditional medicines to treat respiratory disorders, e.g., bronchitis, pertussis and tuberculosis, and also as an anthelmintic agent for domestic animals. The roots of *Stemona* genus (Stemonaceae) have long been prescribed in traditional Chinese medicine as insecticidal and antitussive agents. Extracts from the roots of these plants are used for respiratory disorders, including pulmonary tuberculosis and bronchitis, and externally used against different insect pests (Michael Adams et al. 2005, Li-Gen et al. 2008).

MATERIALS AND METHODS

NCBI, Genbank, PDB, PROSITE, Pubchem databases were used to get the sequence and structure information. Pre ADMET server was used for AMDET calculation of drug molecules. AUTODOCK 4 a docking analysis software was used to study interaction of drug molecules with the target. Chem draw tool was used to draw the structures of drug molecules used for analysis.

Various compounds present in *Stemona tuberosa* was tested for activity against H5N1 neuraminidase *in silico*. Eighty one molecules including stilbenoids, bibenzyls and others were selected as probable compounds for lead molecules.

Neuraminidase has been targeted for structure based enzyme inhibitor drug design programmes as a result of which two drugs Zanamivir and oseltamivir has been designed. The X-ray molecular structure of the influenza virus has already been determined (Varghese et al. 1983, Baker et al. 1987). Structure of Neuraminidase was retrieved from Protein Data Bank and used for analysis.

RESULTS AND DISCUSSIONS

Bioinformatics analysis of neuraminidase sequence

Whole genome analysis result

In the whole genome analysis there was a conserved region in the amino acid region 145-156. This range of amino acid was mainly focused to find the docking site in structural analysis. Genome sequence data also shown that reassortment involving all the segments of the influenza virus genome, is a frequent process and might also facilitate major antigenic changes. Natural selection on the HA protein seemed to operate in a punctuated manner, causing distinct but irregular episodes of phenotypic change that were manifested as antigenic 'cluster jumps'.

Validation of compounds from *S. tuberosa*

Validation of compounds was carried out using pre ADMET server. The Compounds which cleared toxicity assay and follow lipinski rule were chosen for docking analysis. Out of 81 molecules, 33 molecules cleared validation test.

List of validated compounds

From Table 1, molecules which are non mutagen are validated compounds which were used for further analysis. Docking of validated ligands with active site of protein was carried out using Auto dock.

Using Pre ADMER server it was verified that Croomine satisfies Lipinski's Rule of Five and also clears the mutagenecity tests. Molecular weight of Croomine is 307.388800 daltons. Number of Hydrogen bond acceptors and donors are 4 and 1 respectively. Croomine is negative for TA98, TA100, TA1525 strains of Ames mutagenicity Tests.

Table 1 - List of compounds from *Stemona tuberosa* which were subjected to validation tests in pre ADMET server.

Number	Compound Name stilbenoids (S1-S25) & Other compounds	Compound type
1	S1	Mutagen
2	S2	Mutagen
3	S3	Non -Mutagen
4	S4	Mutagen
5	S5	Non -Mutagen
6	S6	Non -Mutagen
7	S7	Non -Mutagen
8	S8	Non -Mutagen
9	S9	Non -Mutagen
10	S10	Non -Mutagen
11	S11	Non -Mutagen
12	S12	Non -Mutagen
13	S13	Mutagen
14	S14	Mutagen
15	S15	Non -Mutagen
16	S16	Mutagen
17	S17	Non -Mutagen
18	S18	Non -Mutagen
19	S19	Mutagen
20	S20	Mutagen
21	S21	Mutagen
22	S22	Mutagen
23	S23	Mutagen
24	S24	Mutagen
25	S25	Mutagen
26	Stenine	Non -Mutagen
27	Tuberostemonine	Non -Mutagen
28	Neotuberostemonine	Non -Mutagen
29	Croomine	Non -Mutagen
30	Stemonine	Non -Mutagen
31	3,5-dihydroxy-4-methyl bibenzyl	Mutagen
32	3,5-dihydroxy-2-methoxy-4-methylbibenzyl	Mutagen
33	3 hydroxy-2,5-dimethoxy-2-methyl bibenzyl	Mutagen
34	Bisdehydrostemonine	Mutagen
35	Iso Bisdehydrostemonine	Non -Mutagen
36	Bisdehydrostemonine A	Non -Mutagen
37	Bisdehydrostemonine B	Non -Mutagen
38	Bisdehydrostemonine C	Mutagen
39	Bisdehydrostemonine D	Mutagen
40	Bisdehydrostemonine E	Mutagen
41	Protostemonine	Mutagen
42	Stemanthraquinone	Mutagen
43	Phenanthraquinone	Mutagen
44	Resveratrol	Mutagen
45	Pinosylvin A	Mutagen
46	Pinosylvin B	Mutagen

Cont. ...

(Cont. Table 1)

Number	Compound Name stilbenoids (S1-S25) & Other compounds	Compound type
47	Pinosylvin C	Mutagen
48	Pinosylvin D	Mutagen
49	Stemofoline	Mutagen
50	Dihydropinosylvin	Mutagen
51	Iso Dihydropinosylvin	Mutagen
52	Stilbostemin A	Mutagen
53	Stilbostemin B	Non -Mutagen
54	Stilbostemin C	Mutagen
55	Stilbostemin D	Non -Mutagen
56	Stilbostemin E	Mutagen
57	Stilbostemin F	Non -Mutagen
58	Stilbostemin G	Non -Mutagen
59	Stemokerrine	Mutagen
60	Stemofuran A	Mutagen
61	Stemofuran B	Mutagen
62	Stemofuran C	Non -Mutagen
63	Stemofuran D	Non -Mutagen
64	Stemofuran E	Mutagen
65	Stemofuran	Mutagen
66	Stemanthrene A	Mutagen
67	Stemanthrene C	Non -Mutagen
68	Stemanthrene D	Non -Mutagen
69	Stemanthrene E	Non -Mutagen
70	Zileuton	Mutagen
71	Parvistemonine A	Mutagen
72	Parvistemonine B	Mutagen
73	6-hydroxycroomine	Mutagen
74	Stemocurtisinol	Mutagen
75	Stemocurtisine	Mutagen
76	Tuberostemonine H	Non -Mutagen
77	Neostemonine	Non -Mutagen
78	Prostemonine	Non -Mutagen
79	Maistemonine	Non -Mutagen
80	Stemonidine	Non -Mutagen
81	Stemotinine	Non -Mutagen

Table 2 - Comparative docking results of ligands having lesser binding energy values among validated compounds in comparison with Zanamivir and Oseltamivir.

Compound Name	ΔG (Kcal/mol)
Croomine	-7.45
Stemonine	-7.41
Tuberostemonie	-7.14
Stilbostemin G	-6.98
Stilbostemin C	-6.93
Zanamivir	-6.60
Oseltamivir	-4.85

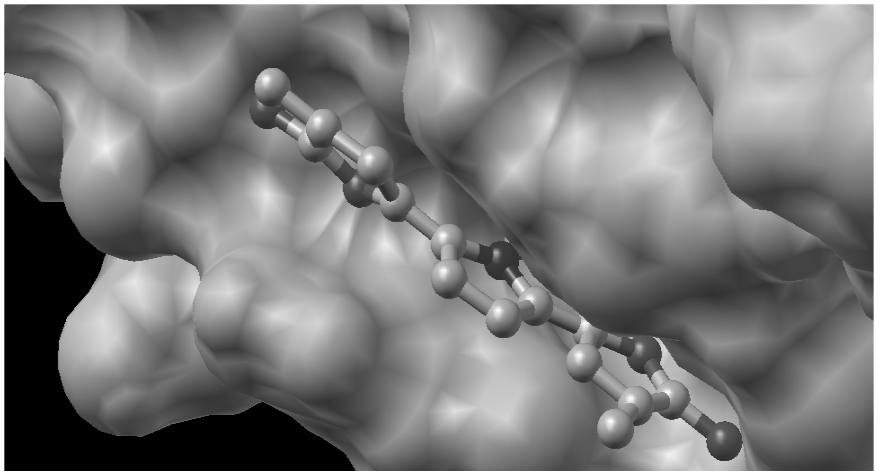

Figure 1 - Interaction of croomine with active site of H5N1 Neuraminidase captured using Autodock 4.

CONCLUSION

The worldwide spread of H5N1 avian influenza virus has raised concerns that this virus might acquire the ability to pass readily among humans, and cause pandemic. Various validated compounds from *S. tuberosa* were used as inhibitor for N1 neuraminidase of H5N1 avian virus. Results showed that some compounds are better than currently used anti-influenza drugs. Hence *S. tuberosa* could be expected as a silver lining for latest threat to mankind, H5N1 Pandemic.

REFERENCES

Rupert J. Russell, The structure of H5N1 avian influenza neuraminidase suggests new opportunities for drug design, *Nature* .2006;443(2):45-49.

Von Itzstein M,Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature*. 1993; 363(4):418-423.

Kim C. U,Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent antiinfluenza activity", *J. Am. Chem. Soc*. 1997; 119(6):681-690.

Aeron C.Hurt, Pina Innello , Neruminidase Inhibiotor-Resistant and –Sensitive Influenza B Viruses Isolated from an Untreated Human Patient,*Antimicrobial Agents and Chemotherapy*, 2006;50(3):1872-1874.

Jackson H.C.,N.Roberts,Z.M. Wang, and R.Belshe, Management of influenza :use of new antivirals and resistance in perspective, *Clin.Drug Investig*.2000; 20(7): 447-454.

Varghese, J.N., Laver, W.G & Colman, P.M,Structure of influenza virus glycoprotein antigen neuraminidase at 2.9 A Resolution, *Nature* 1983;303(5):35-40.

Baker, A.T, Varghese J.N,LaverW.G, & Colman, P.M, Three –dimensional structure of neuraminidase of subtype N9 from an avian influenza virus,*Proteins* 1982; 34(3):111-117.

Michael Adams, Thomas Pacher, Harald Greger and Rudolf Bauer ,Inhibition of Leukotriene Biosynthesis by Stilbenoids from *Stemona* Species, *J. Nat. Prod.* 2005;68(9)*:* 83-85.

Li-Gen Lin , Xin-Zhou Yang , Chun-Ping Tang, Chang-Qiang K,Antibacterial stilbenoids from the roots of *Stemona tuberose*, *Phytochemistry* , 2008;69(1) :457-463.

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