REVIEW

Computational analysis in Influenza virus

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Influenza viruses are major human pathogens accountable for respiratory diseases affecting millions of people worldwide and characterized by high morbidity and significant mortality. Influenza infections can be controlled by vaccination and antiviral drugs. However, vaccines need yearly updating and give limited protection. In addition, the currently available drugs suffer from the rapid and extensive emergence of drug resistance. All this highlights the urgent need for developing new antiviral strategies with novel mechanisms of action and with reduced drug resistance potential. Recent advances in the understanding of Influenza virus replication have discovered a number of cellular drug targets that counteract viral drug resistance. With expanded bioinformatics' knowledge on computational modeling and molecular dynamic stimulations, novel small molecule inhibitors of herbal/ayurvedic origin are being explored due to their non-toxicity and affordability. Using *in-silico* techniques the structural details and information of influenza protein have been studied to identify the potential drugs for inhibition. Further, we have discussed the various computational studies carried out on major protein/targets of Influenza which could provide new clues for a newer class of antiviral (ayurvedic) drugs. In the years to come ahead, the influenza treatment will go through major changes, with advancing our knowledge of pathogenesis as new methods becoming clinically validated.

Keywords: Influenza; Hemagglutinin; Neuraminidase; M2 protein; Ayurvedic Medicines

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Introduction

Influenza is a most important cause of severe respiratory infections foremost to unwarranted hospitalization and deaths globally; annual epidemics (e.g., A/H3N2, A/H1N1 B; due to antigenic drift/shift), pandemics (e.g., A/H1N1$_{pdm09}$; due to genetic re-assortment), and sporadic/endemic avian virus infections (e.g., A/H5N1, A/H7N9; adapted for limited human transmission) arise as a consequence of rapid, constant evolution of influenza virus [1]. The annual epidemics outcome a considerable amount of hospitalizations with an expected 3 to 5 million cases of severe disease and 300,000 to 500,000 deaths. Furthermore, during the 20th century, three major influenza pandemics have taken place

Table 1. Table of influenza databases

Influenza Databases	URL
Influenza Research Database	http:// www.fludb.org
Influenza Virus Resource	http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html
Global Initiative on Sharing Avian Influenza Data (GISAID) EpiFlu Database	platform.gisaid.org
Korea Influenza Sequence & Epitope Database (KISED)	influenza.cdc.go.kr/home/a
Influenza Virus Database (IVDB)	http://influenza.psych.ac.cn/
OpenFlu Database	http://openflu.vital-it.ch

with a total mortality of 50 -100 million people [2]. The recent appearance of the 2009 pandemic influenza A/H1N1 virus has decorated the cost of free and undoes access to influenza virus genome sequence data integrated with information about other significant virus characteristics [3].

Influenza belongs to the genus orthomyxovirus in the family of *Orthomyxoviridae*. It is single stranded RNA enveloped viruses with a helical symmetry. These are enveloped particles 80-120nm in diameter. The RNA is closely associated with the nucleoprotein (NP) to form a helical structure. The genome is segmented; with 8 RNA fragments (7 for influenza C). There are 4 antigens present, the haemagglutinin (HA), neuraminidase (NA), nucleocapsid (NA), the matrix (M) and the nucleocapsid proteins. The NP is a type-specific antigen which arises in 3 forms, A, B, and C, which provide the root for the classification of human influenza viruses. The matrix protein (M protein) surrounds the nucleocapsid and makes up 35-45% of the particle mass. Two surface glycoproteins are seen on the surface as rod-shaped projections. The haemagglutinin (HA) is made up of 2 subunits, HA1 and HA2. HA mediates the attachment of the virus to the cellular receptor. Neuraminidase molecules are present in lesser quantities in the envelope [4].

Influenza is acute respiratory diseases differentiate in its full form by the rapid onset of high fever, coryza, cough, headache, prostration, malaise, and inflammation of the upper respiratory tract and trachea. In the majority cases, the pneumonic association is not clinically well-known. Acute symptoms and fever frequently endure for 7 to 10 days. Weakness and fatigue may remain for weeks. Influenza usually takes place in winter outbreaks or epidemics (in temperate climates). There are three antiviral drugs recommended by the CDC and approved by the FDA. These are oseltamivir (brand name Tamiflu®), zanamivir (brand name Relenza®) and peramivir (brand name Rapivab®). Tamiflu® comes as a pill or liquid, and Relenza® is an inhaled powder. (Relenza should NOT be used in anyone with breathing problems, like asthma or COPD, for example.). Rapivab® is administered intravenously by a health care provider. There are no generic flu antiviral drugs [5].

Influenza databases

The computational approaches serve up to organize numerous sets of data in an ordinary framework, to highlight in these ways significant features and to enlighten associations between special aspects of the system are under study. The replica once built is used to ascertain biological regimes which are confined by the research at hand, to skill the likelihood that are test of replica, and to lead to new insights about concealed components or unexplored relations between known ones [6].

In the infectious disease research, with the commencement of high-throughput new techniques, it has become obvious that publicly open databases and bioinformatics are eventually more needed (Table 1). NIAID has developed the Bioinformatics Resource Centers (BRC) program to sustain the management and analysis of data related to human pathogenic microorganisms [7]. The Influenza Research Database (IRD) is paying attention to data related to influenza virus. IRD is distinctive in the size and intensity of the data and analysis tools provided and in its approach to data and workflow integration that aid in the development of vaccines, therapeutics, and diagnostics [3]. A number of influenza-focused web-accessible databases exist: the NCBI Influenza Virus Resource (IVR) [8], Global Initiative on Sharing Avian Influenza Data (GISAID) EpiFlu Database [9], Korea Influenza Sequence & Epitope Database (KISED) [10], the Influenza Virus Database (IVDB) [11], and the OpenFlu Database [12].

Computational studies

The multiple circulating human influenza A virus subtypes united with the perpetual genomic mutations and segment reassortment events challenge the progress of effective therapeutics. The capacities to drug-most RNAs stimulate the investigation on viral RNA targets [13]. Thus various computational techniques are now paying attention on three important targets out of the 11 known proteins in the viral life cycle: hemagglutinin (HA), neuraminidase (NA) and M2 protein. Protein mechanism of action, substrate binding specificity and drug resistance, ligand-target interactions of substrate binding to these three proteins either wild-type or mutant strains were studied. Progress was made on the novel

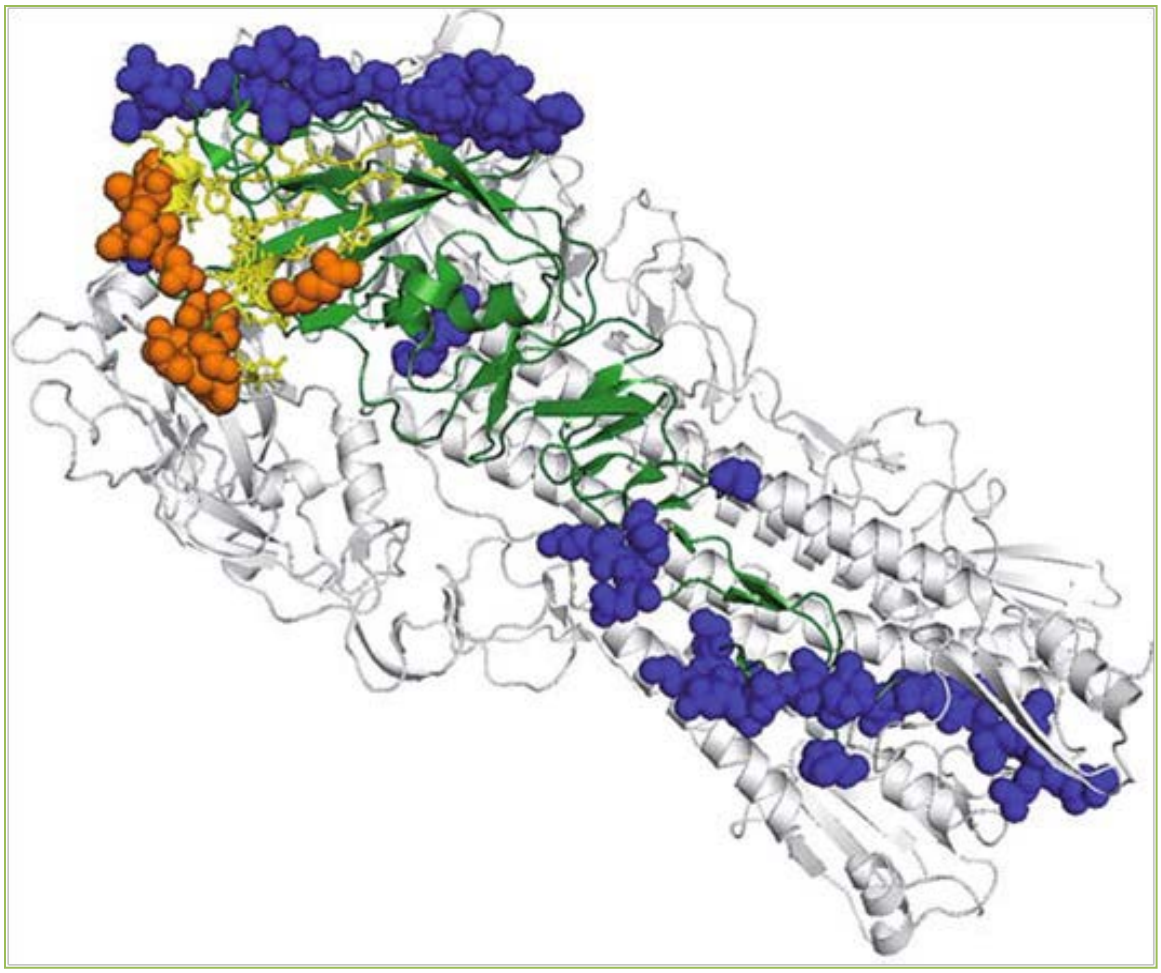

Figure 1. 3D structure of hemagglutinin with highlighted epitope predictions using chain A from the PDB entry 2IBX. (adapted from [14])

anti-influenza agents, intended specifically to combat the avian H5N1 and pandemic H1N1 viruses are introduced. An understanding of molecular inhibition and source of drug resistance, newly designed compounds is greatly useful as a rotational guide for synthetic and medical chemists to build up a new cohort of anti-influenza drugs [14].

Hemagglutinin

Hemagglutinin (HA) and neuraminidase (NA) are two chief viral envelope glycoproteins that identify sialic acid (SA) on the host cell. HA binds to sialylated host cell receptor and intervenes membrane fusion, whereas NA removes sialyl residues from the membrane of infected cells and from viral membranes to facilitate budding and let go of newly synthesized virus particles. Once the influenza virus infects the host, both HA and NA is continuously under fire by neutralizing antibodies and depending on their antigenic properties, they are further classified into 18 HA subtypes (H1 - H16 in wild waterfowl, and H17 and H18 in bats) and into 11NA subtypes (N1-N9 in wild waterfowl, and N10 and N11 in bats) [15-17].

The cell surface receptors that influenza binds to are glycolipids or glycoproteins that include terminal SA moieties [18-20]. The host receptor that influenza virus binds to contain the three common terminal saccharides SA1, galactose (Gal2) and N-acetylglucosamine (GlcNAc3) [21], and the penultimate Gal is lined to either α2,3-SA or α2,6-SA. Modulation of hemagglutinin protein will enable the H1N1 virus to bind to the sialic acid of target cell surface and thus won't be able to enter the cell cytoplasm [22]. The two disulfide-linked subunits (HA1 & HA2) are responsible for low pathogenic influenza virus (LPIV) where HA cleavage site is cleaved by trypsin-like protease enzymes and in high pathogenic influenza virus (HPIV) it is cleaved by pro-protein-processing endoproteases PC6 and furin. Structure analyses suggested that the HA cleavage site of LPIV contains a single basic amino acid residue (Q/E-X-R) while the insertion of polybasic amino acids (R-X-R/KR) leads to the HPIV (H5 and H7 subtypes of HA) [14].

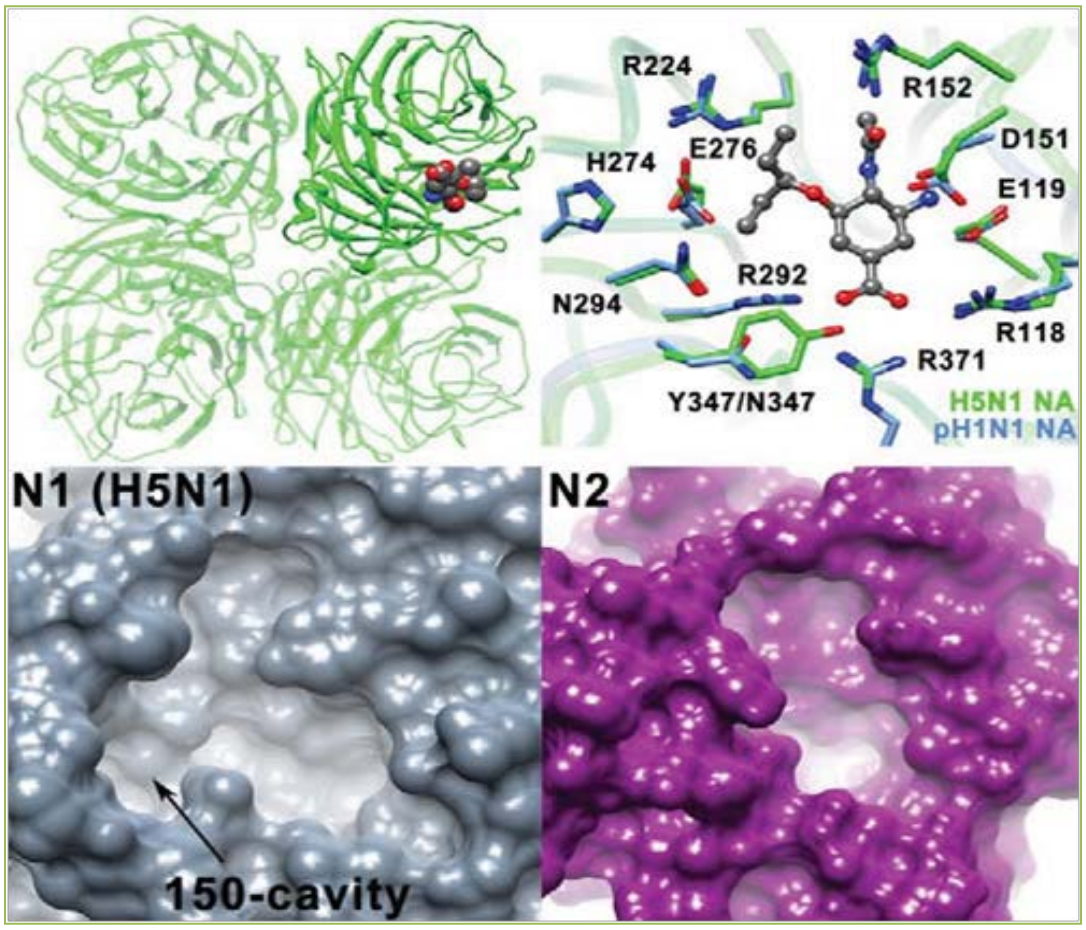

Figure 2. Tetrameric neuraminidase with drug bound. Close up of oseltamivir and its surrounding residues in NA subtype N1 of the influenza H5N1 and pH1N1 viruses. The active site cavity of NA subtypes N1 and N2. (adapted from [14])

Receptor binding preference of HA is a critical factor determining the host range specificity. Li and Wang applied molecular docking and MD simulations to identify the binding characteristics of influenza H5N1 HA with either avian or human receptors. Studies found that the SA- α -2,3-Gal and SA- α -2,6-Gal bound with HA active residues via *trans* and *cis* conformations, respectively. In the study by Iwata and colleagues, *ab initio* fragment molecular orbital method (FMO) at MP2/6-31G level was applied to examine the binding specificity of different HAs of human H1, swine H1, avian H3 and avian H5 to avian and human receptor analogs. In contrast, the avian H3 and H5 HAs exhibited more preferentially favorable to avian receptor than the human analog [14]. In all complexes, the HA binding area is composed of residues member of 190-helix and 130- and 220-loops. Among five glycan units, SA strongly interacted with HA receptor binding residues, thereby exhibiting the highest contribution to stabilize protein-glycan affinity (Figure 1). The human receptor specificity was greatly enhanced in H2, H3 and H5 subtypes in avian influenza virus when mutation occurred in two vital amino acids Q226 and G228 to L226 and S228. Besides the mutation of residues 226 and 228, another double mutation L129V and A134V of avian H5N1 HA was reported to be a potential route for H5 to adapt the human receptor specificity [23]. A novel combination of V135S and A138S was predicted to significantly increase the human receptor binding preference due to its high contribution of electrostatic interaction. Substitutions of serine at both positions 135 and 138 resulted in a conformational rearrangement in the HA-glycan binding area, therefore accommodating the stable hydrogen bonds between HA and SA-α-2,6-Gal receptor [24]. Further research showed that the *tert*-butyl hydroquinone (TBHQ) showed potent activity with *IC50* values of 5 to 10 μM against the conformational rearrangement of H3N2 [25].

QSAR studies were done for Hemagglutinin of Influenza A virus using the available synthetic drugs like Oseltamivir and Zanamivir and also natural products like Shikimic acid for studying the physicochemical/molecular properties using Molinspiration software. It was found that Amantadine, an antiviral drug which is not used in Swine flu treatment;

Shikimic acid, a natural compound show better docking energies in comparison with available synthetic drugs with this protein. We can further go for future modifications of the shikimic acid compound to get a better drug with high efficacy [22].

Progress in the discovery and development of inhibitors against the HA of the virus could bring to the hope of a successful finding new anti-influenza drugs.

Neuraminidase

Neuraminidase is responsible for cleaving the terminal sialic acid from the host receptors in the viral replication cycle. In the treatment and prophylaxis of influenza infections, NA acts as chief target for anti-influenza agents because by blocking the NA activity the new infectious virions cannot be released from the host cell The nine subtypes of NA, named N1-N9, are classified into two groups, group-1 (N1, N4, N5 and N8) and group-2 (N2, N3, N6, N7 and N9). The main discrepancy in the structural feature between the two NA groups was revealed by Russell *et al.* in 2006.[26] The highly conserved active site in all subtypes consists of the catalytic residues (R118, D151, D152, R224, E276, R292, R371 and Y406) and the framework residues (E119, R156, W178, S179, D198, I222, E227, H274, E277, N294, and E425). Up to date, the four anti-influenza drugs-zanamivir, oseltamivir, peramivir and zanamivir targeting NA are available to combat the influenza virus.

In one of the studies [27] of NA substrate specificity for H5N1 virus using SA and two trisaccharides, SA-α2, 3-Gal-Glc (3SL) and SA- α 2, 6-Gal-Glc (6SL), it was experimentally confirmed that the conformational transition was in SA while the distorted boat conformation was adopted for both trisaccharides. With comparable ligand protein binding in the three complexes via the E276, R292, Y347, R371 and Y406 residues, the SA1 units of the two trisaccharides were proposed to stronger interact with the binding pocket than the sialic acid alone. The strength of the substrate binding is in the following order: 3SL > 6SL >> sialic acid. This might be a reason that an avian N1 was experimentally found to cleave the SA-α-2,3-Gal glycoconjugates better than the SA-α-2,6-Gal [27-29].

The efficiency of Oseltamivir (OTV) Toward the Group-1 and Group-2 NA Strains was studied using MD simulations [30]. Lower drug-target interactions at the influenza NA subtype N1 was found relative to those of the N2 and N9 complexes (Figure 2). This may be due to the rotational design of commercial NA inhibitors is primarily based on the NA group-2. Therefore, designs of novel antiviral agents according to the N1 crystal structure are needed and reviewed hereafter [14]. Identification of inhibitor-binding residues is an important factor for understanding the enzyme function as well as for developing the new potent inhibitors. By using MD simulation approach the Key Binding Interactions at the Sialic Acid Site in N1, are as follows:

Avian Influenza A H5N1 Virus

The tamiphosphor and its analogs which are termed as the phosphonate congener of OTV demonstrated major inhibitory potency than OTV against the native NA strains of the H1N1 and H5N1 influenza viruses [31]. The OTV-2 compound with -CH2NH3+ substitution on the C3 position of OTV was suggested to be a drug candidate since it showed highly predicted binding affinity through an additional interaction with E119 as well as good bioavailability [32].

Pandemic Influenza A H1N1 Virus

In February 2009, the pandemic influenza A H1N1 virus (pH1N1) emerged from swine's in Mexico and rapidly spread worldwide had raised a global human health concern. This new influenza virus already contains the adamantine-resistant mutation in M2 protein. After a couple of months of the outbreak, Thanyada *et al.* [14] had modeled the complex structure of the A/California/04/2009 (H1N1) NA strain with the most important anti-influenza drug, OTV, using homology modeling and multiple MD simulations. The comparative study of OTV and ZNV in the N1 cavity of Spanish H1N1, H5N1 and pH1N1 viruses presented a relatively conserved and unique drug binding pattern among all different N1 strains [33]. More intermolecular interaction through hydrogen bonds with ZNV than OTV was detected. Also the discovery of the open N1 conformation with the 150-cavity and 430-cavity adjacent the sialic acid binding site has provided the new opportunity for drug design of anti-influenza inhibitors [26, 34].

M2 Protein Channel

The M2 protein of Influenza A virus is a tetrameric type III integral transmembrane (TM) protein whose structures and functions are revised since 1900s using different methods such as functional mutagenesis [35-37], site-directed infrared dichroism [38], solution NMR (solNMR) [39], solid-state NMR (ssNMR) [40], X-ray crystallography [41-42] and computational modeling [43-47]. It plays an essential role in viral replication by mediating the acidification and uncoating of endosomally entrapped virus [14]. The proton specific conductance of M2 is activated by low *pH*, evidenced by the channel recordings [48-50].

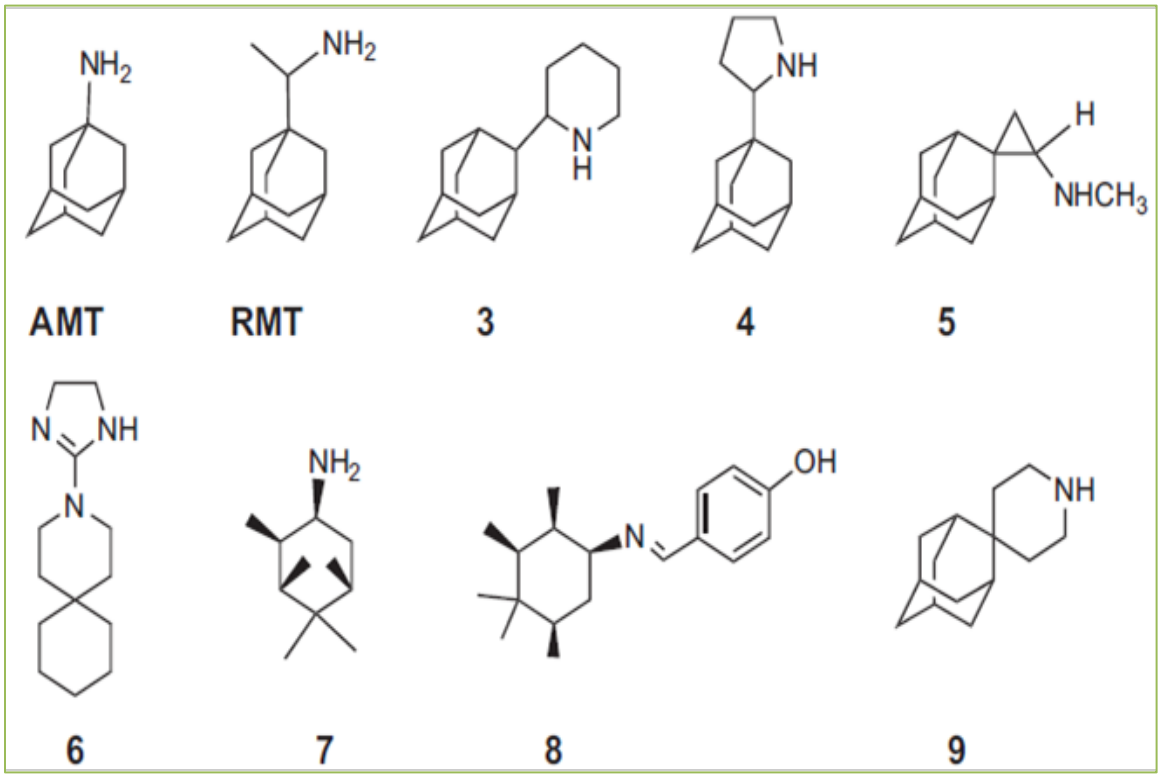

Figure 3. The current M2 drugs, amantadine (amt) and rimantadine (rmt), and the newly designed M2 inhibitors. (adapted from [14])

Molecular Dynamic Stimulation

As said earlier, different techniques used such as ssNMR, solNMR and X-ray led to different M2 structures. In the MD studies using the ssNMR structure, H37/W41 gate was almost found in closed form at low protonation state and open form at high protonation state [44,45,47] , in which the V27 is always opened. Chen *et al.* [45] and Hu *et al.* [51] reported that the highest proton permeability is the triple protonation state of H37. Leonov *et al.* [46] and Khurana *et al.* [47] performed various MD simulations. In one report by Lenov *et al* [46], the water conductance was correlated to the protonation state of the channel since it always closes at V27 in the 0H and 2H states and even at low pH in the 3H state as stated by X-ray which is the most stable one. Khurana *et al.* [47] found that at high *pH* the channel is opened at the V27 and closed at H37 gates, and in *vice versa* at the low pH. Recently, the fragment residues 21-61, 21-51 and 22-46 in *Xenopus* oocytes were studied and compared. The results showed that the assembly, drug binding, and proton translocation in both micelles and bilayer can be studied with confidence by using the short fragment M2/TM only. The mechanism of proton transport through the aqueous pore of the channel is actually not known yet. The two acceptable mechanisms are the shuttling and gating (or shutter). *Hirata group* [52] proposed the new proton transport mechanism describing the low proton conductance of the M2 channel through two histidines, one protonated and another non-protonated.

As known, the proton conductivity can be inhibited by amantadine (AMT) and rimantadine (RMT), the first effective drugs licensed for influenza virus. The drug binding sites are often predicted by the location of drug-resistance to the mutant viruses L26, V27, A30, S31 and G34 [35, 48, 53, 54]. Based on the MD simulations studied by Voth group, the 3H triply protonated H37 was found to be the most likely open state in which the AMT primarily bound at A29, therefore reducing the proton conductance by 99.8% [45] MD studies of the entirely six protonation states of M2 complexed with AMT and RMT by Intharathep *et al.* [55] suggested that the preferential position of the two drugs depends on a number of charged His37. The total number of Influenza A cases are reduced to 60%-70% by using either AMT or RMT as demonstrated by clinical studies but their continual usage lead to neuropsychiatric side effects and the widespread of drug resistance [56].

The recent outbreak of 2009 H1N1influenza A virus appears a combination of double V27A/S31N mutation, fully resistant to both AMT and RMT [57]. In one of the experiments, the use of reverse genetics to describe and generate mutations of L26F, V27A, A30T, S31N, and G34E in M2 gene of recombinant influenza A H1N1 was carried out by Abed *et.al* [58]. The experimental result supported a

high-level resistance of AMT (Figure 3) to A30T in comparison with S31N [59]. In southern California, the early 2009 H1N1 influenza virus, A/California/04/2009 (H1N1) isolated from infected patients, harbors with V28I, S31N and L43T mutations which has strong resistance to AMT, results in loss of hydrogen bonding with the M2 residues; as reported by Thanyada *et al* [14].

Thus, on the urgent need to development of new anti-influenza, the discovery of new types of M2 inhibitor for replacement of the AMT and RMT becomes a crucial issue.

Recently, Hu *et al*. [60] have reported the identification of several new hits as M2 inhibitors through the focused screening of a small primary amine library [61]. Based on non-adamantane compound **6** (Figure 3), Wang *et al* [62] have been searching for new classes of M2 inhibitors. With a structure activity relation, the spiro-piperidine compound **9** (Figure 3) was found as the most active with low *IC50* of 0.92±0.11 μM. They also suggest based on that this spiro-piperidine compound **9**(Figure 3) binds more extensive with M2 channel, thus leading to stronger inhibitory potency.

Hence, an enhanced perceptive of role of protein mechanism as well as drug-target interaction is likely to aid in the design of novel effective drug; particularly ones enable to fight the drug-resistant variants.

Computational tools for epitope vaccine design/strain selection:

Computational tools can now be used for epitope vaccine design and evaluation for viral pathogens like influenza for which empirical approaches have failed. The main objective of a rational vaccine strategy is to design novel immunogens that are capable of inducing long-term protective immunity. In practice, this requires structure-based engineering of the target neutralizing epitopes and a quantitative readout of vaccine-induced immune responses. Therefore, computational tools that can facilitate these two areas have played increasingly important roles in rational vaccine design in recent years [63].

The importance of such computational research is such that Human influenza A viruses continuously change antigenically to circumvent the immune protection evoked by vaccination or previously circulating viral strains. To maintain vaccine protection and thereby reduce the mortality and morbidity caused by infections, regular updates of the vaccine strains are required. Thus developing a data-driven framework for vaccine strain prediction facilitates the computational analysis of genetic and antigenic data and does not rely on explicit evolutionary models. Besides serological analysis, computer-guided analysis is becoming increasingly important in the interpretation of the large amounts of generated data [64].

Herbal/Ayurvedic medicines:

Ayurvedic medicine (also called *Ayurveda*) is one of the world's oldest medical systems. It originated in India more than 3,000 years ago and remains one of the country's traditional health care systems. Its concepts about health and disease promote the use of herbal compounds, special diets, and other unique health practices. The Government of India established the Ministry of AYUSH in November 2014, with a view to providing focused attention to the development of education and research in Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy system. More than 277 Essential drugs list of Ayurveda has been published by Ministry of AYUSH in March 2013 [65]. The increased global travel and rapid urbanization, epidemic outbreaks caused by emerging and reemerging viruses represent a critical threat to public health, particularly when preventive vaccines and antiviral therapies are unavailable. Examples include the recent surfacing of dengue virus, influenza virus, measles virus, severe acute respiratory syndrome (SARS) virus, and West Nile virus outbreaks [66-68]. To date, however, many viruses remain without effectual immunization and only a few antiviral drugs are licensed for clinical practice. The situation is further exacerbated by the potential advance of drug-resistant mutants, especially when using viral enzyme-specific inhibitors, which drastically hampers drug efficacy [69-72]. This calls for the urgency to explore new antivirals which are highly potent and cost-effective which helps in management of viral infections when vaccines and other standard therapies are lacking.

Despite the availability of vaccines based on predicted circulating strains, influenza viruses are known to continuously change their hemagglutinin (HA) and neuraminidase (NA) envelope proteins [73,74]. This difference renders any preexisting circulating antibody from earlier exposure or immunization ineffective at neutralizing the virus, hence making the host susceptible to infection. Furthermore, probable risks of cross-species' transmission and host adaptation of influenza viruses between animals and humans resulting in highly pathogenic strains have also raised concerns [75]. In addition to this, the extensive development of drug resistance which has been observed with earlier anti-influenza medications, specifically with the M2 ion channel blockers amantadine and rimantadine [76]. Resistant strains against the currently approved neuraminidase inhibitors (which prevent the release of mature influenza viruses) including oseltamivir and zanamivir have also already appeared [77]. Due to the drug

resistance problems, the rapid evolution of influenza viruses, and the incidence of several recent outbreaks (e.g., H5N1, H1N1, H7N9) [78], more sophisticated antiviral strategies are right away needed to prevent and control potential pandemics with emerging influenza strains. Several natural products have been examined for their effects against influenza. Standardized elderberry (*Sambucus nigra*) liquid extract exerts *in vitro* antiviral effects against Influenza A (IFA), Influenza B (IFB), as well as respiratory bacterial pathogens [79]. A licensed commercial extract from *Pelargonium sidoides* roots inhibits the entry of IFA, impairs viral hemagglutination as well as neuraminidase activity, and improves the symptoms of influenza-infected mice [80]. The aqueous extracts from dandelion (*Taraxacum officinale*) delay IFA infection and decrease its polymerase activity as well as the nucleoprotein (NP) RNA level [81]. Spirooliganone B from the roots of *Illicium oligandrum* exhibits potent anti-IFA activities [82]. A multitude of secondary plant metabolites have also been recognized as potential influenza NA inhibitors [83], and more recent ones consist of chalcones from *Glycyrrhiza inflate* [84], xanthones from *Polygala karensium* [85], and homoisoflavonoids from *Caesalpinia sappan* [86]. Additional exploration of these natural anti-influenza agents for clinical application will help widen the drug portfolio for prophylactic/therapeutic treatment of potential flu epidemics or pandemics. The phytochemicals or the secondary metabolites also play a major role in the mechanism of inhibition of viral replication. Vasicine a major alkaloid present in the leaves of *Justicia adhatoda* was found to be the most active antiviral agent against Herpes simplex viruses and Influenza viruses [87, 88].

In the light of computational biology systems in hand, majority of the research are paying attention to structure based drug design that engage in improvement of organic molecules or macromolecular scaffolds that are similar in shape and charge to potential ligand-binding pockets of the viral target protein. On the same note, various in-silico subtractive genomics and docking technology models were used and Allicin and Plumbagin were predicted as the potent multi-drug targets against the Neuraminidase, Hemagglutinin and M2 protein channel of H1N1/A/2009 [89]. Further, by in-vitro methodologies, it was confirmed that Plumbagin (*Plumbago indicia)* extracts are good candidates for anti-influenza therapy and should be used in medical treatment after further research [90]. Curcuin, a ribosome-inactivating protein, from *Jatropha curcas Linn*. (Euphorbiaceae), an herbal plant has been used in traditional folk medicine in many tropical countries, was investigated against two proteins of Pandemic Influenza H1N1/2009. Although the structural properties of curcuin are well documented in the literature, the modeling and docking studies by in-silico techniques with photoproteins are limited. With the use of current tools like Molecular operating environment (MOE) to investigate the protein interactions between Curcuin with Hemagglutinin and with Neuraminidase, it was put forward for use as powerful inhibitor against the Influenza virus [91].

Conclusion

As many reports in this field are only preliminary, additional investigation in differentiating the bioactive ingredients, defining the essential machinery, as well as evaluating the efficacy and prospective application *in vivo* is expectant in order to help build up effectual antiviral treatments. The newly designed compounds which is really useful for medical chemist to put together a new group of anti-influenza drugs is by understanding the molecular inhibition and source of drug resistance with the aid of computational studies. Further research is required to look at the option of combination therapies with other natural agents or with standard therapeutics, as a multi-target therapy may reduce the peril of drug-resistant viruses. We believe that natural products will go on to play a central role and add up to antiviral drug development

Conflicting interests

The authors have declared that no conflict of interests exist.

Abbreviations

HA: Haemagglutinin; NA: neuraminidase; NP: nucleocapsid; M: Matrix; GlcNAc3: N-acetylglucosamine; FMO: fragment molecular orbital; TBHQ: *tert*-butyl hydroquinone; OTV: Oseltamivir; AMT: amantadine; RMT: rimantadine.

References

1. Lee,N.; Ison, M.G. Diagnosis, management and outcomes of adults hospitalized with Influenza. Antivir.Ther 2012; 17:143-157.
2. Lambert LC, Fauci AS. Influenza vaccines for the future. N Engl J Med 2010; 363:2036-2044.
3. Squires B, Macken C, Garcia-Sastre A *et al*. BioHealthBase: informatics support in the elucidation of influenza virus host pathogen interactions and virulence. Nucleic Acids Res 2007; 36(Database):D497-D503.
4. Influenza Viruses: Respiratory Viruses Slide Set. Available from [http://virology-online.com/viruses/Influenza.htm accessed on August, 2016].
5. Cent. Dis. Control. Update: influenza activity: United States and worldwide, 1999-2000 season, and composition of the 2000-01 influenza vaccine. MMWR Morb. Mortal. Wkly. Rep 2000; 49:375-381.

6. Elena Zaslavsky, Fernand Hayot, and Stuart C. Sealfon. Computational Approaches to Understanding Dendritic Cell Responses to Influenza Virus Infection, Immunol Res. 2012; 54: 160-168.

7. Greene JM, Collins F, Lefkowitz EJ, Roos D, Scheuermann RH, Sobral B, *et al*. National institute of allergy and infectious diseases bioinformatics resource centers: new assets for pathogen informatics. Infect Immun 2007; 75:3212-3219.

8. Bao Y, Bolotov P, Dernovoy D, Kiryutin B, Zaslavsky L, Tatusova T, *et al*. The Influenza virus resource at the National Center for Biotechnology Information. J Virol 2008; 82:596-601.

9. Bogner, P.; Capua, I.; Cox, N.J.; Lipman, D.J.; Johnson, M.A.; *et al*. A global initiative on sharing avian flu data. Nature 2006; 442:981.

10. Seok Yang, Joo-Yeon Lee, Joon Seung Lee, Wayne P.M, Hee-Bok Oh, Chun Kang, *et al*. Influenza sequence and epitope database. Nucleic Acids Research, 2009; 37, Database: D423-D430.

11. Chang S, Zhang J, Liao X, Zhu X, Wang D, Zhu J, *et al*. Influenza Virus Database (IVDB): an integrated information resource and analysis platform for influenza virus research. Nucleic Acids Res 2007; 35:D376-D380.

12. Liechti R, Gleizes A, Kuznetsov D, Bougueleret L, Le Mercier P, Bairoch A, *et al*. Open Flu DB, a database for human and animal influenza virus Database (Oxford). 2010; 6:baq004.

13. Keng Boon Wee, Raphael Tze Chuen Lee, Jing Lin, Zacharias Aloysius Dwi Pramono, Sebastian Maurer-Stroh. Discovery of Influenza A Virus Sequence Pairs and Their Combinations for Simultaneous Heterosubtypic Targeting that Hedge against Antiviral Resistance. PLoS Comput Biol 2012 : e1004663.

14. Thanyada Rungrotmongkol, Pathumwadee Yotmanee, Nadtanet Nunthaboot and Supot Hannongbua. Computational Studies of Influenza A Virus at Three Important Targets: Hemagglutinin, Neuraminidase and M2 Protein. Current Pharmaceutical Design, 2011; 17:1720-1739.

15. Gamblin, S.J. & Skehel, J.J. Influenza Hemagglutinin and neuraminidase membrane glycoproteins. J. Biol. Chem.2010; 285, 28403-28409.

16. Tong S, Li Y, Rivailler P, Conrardy C, Castillo DA, Chen LM, et.al. A distinct lineage of Influenza A virus from bats. Proc. Natl Acad. Sci.2012; USA 109: 4269- 4274.

17. Tong S, Zhu,X, Li Y, Shi M, Zhang J, Bourgeois M et.al. New world bars harbor diverse Influenza A viruses. PLos Pathogen 2013; 9. e1003657.

18. Sauter, N.K, Bednarski MD, Wurzburg BA, Hanson JE, Whitesides GM, Skehel JJ. et.al. Hemagglutinins from two influenza virus variants bind to sialic acid derivatives with millimolar dissociation constants: a 500- MHz proton nuclear magnetic resonance study. Biochemistry1989; 28, 8388-8396.

19. Takemoto, D.K. Skehel, J.J. & Wiley, D.C. A surface plasma resonance assay for the binding of Influenza virus Hemagglutinin to its sialic acid receptor. Virology 1996; 217:452-458.

20. Gambaryan, A.S .A.B.Tuzikov, Piskarev AB, Yaminkova S.S, Lvov DK, Robertson JS. et.al. Specification of receptor binding phenotypes of Influenza virus isolates from different tools using synthetic sialyglycopolymers: non-egg adapted human H1 and H3 influenza A and influenza B viruses share a common high binding affinity for 6'-sialyl(N-acetlylactosamine).Virology 1997; 232: 245-250.

21. Eisen, M.B, Sabesan, S, Skehel, J.J. & Wiley, D.C. Binding of Influenza A virus to cell surface receptor: structures of five Hemagglutinin-sialyloligosaccharides complexes determined by X-ray crystallography. Virology 1997; 232:19-31.

22. A Bhattacharjee, P Yadav, BJ Mylliemngap, E Annipindi. Homology modeling and Docking Studies of Hemagglutinin protein of influenza A(H1N1) virus with selected ligands: A computer aided structure based drug design approach to find suitable inhibitor. Advanced Biotech 2009; 28-30.

23. Auewarakul P, Suptawiwat O, Kongchanagul A, Sangma C, Suzuki Y, Ungchusak K, *et al*. An avian influenza H5N1 virus that binds to a human-type receptor. J Virol 2007; 81: 9950-9955

24. Lee AN, Hartono YD, Sun T, Leow ML, Liu XW, Huang X *et al*. Molecular dynamics studies of human receptor molecule in hemagglutinin of 1918 and 2009 H1N1 influenza viruses. J Mol Model 2011; 17: 1635-1641.

25. Russell RJ, Kerry PS, Stevens DJ, Steinhauer DA, Martin SR, Gamblin SJ, *et al*. Structure of influenza hemagglutinin in complex with an inhibitor of membrane fusion. Proc Natl Acad Sci USA 2008; 105: 17736-17741.

26. Russell RJ, Haire LF, Stevens DJ, Collins PJ, Lin YP, Blackburn GM, *et al*. The structure of H5N1 avian influenza neuraminidase suggests new opportunities for drug design. Nature 2006; 443: 45-49.

27. Kobasa D, Kodihalli S, Luo M, Castrucci MR, Donatelli I, Suzuki Y, *et al*. Amino acid residues contributing to the substrate specificity of the influenza A virus neuraminidase. J Virol 1999; 73: 6743-6751.

28. Fran de Barros Jr J, Sales Alviano D, da Silva MH, Dutra Wigg M, Sales Alviano C, Schauer R. *et al*. Characterization of sialidase from an influenza A (H3N2) virus strain: kinetic parameters and substrate specificity. Intervirology 2003; 46: 199-206.

29. Mochalova LV, Korchagina EY, Kurova VS, Shtyria JA, Gambaryan AS, Bovin NV. Fluorescent assay for studying the substrate specificity of neuraminidase. Anal Biochem 2005; 341: 190-193.

30. Aruksakunwong O, Malaisree M, Decha P, Sompornpisut P , Parasuk V , Pianwanit S, *et al*. On the lower susceptibility of oseltamivir to influenza neuraminidase subtype N1than those in N2 and N9. Biophys J 2007; 92: 798-807.

31. Shie JJ, Fang JM, Wang SY, Tsai KC, Cheng YS, Yang AS, et.al. Synthesis of tamiflu and its phosphonate congeners possessing potent anti-influenza activity. J Am Chem Soc 2007; 129:11892-11893.

32. Park JW, Jo WH. Computational design of novel, high-affinity neuraminidase inhibitors for H5N1 avian influenza virus. Eur J Med Chem 2010; 45: 536-41.

33. Le L, Lee E, Schulten K, Truong TN. Molecular modeling of swine influenza A/H1N1, Spanish H1N1, and avian H5N1 flu N1 neuraminidases bound to Tamiflu and Relenza. PLoS curr 2011; doi:10.1371/currents.RRN1015.

34. Amaro RE, Minh DDL, Cheng LS, Lindstrom WM Jr, Olson AJ, Lin JH, *et al*. Remarkable loop flexibility in avian influenza N1 and its implications for antiviral drug design. J Am Chem Soc 2007; 129: 7764-7765.

35. Pinto LH, Dieckmann GR, Gandhi CS, Papworth CG, Braman J, Shaughnessy MA *et al*. A functionally defined model for the M2 proton channel of influenza A virus suggests a mechanism for its ion selectivity. Proc Natl Acad Sci USA 1997; 94: 11301-11306.

36. Sugrue RJ, Hay AJ. Structural characteristics of the M2 protein of influenza A viruses: evidence that it forms a tetrameric channel. Virology 1991; 180: 617-624.

37. Gandhi CS, Shuck K, Lear JD, Dieckmann GR, DeGrado WF, Lamb RA, *et al*. Cu(II) inhibition of the proton translocation machinery of the influenza A virus M2 protein. J Biol Chem 1999; 274: 5474-5482.

38. Kukol A, Adams PD, Rice LM, Brunger AT, Arkin IT. Experimentally based orientational refinement of membrane protein models: a structure for the influenza A M2 H+ channel. J Mol Biol 1999; 286: 951-962.

39. Schnell JR, Chou JJ. Structure and mechanism of the M2 proton channel of influenza A virus. Nature 2008; 451: 591-U512.

40. Kovacs FA, Denny JK, Song Z, Quine JR, Cross TA. Helix tilt of the M2 transmembrane peptide from influenza A virus: an intrinsicproperty. J Mol Biol 2000; 295: 117-125.

41. Stouffer AL, Acharya R, Salom D, Levine AS, Di Costanzo L, Soto CS, *et al*. Structural basis for the function and inhibition of an influenza virus proton channel. Nature 2008; 451: 596-U513.

42. Acharya R, Carnevale V, Fiorin G, Levine BG, Polishchuk AL, Balannik V, *et al*. Structure and mechanism of proton transport through the transmembrane tetrameric M2 protein bundle of the influenza A virus. Proc Natl Acad Sci USA 2010; 107: 15075-15080.

43. Astrahan P, Kass I, Cooper MA, Arkin IT. A novel method of resistance for influenza against a channel-blocking antiviral drug. Proteins 2004; 55: 251-257.

44. Kass I, Arkin IT. How pH opens a H+ channel: the gating mechanism of influenza a M2. Structure 2005; 13: 1789-1798.

45. Chen HN, Wu YJ, Voth GA. Proton transport behavior through the influenza a M2 channel: insights from molecular simulation. Biophys J 2007; 93: 3470-3479.

46. Khurana E, Dal Peraro M, DeVane R, Vemparala S, DeGrado WF, Klein ML. Molecular dynamics calculations suggest a conduction mechanism for the M2 proton channel from influenza A virus. Proc Natl Acad Sci USA 2009; 106: 1069-1074.

47. Leonov H, Arkin IT. Structure and dynamics of the influenza A M2 channel: a comparison of three structures. J Mol Model 2009; 15:1317-1728.

48. Pinto LH, Holsinger LJ, Lamb RA. Influenza virus M2 protein has ion channel activity. Cell 1992; 69: 517-5128.

49. Lin T-I, Schroeder C. Definitive assignment of proton selectivity and attoampere unitary current to the M2 ion channel protein of influenza A virus. J Virol 2001; 75: 3647-3656.

50. Pielak RM, Schnell JR, Chou JJ. Mechanism of drug inhibition and drug resistance of influenza A M2 channel. Proc Natl Acad Sci USA 2009; 106: 7379-7384

51. Hu J, Fu R, Nishimura K, Li Zhang, Huan-Xiang Zhou, David D *et al*. Histidines, heart of the hydrogen ion channel from influenza A virus: toward an understanding of conductance and proton selectivity. Proc Natl Acad Sci USA 2006; 103: 6865-6870.

52. Phongphanphanee S, Rungrotmongkol T, Yoshida N, Hannongbua S, Hirata F. Proton transport through the influenza A M2 channel: three-dimensional reference interaction site model study. J Am Chem Soc 2010; 132: 9782-9788.

53. Chizhmakov IV, Geraghty FM, Ogden DC, Hayhurst A, Antoniou M, Hay AJ. Selective proton permeability and pH regulation of the influenza virus M2 channel expressed in mouse erythroleukaemia cells. J Physiol 1996; 494: 329-336.

54. Wang C, Lamb RA, Pinto LH. Direct measurement of the influenza A virus M2 protein ion channel activity in mammalian cells. Virology 1994; 205: 133-140.

55. Intharathep P, Laohpongspaisan C, Rungrotmongkol T, Loisruangsin A, Malaisree M, Decha P, *et al*. How amantadine and rimantadine inhibit proton transport in the M2 protein channel. J Mol Graph Model 2008; 27: 342-348.

56. Jefferson T, Demicheli V, Pietrantonj CD, Rivetti D. Amantadine and rimantadine for influenza A in adults. Cochrane Database Syst Rev 2006; 19: CD001169.

57. Shinde V, Bridges CB, Uyeki TM, Shu B, Balish A, Xu X, *et al*. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005-2009. N Engl J Med 2009; 360: 2616-2625.

58. Abed Y, Goyette N, Boivin G. Generation and characterization of recombinant influenza A (H1N1) viruses harboring amantadine resistance mutations. Antimicrob Agents Chemother 2005; 49: 556-559.

59. Laohpongspaisan C, Rungrotmongkol T, Intharathep P, Malaisree M, Decha P, Aruksakunwong O, *et al*. Why amantadine loses its function in influenza M2 mutants: MD simulations. J Chem Inf Model 2009; 49: 847-852.

60. Hu WH, Zeng SG, Li CF, Jie YL, Li ZY, Chen L. Identification of Hits as matrix-2 protein inhibitors through the focused screening of a small primary amine library. J Med Chem 2010; 53: 3831-3834.

61. Zoidis G, Kolocouris N, Naesens L, De Clercq E. Design and synthesis of 1,2-annulated adamantane piperidines with anti-influenza virus activity. Bioorg Med Chem 2009; 17: 1534-1541.

62. Wang J, Cady SD, Balannik V, Pinto LH, DeGrado WF, Hong M. Discovery of spiro-piperidine inhibitors and their modulation of the dynamics of the M2 proton channel from influenza A virus. J Am Chem Soc 2009; 131: 8066-8076.

63. Linling He, Jiang Zhu. Computational tools for epitope vaccine design and evaluation. Curr Opin Virol 2015 April; 11: 103-112.

64. Steinbrück L, McHardy AC. 2011. Allele dynamics plots for the study of evolutionary dynamics in viral populations. Nucleic Acids Res. 39:e4.Available at: http://dx.doi.org/10.1093/nar/gkq909.

65. Essential Drug List. Department of AYUSH (Drug Control Cell) Ministry of Health and Family Welfare, Government of India March 2013.

66. Christou L. The global burden of bacterial and viral zoonotic infections. Clin Microbiol Infect 2011; 17:326-330.

67. Cascio A, Bosilkovski M, Rodriguez Morales AJ, Pappas G. The socio ecology of zoonotic infections. Clin Microbiol Infect 2011;17:336-342.

68. Grais RF, Strebel P, Mala P, Watson J, Nandy R, Gayer M. Measles vaccination in humanitarian emergencies: A review of recent practice. Confl Health 2011;5:21.

69. Sheu TG, Deyde VM, Okomo Adhiambo M, Garten RJ, Xu X, Bright RA, *et al*. Surveillance for neuraminidase inhibitor resistance among human influenza A and B viruses circulating worldwide from 2004 to 2008. Antimicrob Agents Chemother 2008; 52:3284 92.

70. Geretti AM, Armenia D, Ceccherini Silberstein F. Emerging patterns and implications of HIV 1 integrase inhibitor resistance. Curr Opin Infect Dis 2012; 25:677 686.

71. Locarnini SA, Yuen L. Molecular genesis of drug resistant and vaccine escape HBV mutants. Antivir Ther 2010; 15:451 461.

72. Wyles DL. Antiviral resistance and the future landscape of hepatitis C virus infection therapy. J Infect Dis 2013;207 Suppl 1:S33 s39

73. Yamada S, Suzuki Y, Suzuki T, Le MQ, Nidom CA, Sakai Tagawa Y, *et al*. Haemagglutinin mutations responsible for the binding of H5N1 influenza A viruses to human type receptors. Nature 2006; 444:378 382.

74. van der Vries E, Collins PJ, Vachieri SG, Xiong X, Liu J, Walker PA, *et al*. H1N1 2009 pandemic influenza virus: Resistance of the I223R neuraminidase mutant explained by kinetic and structural analysis. PLoS Pathog 2012; 8:e1002914.

75. Mak PW, Jayawardena S, Poon LL. The evolving threat of influenza viruses of animal origin and the challenges in developing appropriate diagnostics. Clin Chem 2012; 58:1527 1533.

76. 76. Fiore AE, Fry A, Shay D, Gubareva L, Bresee JS, Uyeki TM. Antiviral agents for the treatment and chemoprophylaxis of influenza recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2011; 60:1 24.

77. Samson M, Pizzorno A, Abed Y, Boivin G. Influenza virus resistance to neuraminidase inhibitors. Antiviral Res 2013;98:174 185.

78. World Health Organization: Available from: http://www.who.int. [Last accessed on Sep 18, 2013]

79. Krawitz C, Mraheil MA, Stein M, Imirzalioglu C, Domann E, Pleschka S, *et al*. Inhibitory activity of a standardized elderberry liquid extract against clinically relevant human respiratory bacterial pathogens and influenza A and B viruses. BMC Complement Altern Med 2011; 11:16.

80. Theisen LL, Muller CP. EPs (R) 7630 Umckaloabo (R), an extract from Pelargonium sidoides roots, exerts anti influenza virus activity in vitro and in vivo. Antiviral Res 2012; 94:147 156.

81. He W, Han H, Wang W, Gao B. Anti influenza virus effect of aqueous extracts from dandelion. Virol J 2011; 8:538.

82. Ma SG, Gao RM, Li YH, Jiang JD, Gong NB, Li L, *et al*. Antiviral Spirooliganones A and B with Unprecedented Skeletons from the Roots of Illicium oligandrum. Org Lett 2013; 15:4450 4453.

83. Grienke U, Schmidtke M, von Grafenstein S, Kirchmair J, Liedl KR, Rollinger JM. Influenza neuraminidase: A druggable target for natural products. Nat Prod Rep 2012;29:11 36.

84. Dao TT, Nguyen PH, Lee HS, Kim E, Park J, Lim SI, *et al*. Chalcones as novel influenza A (H1N1) neuraminidase inhibitors from Glycyrrhiza inflata. Bioorg Med Chem Lett 2011; 21:294 298.

85. Dao TT, Dang TT, Nguyen PH, Kim E, Thuong PT, Oh WK. Xanthones from Polygala karensium inhibits neuraminidases from influenza A viruses. Bioorg Med Chem Lett 2012; 22:3688 3692.

86. Jeong HJ, Kim YM, Kim JH, Kim JY, Park JY, Park SJ, *et al*. Homoisoflavonoids from Caesalpinia sappan displaying viral neuraminidases inhibition. Biol Pharm Bull 2012;35:786 790.

87. Chavan R, Chowdhary AS. In Vitro Inhibitory activity of Justicia adhatoda extracts against Influenza Virus Infection and Hemagglutination. Int. J. Pharm. Sci. Rev. Res 2014; 43: 231-236.

88. Chavan R, Gohil D, Shah V, Kothari S, Chowdhary AS, Antiviral activity of Indian medicinal plant Justicia Adhatoda against herpes simplex virus: an in-vitro study, Int J Pharm Bio Sci 2013; 4: 769-778.

89. Chavan R, Shinde P, Girkar K, Mandge R, Chowdhary AS. Identification of Potent Natural Inhibitors against H1N1/A/2009 virus using in silico Subtractive Genomics approach and Docking Technology. Int. Jrn. Phrm. Res., July-September 2014; 6:105-113

90. Chavan R, Shinde P, Girkar K, Mandge R, Chowdhary AS. Assessment of anti-influenza activity and heamagglutination inhibition of Plumbago Indica and Allium Sativum Extracts. Jrn of Pharm Res. April-June 2016; 8:105-111.

91. Chavan R, Samant L, Bapat S, Chowdhary AS. Protein Modeling and Docking Of Curcurin against Neuraminidase, Hemagglutinin Proteins of Pandemic Influenza H1N1/2009. J. Pharm. Sci. & Res 2015; 7:70-75.

92. Frishman, D., Valencia, Alfonso (Eds.). Modern Genome Annotation: The Biosapiens Network. Book. Springer Science and Business media, 2008. doi:10.1007/978-3-211-75123-7.