Chapter 1

Introduction

Influenza infection is a major threat to human life, which killed millions of life (Neumann et al., 2009). Influenza virus is a negative-strand segmented RNA virus, which belongs to the family Orthomyxoviridae. It contains three major surface membrane proteins hemagglutinin (HA) and neuraminidase (NA) and M2 proton channel (Li et al., 2011). Hemagglutinin binds to the sialic acid receptor on the cell surface and facilitates the entry of the virus (Takeda et al., 2003). The neuraminidase is responsible for cleaving of sialic acid residues on newly formed virions and plays an essential role in the release and spread of progeny virions. Neuraminidase may facilitate the early processing of influenza virus infection in lung epithelial cells (McKimm-Breschkin, 2000). The M2 proton channel is a homotetrameric protein which contains 97 residues per subunit, each of which consist of 54 residues in intracellular C-terminal domain, 19 residues in transmembrane domain and 24 residues in extracellular N-terminal domain (Wang et al., 2010). The M2 protein functions as a proton channel which is important for viral replication (Pinto et al., 2006). The influx of proton causes the pH of interior viral surface to get acidic thereby causing the disintegration of viral RNA and allows the replication of viral genetic material to the cytoplasm for replication (Balgi et al., 2013). Because of their essential roles Neuraminidase and M2 proton channel are considered to be potential target for the treatment of influenza virus infection (Wang et al., 2010).

The neuraminidase inhibitors (NAIs), oseltamivir (Tamiflu), zanamivir (Relenza) and peramivir, are representatives of the most effective class of anti-viral drugs for the treatment and prevention of influenza A and B infections (Gubareva et al., 2000). These inhibitors prevent the hydrolysis of the connections found in sialic acid, preferably N-acetylated and the adjacent carbohydrate molecule, of cellular glycoprotein (Bucher and Kilbourne, 1972). Oseltamivir is more commonly used than zanamivir in both adults and children. Resistance to oseltamivir is also much more common than resistance to
zanamivir. Although some of the discrepancy in resistance prevalence may represent differences in the ease with which drug-specific resistance mutations can occur, it seems difficult to escape the notion that, as is true for all other antimicrobial agents, increased use provides the selection pressure for resistance selection (Baum, 2009). Structural analysis predicted that aspects of the chemical structure of oseltamivir (not present in zanamivir) could facilitate the development of resistance mutations that would permit neuraminidase to function, allowing drug-resistant virus to survive and propagate (Varghese et al., 1998).

Peramivir is the another drug which has long-lasting anti-influenza activity and it binds strongly to the influenza neuraminidase and inhibits activation of neuraminidase much longer than oseltamivir or zanamivir (Sugaya, 2011). It also tested in number of toxicological studies and demonstrated good safety profile (Bantia et al., 2006). As of 2009 the U.S. Food and Drug Administration granted Emergency Use Authorization for using peramivir against the treatment of influenza infection. Yet, the emergence of some drug resistant strains of influenza decreased the efficacy of peramivir (Duan et al., 2011).

Amantadine and rimantadine have also been recommended for the treatment of influenza A infection which targets M2 proton channel (Pielak and Chou, 2011). These inhibitors block the ion channel formed by the M2 protein that covers the viral membrane and inhibit the replication of virus (Hay et al., 1985). Although these inhibitors are similar in their antiviral activities, they differ from each other in their metabolic and safety profiles. Amantadine is excreted by renal tubular excretion, whereas rimantadine is metabolized in liver. Use of amantadine will result in neurological side effects, which is not present in rimantadine (Monto, 2003). Because of its safety profiles, rimantadine is used more for the treatment of influenza A infection than amantadine for targeting M2 proton channel.

Although these drugs are efficient in treating influenza infection, drug resistance often reduces the efficacy of the drug. The principal mutations responsible for conferring high clinical resistance in neuraminidase are H274Y, N294S and R292K, whereas the
mutation responsible for drug resistance in M2 proton channel is S31N. Hence, a normal mode analysis (NMA) and molecular dynamics (MD) have been carried out alongside docking studies for the native and mutant types of NA and M2 so as to provide detailed information on the primary source of drug resistance due to these mutations.

To overcome this drug resistance problem, we have carried out virtual screening techniques to screen potential lead compounds. Literature evidences also showed that the virtual screening is a widely used method that has been shown to be successful in a variety of studies, although it also has many shortcomings (Oprea and Matter, 2004; Chen, 2008). In the past few years, many reports indicated that VS techniques proved to be effective in making qualitative predictions that discriminated active from inactive compounds (Kitchen et al., 2004). The use of experimentally derived protein structures and a hybrid computational method that combines the advantages of docking algorithms with dynamic structural information provided by normal mode analysis (NMA) has been successfully applied to a number of systems (Wei et al., 2004; Bowman et al., 2007). Several databases are available for virtual screening.

Hence, in this study, we have used Pubchem, DrugBank and TCMD databases for virtual screening techniques. Hopefully, we have proposed some useful candidates for influenza and put forward a constructive concept of designing influenza inhibitors.
Objectives of the study

i. To analyze the drug resistant mutations involved in influenza virus.

ii. To elucidate influenza drug resistance mechanism using molecular docking and molecular dynamics simulation.

iii. To analyze the drug resistant mutants in terms of drug-target interactions.

iv. To distinguish the flexibility behavior of native and mutant influenza strains in terms of RMSD and RMSF analysis.

v. To identify the stability of drug binding to the target in terms of hydrogen bond analysis.

vi. To screen potent lead compounds against both native and mutant strains of influenza virus with the help of virtual screening techniques.

vii. To evaluate the safety profiles of the screened compound using *insilico* ADME filtering and toxicity analysis.