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Drug Design for Influenza a Pandemic (H1N1) 2009 Virus Isolates from India

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ABSTRACT

The membranes of influenza A virus subtype H1N1 contain two functional surface glycoprotein's hemagglutinin (HA) and neuraminidase (NA) to initiate and spreading of infection to target cells. By inhibiting HA and NA proteins could prevent virus infection to host cells. In order to inhibit HA and NA proteins, the amino acid sequence of HA (Accession No: ACZ97508) and NA(Accession No: ACZ97471) of influenza A virus subtype H1N1 of A/Pune/NIV6196/2009(H1N1) were retrieved from influenza virus resource database. The 3D model of HA and NA proteins were built using comparative homology modeling program Modeller9v7. The computed models of HA and NA were optimized by using molecular dynamics approach through same program Modeller and eventually validated using PROCHECK program. The model of HA and NA were submitted in protein model database (PMID-ID: PM0076654 for HA and PM0076653 for NA). Homology models of hemagglutinin and neuraminidase were used for virtual screening against 131 drug like compounds using AutoDock3.0.5. These 131 compounds were screened from ZINC (a database of commercially-available compounds) on the basis of structure base similarity search of known drugs Oseltamivir and Zanamivir. The docked complexes were validated and enumerated based on docked energy. Six potent inhibitors were found and suggested as potent dual target candidate drugs with lowest docked energy. These inhibitors were designed with computational tools having greater binding affinity with HA and NA proteins than known drugs Oseltamivir and Zanamivir. The results may help to solve the drug-resistant problem and stimulate designing more effective drugs against 2009-H1N1 influenza pandemic, yet pharmacological studies have to confirm it.

Key words: Influenza A virus, hemagglutinin, neuraminidase, swine flu, molecular docking

INTRODUCTION

The Swine flu is an infectious disease of swine and human, caused by influenza A virus subtype H1N1 (Itoh *et al*., 2009). The World Health Organization figures shows that worldwide more than 214 countries have reported laboratory confirmed cases of H1N1, including over 18449 deaths (WHO, 2010). The virus was first detected in India in May 2009 (MHFW, 2009a). Since then outbreaks have been reported from many parts of the country. As of December 6, 2009, the

total number of confirmed cases in India were 19,632 with 621 deaths (MHFW, 2009b). Malaysia proposed a mathematical model to help understand the dynamics of an epidemic, to design treatment and develop control strategies such as a vaccination program or quarantine policy (Karim and Razali, 2011). The first case of Swine influenza was reported in Nigeria by the country's main health agency, the Federal Ministry of Health (FMH) on 4/11/2009 and involved an American child returning to Lagos from holidaying abroad (Sar *et al*., 2010).

Swine influenza A virus belong to the viral family of Orthomyxoviridae. They are RNA viruses with a segmented genome that is comprised of eight negative-sense, single-stranded RNA segments. These eight segments encode eleven proteins (Brockwell-Staats *et al*., 2009) in which two are surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Hemagglutinin has 16 subtypes (H1, H2, H3, ..., H16) and neuraminidase has 9 subtypes (N1, N2, N3, ..., N9) and this novel virus consists of subtype H1 and N1(Mukhtar *et al*., 2007; Shirvan *et al*., 2007). HA binds with sialic acid located on the surface of the targeted host cell to initiate virus infection and sialic acid was removed from virus by NA (Chen *et al*., 2010). By the above two steps process, HA and NA improve virus releasing and the spread of infection to new cells, respectively (Raymond and Leach, 2007; Takabatake *et al*., 2007). By blocking hemagglutinin or neuraminidase could prevent virus from invading into host cells (Russell *et al*., 2006; Shimbo *et al*., 2007). Both zanamivir (Relenza) and oseltamivir (Tamiflu) are neuraminidase inhibitors (Collins *et al*., 2008; Ho *et al*., 2007). All the Indian isolates possessed residue H274 (position 275 in NA numbering) a known marker for sensitivity to the neuraminidase inhibitor, Oseltamivir (Potdar *et al*., 2010). Hence, a new drug is required against this epidemic. In this study, Homology models were built for hemagglutinin (Accession No: ACZ97508) and neuraminidase (Accession No:ACZ97471) proteins of influenza A virus subtype H1N1 of A/Pune/NIV6196/2009(H1N1) isolated from a patient of Pune, India on August 16, 2009 (Potdar *et al*., 2010). Reliability of models was checked by Ramachandran plot. 131 compounds were screened from ZINC database (Irwin and Shoichet, 2005) using the criteria drug like compounds having structural similarity greater than 80% with existing inhibitors Oseltamivir and Zanamivir of NA proteins. These screened compounds are docked with homology model of HA and NA, respectively. The aim was to figure out potent candidates for HA and NA proteins for the 2009 out break of influenza A virus sub-type H1N1.

MATERIALS AND METHODS

Retrieval of the target protein sequences and alignment with template sequences: The amino acid sequences of hemagglutinin (ACZ97508) and neuraminidase (ACZ97471) of H1N1 virus were retrieved from NCBI influenza virus resource (Bao *et al*., 2008). The templates of HA and NA were downloaded from protein DataBank (www.rcsb.org/pdb) with PDB ID 2WR1 and 3B7E, respectively. The sequence alignment of targets with corresponding templates was performed by using dynamics programming based align2D module in Modeller (Sali *et al*., 1995).

Protein homology modeling: Homology model of HA and NA proteins were constructed using program Modeller9v7. Modeller is simply an implementation of automated approach to comparative modeling by satisfaction of the spatial restraints. After aligning queries HA and NA with templates 2WR1-A and 3B7E-A using align2D script were used as input in Modeller program and five comparative models were generated for each target, respectively. The model of HA and NA were validated with the help of Modeller objective function and DOPE score, which are the statistical parameter for the assessment of model using the standard Modeller energy function. The validated HA and NA models were chosen for further studies and refinement.

Model optimization and evaluation: The newly built homology models often produce unfavorable atomic distances, bond angles, van der Waals radius overlapping and undesirable torsion angles. Therefore, it was essential to minimize the energy to regularize local bond and angle geometry as well as to relax close contacts in geometric chain. Models of HA and NA proteins were optimized with the Variable Target Function Method (VTFM) with Conjugate Gradients (CG). Further it was refined by using the Molecular Dynamics (MD) with Simulated Annealing (SA) method (Sali and Blundell, 1993) in Modeller program. Among the above models, the most acceptable model was finalized by Ramachandran plot, which provides the residue position in particular segments based on phi (φ) and psi (ψ) angles between N-C_α and C_α-C atoms of residue. After the optimization procedure, the stereochemical qualities of the models are checked by PROCHECK (Laskowski *et al.*, 1993).

Binding site analysis: The ligand binding site of HA and NA proteins were predicted using Q-SiteFinder program (Laurie and Jackson, 2005), which is an energy-based sites. It uses the interaction energy between the protein and a simple van der Waals probe to locate energetically favorable binding sites.

ZINC database screening: ZINC database (Irwin and Shoichet, 2005) contains over 13 million commercially available compounds in ready-to-dock, 3D formats for structure based virtual screening. ZINC database was screened using the criteria drug like (xlogP = 5, Rotatable bonds = 8, H-Acceptors = 10, Polar surface area = 150 and 150 = Molecular weight = 500) compounds having similarity value from 80 to 99% with existing antiflu drugs Zanamivir and Oseltamivir 3D structures. A total of 131 (77 similar to Oseltamivir+54 similar to Zanamivir) compounds were screened using the above criteria for docking studies.

Virtual screening: Virtual screening of the entire 131 compounds screened against HA and NA model structures were done using molecular docking program AutoDock 3.0.5 (Goodsell and Olson, 1990; Morris *et al.*, 1998). Gasteiger charges are added to the ligand and maximum 6 numbers of active torsions are given to the lead compounds using AutoDock Tool (http://autodock.scripps.edu/resources/adt). The Kollman charges and the solvation term were then added to the modeled protein structure using the AutoDock Tool. A grid-box was generated that was large enough to cover the entire protein catalytic site and accommodate ligands to move freely. Lamarckian genetic search algorithm was employed and thirty search attempts (ga_run parameter) were performed for each ligand with a population size of 150. Other docking parameters were set to the software's default values. After docking, the ligands were ranked according to their docked energy as implemented in the AutoDock program.

RESULTS AND DISCUSSION

Homology modeling of HA protein: The sequence alignment of the query HA sequence (ACZ97508) of 2009-H1N1 virus and template HA (2WR1-A) of 1957 Asian influenza virus (Liu *et al.*, 2009) was shown in Fig. 1. The query HA sequence of 2009-H1N1 virus was consisting of 566 residues, however, the structure of template HA protein 2WR1-A was a segment containing 509 residues. Query is modeled from 18 to 513 residues. The sequence identity and similarity were 64.49 and 80%, respectively. The result of alignment was employed to build new homology model. Reliability of new homology model for HA was identified by Ramachandran plot. After the optimization and energy minimization process, the best model was selected among five 3D models generated for HA protein on the basis of Modeller scores. Energy minimization of 3D structure is

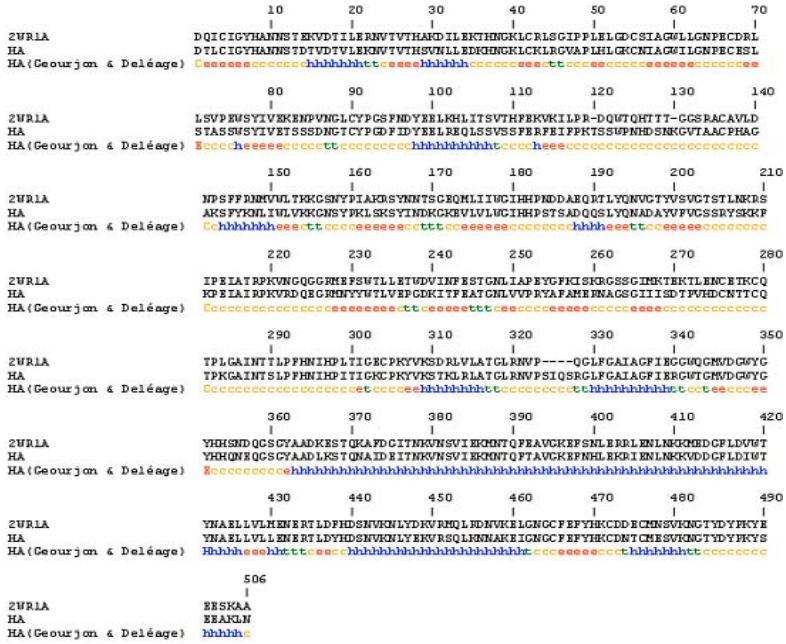

Fig. 1: The sequence alignment of the query HA sequence (ACZ97508) of influenza A virus and the template HA (2WR1) of 1918-H1N1 virus. Secondary structures of the query HA protein was predicted using program SOPMA [26] at ExPASy server

vital for providing the maximum stability to the protein. Ramachandran plot drawn through PROCHECK (Laskowski *et al.*, 1993) program validated the model with 90.7% of the total residues in most favoured region and residues in additional allowed regions was 7.1 and 1.6% in the generously allowed region (Fig. 3a). This stipulates that protein backbone dihedral angles phi(ψ) and psi (φ) occupied reasonably accurate positions in the selected 3D model. Only three residues, ALA139, GLN193 and TYR361 were located in the disallowed region, which constituted 0.7% of the total protein. Further, it was not a part of the binding site under investigation. Model of HA protein (PMID-ID: PM0076654) was submitted in protein model database (Castrignano *et al.*, 2006).

Homology modeling of NA protein: The sequence alignment of the query NA sequence (ACZ97471) of 2009-H1N1 virus and template NA (3B7E-A) of 1918-H1N1 virus (Xu *et al.*, 2008) was shown in Fig. 2. The query NA sequence of 2009-H1N1 virus was consisting of 469 residues, however, the structure of template NA protein 3B7E -A is a segment containing 385 residues. Query is modeled from 83 to 467 residues. The sequence identity and similarity were 88.83 and 96%, respectively. The result of alignment was employed to build new homology model. Reliability of new homology model for NA was identified by Ramachandran plot. 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. Ramachandran plot drawn through PROCHECK (Laskowski *et al.*, 1993) program validated the model with 87.3% of the total residues in most favoured region and residues in additional allowed regions was 11.5 and 1.2% in the generously allowed region (Fig. 3b). Model of NA protein (PMID-ID: PM0076653) was submitted in protein model database (Castrignano *et al.*, 2006).

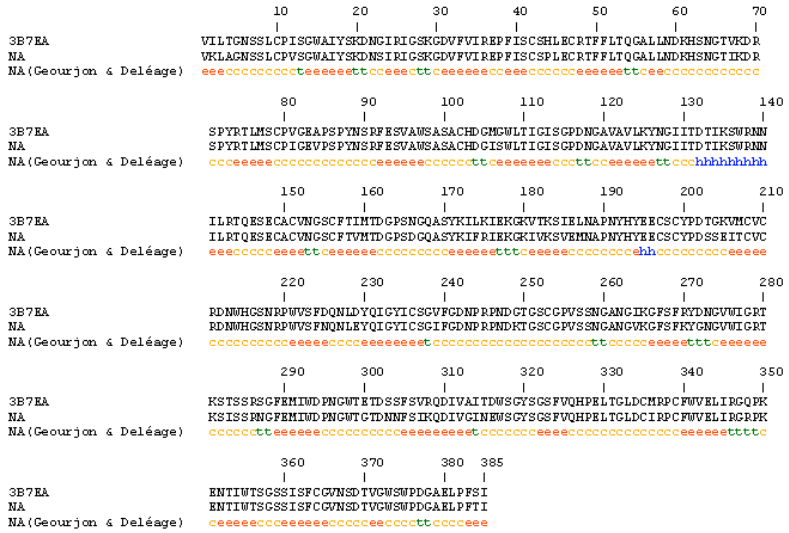

Fig. 2: The sequence alignment of the query NA sequence (ACZ97471) of influenza A virus and the template HA (3B7E) of 1918-H1N1 virus. Secondary structures of the query NA protein was predicted using program SOPMA [26] at ExPASy server

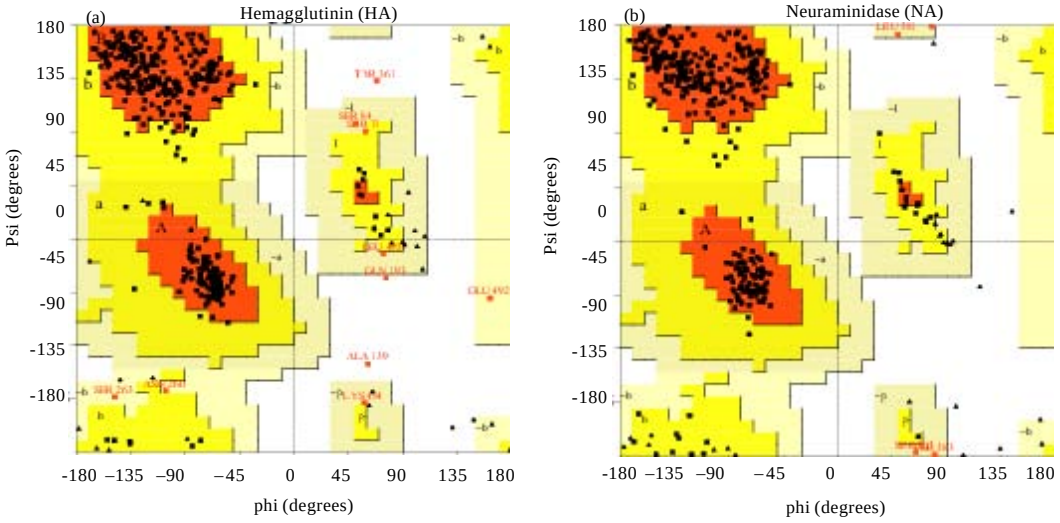

Fig. 3(a-b): (a) Ramachandran plot of modeled HA protein of influenza A virus subtype H1N1 and (b) Ramachandran plot of modeled NA protein of influenza A virus subtype H1N1

Binding site analysis

Binding site analysis of HA protein: The result of Q-SiteFinder shows that predicted binding site cavity volume of modeled HA protein was 409 cubic angstroms and the coordinates of the binding-box around predicted site had min. cords. (10, -2, 4) and max (28, 19, 25). Binding site of HA was constituted by amino acid residues HIS293, HIS296, PRO297, THR299, GLY301, LYS302,

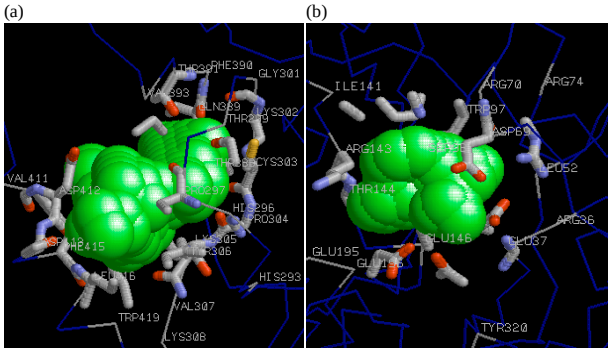

Fig. 4(a-b): (a) The 3D structure of homology model HA with predicted ligand binding site. The predicted binding site surface is shown in green and residues in binding region are shown in stick and ball drawing, (b) The 3D structure of homology model NA with predicted ligand binding site. The predicted binding site surface is shown in green and residues in binding region are shown in stick and ball drawing

CYS303, PRO304, LYS305, TYR306, VAL307, LYS308, THR388, GLN389, PHE390, THR391, VAL393, VAL411, ASP412, ASP413, PHE415, LEU416 and TRP419 shown in Fig. 4a.

Binding site analysis of NA protein: The result of Q-SiteFinder shows that predicted binding site cavity volume of modeled NA protein was 216 cubic angstroms and the coordinates of the binding-box around predicted site had min. cords. (-34, 6,-30) and max (-17, 22,-13). Binding site of NA was constituted by amino acid residues ARG36, GLU37, LEU52, ASP69, ARG70, ARG74, TRP97, SER98, ILE141, ARG143, THR144, GLU146, GLU195, GLU196, and TYR320 shown in Fig. 4b. The confirmed drug-resistant mutation H274Y is outside the active region. This mutation blocks the bioactivity of Oseltamivir drug by a complex allosteric inhibition mechanism (Collins *et al.*, 2008).

The results of virtual screening: Docking studies predicted the interaction of ligands with protein and residues involved in this complex. For such interaction studies, the most important requirement was the proper orientation and conformation of ligand which fitted to the enzyme binding site appropriately and formed protein-ligand complex. Therefore, optimal interactions and the best AutoDock score were used as criteria to interpret the best conformation among the 30 conformations, generated by AutoDock program. All the 131 (77 similar to Oseltamivir+54 similar to Zanamivir) were docked into modeled structures of HA and NA, respectively. The docking results of 77 compounds and 1 known drug Oseltamivir with HA and NA models were shown in Table 1. Among the above docked compounds ZINC03929508 and ZINC36451498 had the lowest docking energy with both HA and NA, respectively. In Table 2, the docking results of 54 compounds and Zanamivir with HA and NA models were shown. Compounds ZINC02043006, ZINC34466451, ZINC34532900 and ZINC39158384 had lowest energy with both HA and NA models than other docked compounds.

On screening the docked complex results on the basis of docking energy, it was predicted that there were six drug candidates (Fig. 5), which inhibits both HA and NA structures. These

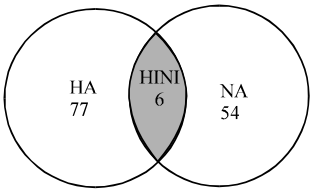

Fig. 5: The screening results of HA and NA by docking study, There are 131 compounds listed in HA and NA docking results, respectively, There are six compounds overlapped in the set-theoretic intersection

Table 1: The docking results of the 77 compounds and 1 known drng with HA and NA model structures

								Docked energy (kcal mol^{-1})		Ref RMS	
Name	MWT	xlogP	HBD	HBA	psa	change	rb	HA	NA	HA	NA
Oseltamivir	312.404	1.10	2	5	90.6	0	8	-2.61	-3.95	34.13	24.78
ZINC11592802	313.418	0.85	4	6	92	1	8	-16.23	-16.99	23.63	32.41
ZINC03874568	313.418	0.85	4	6	92	1	8	-16.10	-15.81	27.00	32.77
ZINC03874569	313.418	0.85	4	6	92	1	8	-15.96	-17.74	26.09	34.22
ZINC03874570	313.418	0.85	4	6	92	1	8	-15.64	-16.57	27.97	33.93
ZINC03874571	313.418	0.85	4	6	92	1	8	-15.03	-16.95	26.43	34.25
ZINC03929508	313.418	0.85	4	6	92	1	8	-16.65	-17.45	27.69	32.97
ZINC36451498	299.391	0.48	4	6	92	1	7	-15.96	-17.75	26.98	33.14
ZINC04134497	298.383	0.41	4	6	106	0	6	-11.25	-10.72	27.02	34.39
ZINC06777828	284.356	-0.14	4	6	106	0	6	-9.28	-9.56	23.41	32.42
ZINC06777829	284.356	-0.14	4	6	106	0	6	-11.19	-8.85	27.50	32.75
ZINC06777830	284.356	-0.14	4	6	106	0	6	-10.13	9.21	20.85	37.65
ZINC34817612	270.329	-0.64	4	6	106	0	5	-9.96	-10.07	25.19	37.05
ZINC03929509	284.356	-0.14	4	6	106	0	6	-11.21	-10.96	22.68	35.06
ZINC04134486	270.329	-0.64	4	6	106	0	5	-10.19	-10.71	25.10	36.40
ZINC34083570	270.329	-0.64	4	6	106	0	5	-10.27	-10.03	24.42	38.05
ZINC34083568	270.329	-0.64	4	6	106	0	5	-8.44	-10.09	27.07	33.34
ZINC06411782	270.329	-0.64	4	6	106	0	5	-10.45	-10.71	22.71	34.27
ZINC06777826	284.356	-0.14	4	6	106	0	6	-8.53	-9.24	22.18	34.08
ZINC14944898	282.340	-0.48	4	6	106	0	4	-10.97	-10.86	23.91	35.01
ZINC34083579	296.367	0.02	4	6	106	0	4	-11.11	-10.49	25.52	35.75
ZINC12404506	296.367	0.02	4	6	106	0	4	-11.26	-10.82	25.06	36.63
ZINC04134490	296.367	-0.10	4	6	106	0	7	-11.42	-11.27	22.50	37.33
ZINC04134489	296.367	-0.10	4	6	106	0	7	-11.36	-10.94	27.52	30.47
ZINC40865651	282.340	-0.37	4	6	106	0	6	-11.16	-10.97	21.15	39.15
ZINC34083576	298.383	0.56	4	6	106	0	8	-11.19	-10.70	28.18	34.01
ZINC04134488	298.383	0.56	4	6	106	0	8	-12.11	-10.97	21.16	36.57
ZINC04134483	256.302	-1.01	4	6	106	0	5	-10.38	-10.24	27.27	33.61
ZINC04134484	270.329	-0.45	4	6	106	0	6	-11.17	-9.99	22.27	35.94
ZINC34083573	256.302	-1.01	4	6	106	0	5	-10.12	-9.94	24.46	38.66
ZINC04134487	284.356	0.06	4	6	106	0	7	-11.94	-10.68	22.07	36.8
ZINC14944926	300.355	-1.38	5	7	126	0	7	-10.66	-10.70	21.74	36.78
ZINC14944928	300.355	-1.38	5	7	126	0	7	-10.0	-10.36	22.48	35.82
ZINC14944900	270.329	-0.17	4	6	106	0	6	-11.11	-10.83	21.46	38.25
ZINC04134482	242.275	-1.51	4	6	106	0	4	-9.86	-9.57	25.88	36.19

Table 1: Continue

								Docked energy (kcal mol^{-1})		Ref RMS	
Name	MWT	xlogP	HBD	HBA	psa	change	rb	HA	NA	HA	NA
ZINC14944922	313.330	-1.50	4	8	146	-1	7	-6.23	-4.09	21.14	35.98
ZINC14944924	313.330	-1.50	4	8	146	-1	7	-6.57	-4.63	21.27	36.69
ZINC14944897	254.286	-1.24	4	6	106	0	5	-10.27	-10.27	27.67	33.63
ZINC13780043	366.502	1.99	4	6	106	0	8	-12.82	-12.88	26.72	33.47
ZINC13780042	352.475	1.72	4	6	106	0	7	-12.80	-11.17	22.76	36.24
ZINC04134485	270.329	-0.76	4	6	106	0	5	-9.53	-10.10	26.76	33.09
ZINC35963009	243.283	-1.51	5	6	103	1	4	-15.19	-16.36	27.82	36.77
ZINC03833967	298.383	0.34	4	6	106	0	6	-9.95	-11.27	20.89	37.94
ZINC14944899	310.272	-0.12	4	6	106	0	6	-10.98	-10.23	27.84	33.39
ZINC04134481	228.248	-1.88	4	6	106	0	3	-9.40	-8.94	24.41	34.96
ZINC14944896	310.272	-0.69	4	6	106	0	6	-10.86	-10.03	26.81	34.41
ZINC14944902	283.328	-0.49	4	7	116	0	7	-4.79	-2.29	26.25	32.90
ZINC32062406	268.333	1.27	1	5	78	-1	6	-3.34	-1.44	23.86	36.34
ZINC03833965	360.454	1.30	4	6	106	0	8	-12.46	-12.81	25.34	31.70
ZINC34083583	360.454	1.30	4	6	106	0	8	-11.75	-10.57	21.82	36.42
ZINC08552722	360.454	1.30	4	6	106	0	8	-11.50	-12.15	24.88	31.05
ZINC38392952	253.342	2.48	1	4	57	0	7	-14.49	-16.16	28.14	30.40
ZINC38340093	253.342	2.48	1	4	57	0	7	-15.35	-15.89	27.80	33.90
ZINC03833966	408.498	2.23	4	6	106	0	8	-10.68	-11.88	23.11	36.74
ZINC03833964	304.346	-0.29	4	6	106	0	5	-12.46	-10.66	25.34	34.16
ZINC14944917	318.801	0.19	4	6	106	0	6	-11.40	-10.81	26.88	32.34
ZINC13780047	312.370	-0.66	6	8	142	0	7	-10.87	-10.27	22.14	37.88
ZINC13780049	326.397	-0.15	6	8	142	0	8	-8.78	-9.48	25.15	32.43
ZINC13780048	312.370	-0.66	6	8	142	0	7	-10.56	-10.06	22.73	38.56
ZINC40863125	346.427	0.79	4	6	106	0	7	-11.46	-12.22	21.6	38.78
ZINC13780050	408.498	1.71	4	6	106	0	8	-9.62	-11.07	20.14	35.81
ZINC04134495	312.370	-0.79	6	8	144	0	6	-10.15	-11.02	23.53	34.11
ZINC04134496	312.370	-0.79	6	8	144	0	6	-10.56	-9.34	22.04	36.41
ZINC38393066	256.278	-0.65	2	6	99	-1	4	-3.01	-0.92	26.56	32.62
ZINC13780045	298.343	-1.02	6	8	142	0	7	-10.66	-10.34	22.23	39.68
ZINC13780046	312.370	-0.46	6	8	142	0	8	10.01	-10.57	26.41	34.74
ZINC04134493	298.343	-1.15	6	8	144	0	6	-10.40	-10.52	22.25	36.46
ZINC34031776	298.343	-1.15	6	8	144	0	6	-11.27	-10.42	25.71	36.99
ZINC34031775	298.343	-1.15	6	8	144	0	6	-10.13	-10.95	24.56	34.21
ZINC34031774	298.343	-1.15	6	8	144	0	6	-10.00	-9.48	21.77	33.89
ZINC34031773	298.343	-1.15	6	8	144	0	6	-10.37	-9.98	22.73	35.16
ZINC04134494	312.370	-0.59	6	8	144	0	7	-10.62	-10.99	26.87	33.34
ZINC04134498	302.346	-0.11	4	6	106	0	6	-11.34	-11.05	26.95	32.39
ZINC14944895	258.274	-1.91	4	7	115	0	5	-9.37	-9.85	23.09	37.29
ZINC13860569	312.342	0.19	1	7	105	-1	7	-2.81	-0.24	24.53	32.49
ZINC38393596	281.352	1.62	1	5	65	0	6	-9.95	-8.41	25.87	31.36
ZINC14944903	312.370	-0.77	5	8	130	0	7	-9.55	-9.81	20.10	35.88
ZINC03833958	214.221	-2.5	5	6	117	0	2	-8.92	-8.34	26.57	34.75

MWT: Molecular weight, HBD: Number of hydrogen bond donors, HBA: Number of hydrogen bond acceptors; psa: Polar surface area, rb: Rotatable bonds, HA: Hemagglutinin, NA: Neuraminidase, Ref RMS: Ref root mean square deviation

Table 2: The Docking results of the 54 compounds with HA and NA model structures

								Docked energy (kcal mol^{-1})		Ref RMS	
ZINC code	MWT	xlogP	HBD	HBA	psa	charge	rb	HA	NA	HA	NA
Zanamivir	332.309	-3.2	7	10	201	0	6	-14.60	-16.77	24.19	31.08
ZINC34126688	302.287	-3.21	8	10	185	0	5	-10.00	-9.84	26.92	36.54
ZINC13443807	316.314	-2.81	8	10	183	0	7	-10.48	-9.53	24.51	33.53
ZINC13487809	326.353	-0.93	6	9	151	0	5	-11.32	10.56	28.06	27.76
ZINC13443835	342.396	-0.08	6	9	151	0	9	-9.86	10.67	20.63	36.79
ZINC13443833	328.369	-0.58	6	9	151	0	8	-9.48	10.49	25.08	36.41
ZINC34124979	272.261	-2.57	7	9	165	0	4	-9.07	-9.60	26.76	37.80
ZINC13604075	290.272	-3.50	7	9	167	0	5	-9.63	-8.86	26.65	31.43
ZINC03870992	290.272	-3.50	7	9	166	0	5	-10.14	-9.39	22.69	35.17
ZINC03870991	290.272	-3.50	7	9	166	0	5	-10.22	-9.36	25.07	33.60
ZINC02043007	290.272	-3.50	7	9	166	0	5	-9.71	-9.35	23.87	34.55
ZINC12503458	290.272	-3.50	7	9	167	0	5	-9.24	-9.37	23.75	35.07
ZINC12503456	290.272	-3.50	7	9	167	0	5	-9.40	-10.47	21.75	34.75
ZINC12503454	290.272	-3.50	7	9	167	0	5	-9.05	-9.99	22.92	35.35
ZINC12503452	290.272	-3.50	7	9	167	0	5	-9.89	-9.90	24.07	34.02
ZINC03590792	290.272	-3.50	7	9	166	0	5	-8.98	-9.46	25.43	34.43
ZINC40747111	290.272	-3.50	7	9	167	0	5	-9.77	-9.01	26.31	33.19
ZINC40747113	290.272	-3.50	7	9	167	0	5	-10.17	-9.53	26.80	31.96
ZINC05884083	290.272	-3.50	7	9	166	0	5	-9.43	-10.17	22.88	35.55
ZINC39156769	304.299	-2.66	7	9	167	0	6	-9.52	-10.30	27.33	35.76
ZINC02043006	290.296	-1.22	9	9	174	1	5	-15.97	-18.00	23.93	34.96
ZINC05884077	290.296	-1.22	9	9	174	1	5	-8.91	-17.56	23.74	35.55
ZINC39086001	304.299	-3.25	6	9	158	0	5	-9.22	-10.51	25.57	34.09
ZINC39176066	316.310	-2.89	6	9	158	0	5	-10.80	-10.91	23.68	38.82
ZINC34369778	331.301	-3.23	5	10	168	-1	6	-3.90	-1.83	26.33	32.68
ZINC34369779	331.301	-3.23	5	10	168	-1	6	-4.16	-1.98	25.73	34.71
ZINC34369777	331.301	-3.23	5	10	168	-1	6	-3.69	-1.95	26.03	33.19
ZINC38437817	260.246	-3.07	6	8	147	0	4	-3.43	-9.51	26.71	33.64
ZINC34466451	318.326	-2.04	6	9	151	0	7	-15.17	-17.31	25.49	37.55
ZINC32062404	312.326	-1.65	6	9	162	0	5	-10.29	-10.49	22.22	35.71
ZINC34532900	318.326	-2.64	5	9	143	0	6	-15.10	-17.74	25.68	37.11
ZINC39158384	330.337	-2.27	5	9	143	0	6	-16.02	-18.09	26.21	38.29
ZINC39130796	316.310	-2.67	7	9	167	0	6	-9.08	-10.07	27.2	35.39
ZINC38680321	270.245	-2.12	6	9	162	0	4	-8.89	-9.27	26.05	36.35
ZINC34228698	373.382	-1.61	5	10	168	-1	8	-4.77	-2.65	26.89	34.58
ZINC34228697	373.382	-1.61	5	10	168	-1	8	-4.98	-1.02	27.29	33.59
ZINC34465931	315.282	-2.82	6	10	180	0	6	-10.29	-8.58	26.80	36.64
ZINC38979910	276.245	-3.48	7	9	167	0	5	-9.25	-9.49	22.50	37.66
ZINC39005741	330.337	-2.05	6	9	151	0	7	-15.75	-15.98	26.76	37.20
ZINC38957373	290.272	-2.86	6	9	151	0	6	-15.12	-16.19	26.19	38.36
ZINC13778721	327.341	-1.82	7	10	171	0	7	-10.28	-11.53	25.16	36.10
ZINC34817410	327.341	-1.95	7	10	174	0	6	-11.85	-10.86	24.70	37.27
ZINC13778742	369.422	-0.70	6	10	162	0	9	-8.40	-11.55	21.39	36.82
ZINC28091013	341.368	-1.71	6	10	165	0	6	-10.10	-9.92	21.05	35.94
ZINC33836689	369.422	-0.83	6	10	165	0	8	-12.22	-9.97	25.07	35.27
ZINC13778743	341.368	-1.71	6	10	162	0	7	-10.38	-10.38	25.98	34.39
ZINC06380100	341.368	-1.71	6	10	164	0	6	-11.11	-11.11	21.97	36.58

Table 2: Continue

								Docked energy (kcal mol^{-1})		Ref RMS	
ZINC code	MWT	xlogP	HBD	HBA	psa	charge	rb	HA	NA	HA	NA
ZINC06778876	341.368	-1.71	6	10	164	0	6	-11.04	-10.29	23.42	31.97
ZINC06778874	341.368	-1.71	6	10	164	0	6	-11.02	-10.79	21.67	34.19
ZINC33360168	341.368	-1.71	6	10	165	0	6	-11.94	-10.13	23.67	34.24
ZINC26739500	299.287	-2.83	7	10	174	0	4	-10.04	-10.47	21.63	35.12
ZINC13778726	313.314	-2.90	6	10	162	0	5	-10.51	-9.99	23.48	34.91
ZINC34817412	313.314	-3.03	6	10	165	0	4	-11.63	-8.94	25.35	33.84
ZINC38438778	274.249	-2.09	4	8	139	-1	5	-3.78	-1.75	26.80	34.91
ZINC33977465	302.259	-2.40	5	9	159	-1	6	-3.13	-0.94	27.05	34.63

MWT: Molecular weight, HBD: Number of hydrogen bond donors, HBA: Number of hydrogen bond acceptors, psa: Polar surface area, rb: Rotatable bonds, HA: Hemagglutinin, NA: Neuraminidase, Ref RMS: Ref root mean square deviation

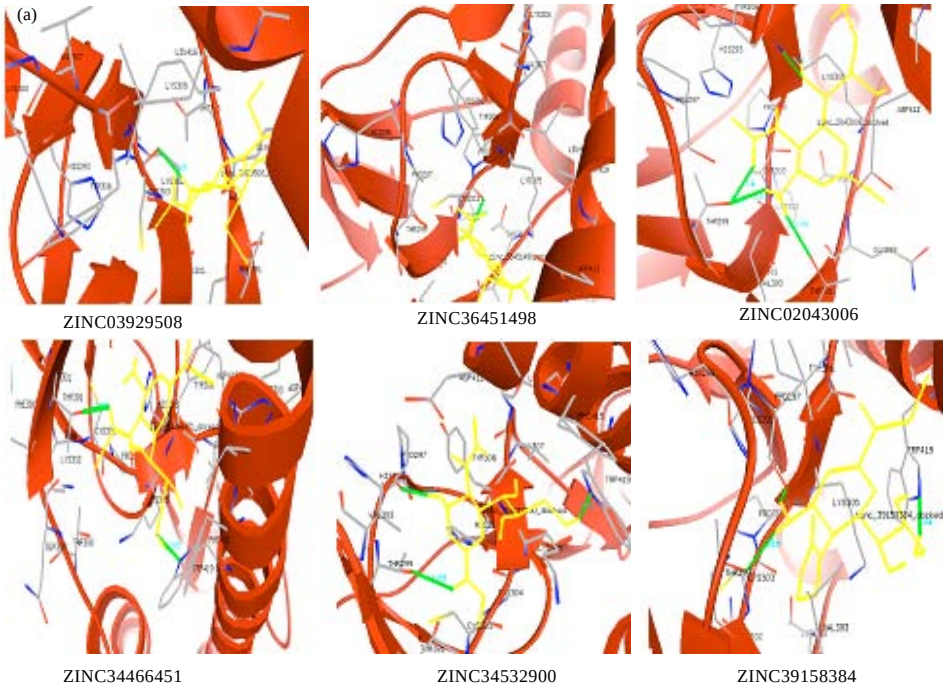

Fig. 6(a): The docking poses of the six candidates in HA

compounds had lower docked energy, even lower than the standard controls, Oseltamivir and Zanamivir. In fact Oseltamivir and Zanamivir were commonly used as inhibitors for NA; drugs for previous H1N1.

Docking poses of the best conformation of six compounds ZINC03929508, ZINC36451498, ZINC02043006, ZINC34466451, ZINC34532900 and ZINC39158384 in the binding sites of modeled HA and NA proteins were shown in Fig. 6a, b. Residues of hemagglutinin protein involved in the formation of hydrogen bonds with six compounds are CYS303, TYR306, PRO297,

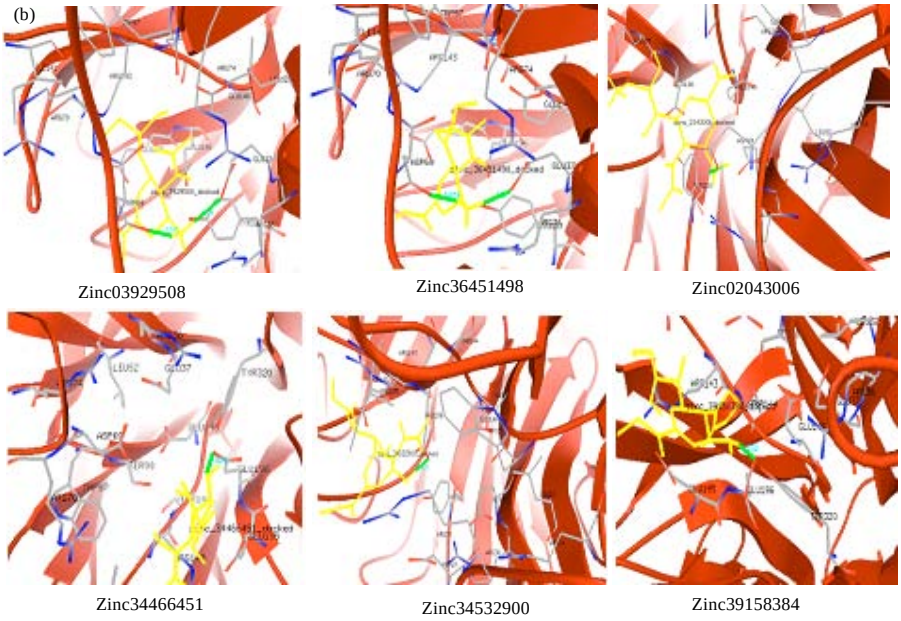

Fig. 6(b): The docking poses of the six candidates in NA

THR299, THR391 and TRP419 are shown in Fig. 6a. Residues of neuraminidase protein involved in the formation of hydrogen bonds with six compounds are ASP69, GLU37 and GLU196 are shown in Fig. 6b.

CONCLUSION

The hemagglutinin (HA) and neuraminidase (NA) of influenza A viruses are the two drug targeting proteins for the drug discovery fighting with the current influenza pandemic. Homology models were built for HA (Accession No: ACZ97508) and NA (Accession No: ACZ97471) proteins of influenza A virus subtype H1N1. Models built had high reliability showed by Ramachandran plot. 131 compounds screened from ZINC database were docked with homology model of HA and NA proteins, respectively. After docking six compounds ZINC03929508, ZINC36451498, ZINC02043006, ZINC34466451, ZINC34532900 and ZINC39158384 were predicted as potent dual-target candidate drugs for H1N1. Yet pharmacological studies have to confirm it.

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