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Research Article
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INHIBITORY ACTIVITY OF HEMAGGLUTININ AND NEURAMINIDASE PROTEIN CASEPASE ACTIVITY IN SWINE FLU [H1N1]

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ABSTRACT

Influenza A virus (H1N1) currently prevailing in Asia causes fatal pneumonia and multiple organ failure in humans. The two principle polypeptides, the Hemagglutinin (HA) and the Neuraminidase (NA), which are the target for the neutralizing antibodies immune response. Despite intensive research, understanding of the characteristics of influenza A virus that determine its virulence is incomplete. There are various immune cells inactivation is causing swine flu. Therefore, current, hot task of influenza virus research is to look for a way how to get us closer to a universal vaccine. In this study aims to identify the 3D structure of H1N1-M2 channels, HA2 gp and eM2 protein structures of patient samples from swine flu. With an explicit water-membrane environment, the molecular docking studies were performed for Oseltamivir and Zanamivir, these two commercial drugs generally used to treat influenza A virus infection. It was found that their binding affinity to the H1N1-M2 channel is significantly lower than that to the H5N1-HA2 gp, M2 and eM2 protein channel, fully consistent with the recent report that the H1N1 swine virus was resistant to the 2 drugs. The findings the relevant analysis reported here might provide useful structural insights for developing effective drugs against the new swine flu virus.

KEY WORDS

Swine Flu, Hemagglutinin, Neuraminidase, Influenza A virus, Molecular Docking.

1. INTRODUCTION

The Swine flu is an infectious disease of swine and human, caused by influenza A virus subtype H1N1 [1]. The World Health Organization shows that worldwide more than 214 countries have reported laboratory confirmed cases of H1N1, including over 18,439 deaths. The virus was first detected in India in May 2009[2]. Since then outbreaks have been reported from many parts of the country. As of September 18, 2009, the total number of confirmed cases in India was 20,632 with 521 deaths.

Swine influenza A virus belongs to the viral family of Orthomyxoviridae. RNA virus with a segmented genome that is comprised of eight negative-sense and single-stranded RNA segments. These 8 segments encode eleven proteins [3] 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 Hl and N1[4][5].

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[6]. By the above two steps process, Hemagglutinin and Neuraminidase improve virus releasing and the spread of infection to new cells, respectively [7][8]. By blocking Hemagglutinin or Neuraminidase could prevent virus from invading into host cells [9][10]. Both Zanamivir (Relenza) and Oseltamivir (Tamiflu) are neuraminidase inhibitors [11]. All the Indian isolates possessed residue H274 (position 275 in NA numbering) a known marker for sensitivity to the Neuraminidase inhibitor, Oseltamivir

[12]. 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[12]. Reliability of models was checked by Ramachandran plot. The 131 compounds were screened from ZINC database[13] using the criteria drug like compounds having structural similarity greater than 80% with existing inhibitors Oseltamivir and Zanamivir of Neuraminidase protein. These screened compound are docked with homology model of HA and NA protein respectively. The aim was to find out potent candidates for HA and NA proteins for the 2009 outbreak of influenza A virus sub-type H1N1.

2. MATERIALS AND METHODS

2.1 SELECTION OF TARGET PROTEIN SEQUENCE:

The sequences of the nucleocapsid protein that is Hemagglutinin (HA), and Neuraminidase (NA) were taken from influenza database of NCBI [14]. The NCBI influenza virus sequence database contains protein sequences as well as nucleotide sequences, and their encoding regions derived from the nucleotide sequences. The Neuraminidase (NA) protein sequences were retrieved by putting the keywords Type: A, Host: Human, Country: India, Subtype: H1 and N1, Protein: NA, Sequence type: Protein. We got a list of 360 protein sequence of Neuraminidase (NA) of different regions of Indian strain from which sequences of eastern India were considered for research. Similar search was performed for HA (Hemagglutinin) and a list of 95 protein sequences from different regions of India of which sequences of Eastern India were considered.

About 15 sequences of Neuraminidase and 95 protein sequences of Hemagglutinin were downloaded in FASTA format for analysis. For model development, the sequences were analyzed in the Bio Edit program. It was found that all sequences were approximately of the same length. The longest sequence of NA with Accession No: ACZ97471 was selected for 3D model development that contains 469 amino acid residues with molecular weight 49954.19 Daltons. Similarly, the longest sequence of HA with Accession No: ACZ97508 was selected for model development that contains 566 amino acid residues with a molecular weight of 37190.18 Daltons.

2.2 TEMPLATE IDENTIFICATION

Hemagglutinin (HA) and Neuraminidase (NA) phylogenetic analyses were performed by the neighbor-joining method with the coding regions of HA and NA nucleotide sequences containing only unambiguous sequences by using MEGA5.10 software as shown in **Figure-1**. We have to find the template sequences for more similar sequences with >30% similarity. The templates of HA and NA were downloaded from protein databank with PDB ID 2WR1 and 3B7E. The sequence alignment of target protein with corresponding templates was performed by using Clustal W program.

2.3 HOMOLOGY MODELING

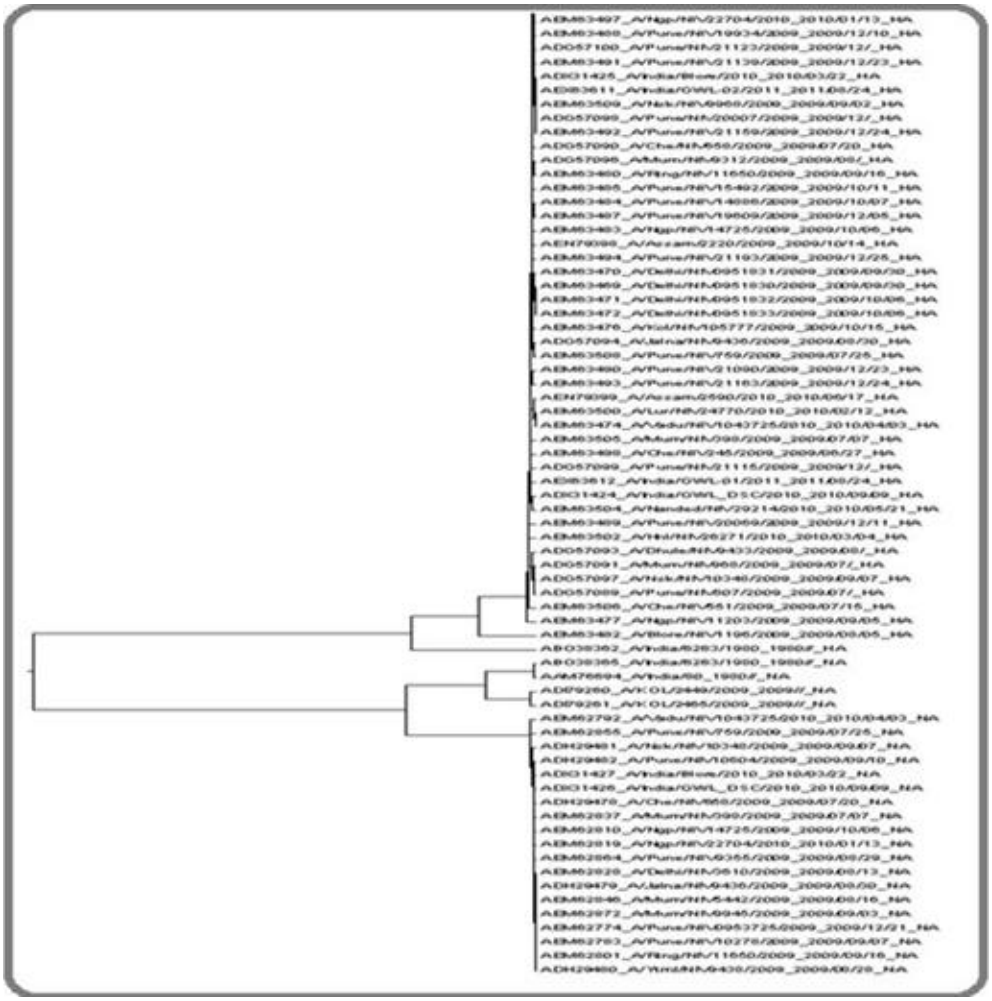
Homology Model of HA (Hemagglutinin) and NA (Neuraminidase) proteins were constructed by using Swiss Model program. After aligning queries with templates of HA protein 2WR1-A and template of NA protein 3B7E-A was used as input in Swiss model program and five comparative models were generated for each target. The model of HA (Hemagglutinin) and NA (Neuraminidase) was validated with the help of Molprobity program. Molprobity provides a detail of all atom contact analysis of any steric problems within the molecules and can calculate and display the H-bond and Vander Waals contacts in the interfaces between components. The validated HA (Hemagglutinin) and NA (Neuraminidase) models were chosen for further studies and refinement.

2.4 STRUCTURAL VALIDATION AND ANALYSIS

The newly built homology models often produce bond angles, unfavorable atomic distances and Vander Waal's radius overlapping and undesirable torsion angles. Therefore, it is more essential to minimize the energy to regularize local angle geometry and bond as well as to relax close contacts in a geometric chain [15]. Among the above models, the acceptable model was finalized by Ramachandran Plot, which provides the residue's position, in particular, segments based on PHI (ϕ) and PSI (ψ) angles between N-Ca and Ca-C atom of residues. After

the optimization procedure, the stereo chemical qualities of the models are checked by PROCHECK [16].

Figure-1: The Evolutionary analysis of HA and NA using NJ method



2.5 ACTIVE SITE PREDICTION

The Ligand binding site of HA (Hemagglutinin) and NA (Neuraminidase) proteins were predicted using Q-Site Finder program [17]. Q-Site Finder uses the interaction energy between the simple Vander Waal's probe and protein to locate energetically favorable binding sites.

2.6 ZINC DATABASE SCREENING

ZINC database contains over 15 million commercially available compounds in ready to dock, three-dimensional formats for structure based virtual screening. ZINC databases was screened using criteria drugs like,(XlogP should be less than or equal to 5, Hydrogen Bond Donor should be less than or equal to 5, Hydrogen Bond Accepter should be less than or equal to 10, Rotatable Bonds= 8, Polar surface Area= 150, Molecular Weight is less than 500). These compounds having similarity values from 80 to 99% with existing antiflu drug Oseltamivir and Zanamivir 3D structures. A total of 131compounds (77 compounds which are similar to Oseltamivir and 54 compounds, which are similar to Zanamivir) was screened using the above criteria for docking studies.

2.7 VIRTUAL SCREENING

Virtual screening of the entire all 131 compounds screened against HA (Hemagglutinin) and NA (Neuraminidase) model structures were done using molecular docking program AutoDock4 [18][19]. Gasteiger charges are added to the Ligand and maximum six numbers of active torsions are given to the lead compounds using AutoDock4 tool. The

salvation term and kollman charges were added to the modeled protein structure using AutoDock4 tool. A grid box was generated that was large enough to cover all protein catalytic sites and accommodate ligands to move freely. The genetic search algorithm was employed, and thirty search attempts were performed for each ligand with a population size of 150. Remaining docking parameters were set to the software default values. After docking as been done, the ligands were ranked according to their docked energy as implemented into the AutoDock4 program.

3. RESULTS AND DISCUSSION

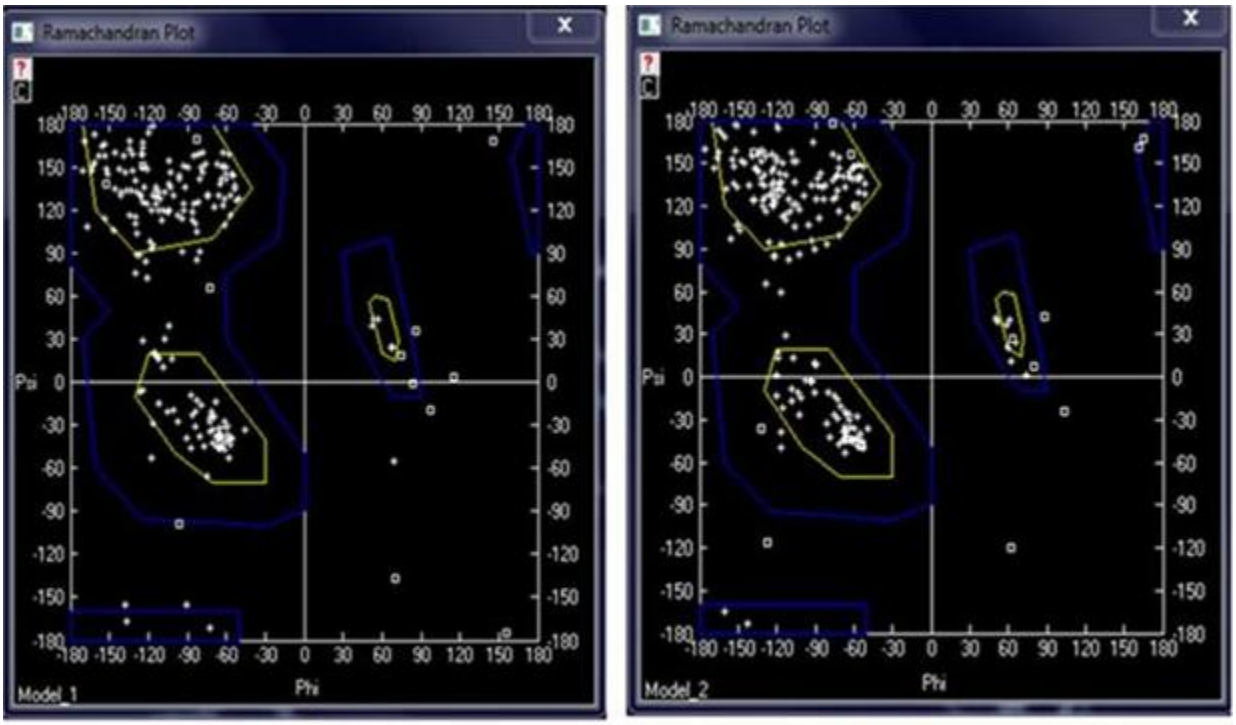
3.1 HOMOLOGY MODELING OF HEMAGGLUTININ PROTEIN

The sequence alignment of the query HA sequence (ACZ97508) of 2009-H1N1 virus and template HA (2WR1-A) of Indian influenza A virus [20]. 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 488 residues. Query sequence is modeled from 18 to 511 residues as shown in **Figure-2**.

Figure-2: 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 at ExPASy Server.

```
>[Template]|2wr1A|2.1| STRUCTURE OF INFLUENZA H2 HEMAGGLUTININ WITH HUMAN
          RECEPTOR
         Length = 490

 Score =  689 bits (1778), Expect = 0.0,   Method: Composition-based stats.
 Identities = 316/494 (63%), Positives = 391/494 (79%), Gaps = 6/494 (1%)

Query: 18   DTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNGKLCKLRGVAPLHLGKCNIAGW 77
            D +CIGYHANNST+ VDT+LE+NVTVTH+ ++LE  HNGKLC+L G+ PL LG C+IAGW
Sbjct: 1    DQICIGYHANNSTEKVDTILERNVTVTHAKDILEKTHNGKLCRLSGIPPLELGDCSIAGW 60

Query: 78   ILGNPECESLSTASSWSYIVETSSSDNGTCYPGDFIDYEELREQLSSVSSFERFEIFPKT 137
            +LGNPEC+ L +   WSYIVE  +  NG CYPG F DYEEL+  ++SV+ FE+ +I P+
Sbjct: 61   LLGNPECDRLLSVPEWSYIVEKENPVNGLCYPGSFNDYEELKHLITSVTHFEKVKILPR- 119

Query: 138  SSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLSKSYINDKGKEVLVLWGIH 197
              W  H +  G + AC      SF++N++WL KKG++YP   +SY N  G+++L++WGIH
Sbjct: 120  DQWTQHTTTGG-SRACAVLDNPSFFRNMVWLTKKGSNYPIAKRSYNNTSGEQMLIIWGIH 178

Query: 198  HPSTSADQQSLYQNADAYVFVGSSRYSKKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKI 257
            HP+  A+Q++LYQN    YV VG+S  +K+  PEIA RPKV  Q GRM + WTL+E  D I
Sbjct: 179  HPNDDAEQRTLYQNVGTYVSVGTSTLNKRSIPEIATRPKVNGQGGRMEFSWTLLETWDVI 238

Query: 258  IFEATGNLVVPRYAFAMERNAGSGIIISDTPVHDCNTTCQTPKGAINTSLPFHNIHPITI 317
             FE+TGNL+ P Y F + +   SGI+ ++  + +C T CQTP GAINT+LPFHNIHP+TI
Sbjct: 239  NFESTGNLIAPEYGFKISKRGSSGIMKTEKTLENCETKCQTPLGAINTTLPFHNIHPLTI 298

Query: 318  GKCPKYVKSTKLRLATGLRNVPSIQSRGLFGAIAGFIERGWTGMVDGWYGYHHQNEQGSG 377
            G+CPKYVKS +L LATGLRNVP     +GLFGAIAGFIE GW GMVDGWYGYHH N+QGSG
Sbjct: 299  GECPKYVKSDRLVLATGLRNVP----QGLFGAIAGFIEGGWQGMVDGWYGYHHSNDQGSG 354

Query: 378  YAADLKSTQNAIDEITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFLDIWT 437
            YAAD +STQ A D ITNKVNSVIEKMNTQF AVGKEF++LE+R+ENLNKK++DGFLD+WT
Sbjct: 355  YAADKESTQKAFDGITNKVNSVIEKMNTQFEAVGKEFSNLERRLENLNKKMEDGFLDVWT 414

Query: 438  YNAELLVLLENERTLDYHDSNVKNLYEKVRSQLKNNAKEIGNGCFEFYHKCDNTCMESVK 497
            YNAELLVL+ENERTLD+HDSNVKNLY+KVR QL++N KE+GNGCFEFYHKCD+ CM SVK
Sbjct: 415  YNAELLVLMENERTLDFHDSNVKNLYDKVRMQLRDNVKELGNGCFEFYHKCDDECMNSVK 474

Query: 498  NGTYDYPKYSEEAK 511
            NGTYDYPKY EE+K
Sbjct: 475  NGTYDYPKYEEESK 488
```

Figure-3: Ramachandran Plot of modeled HA protein of influenza A virus subtypes H1N1.



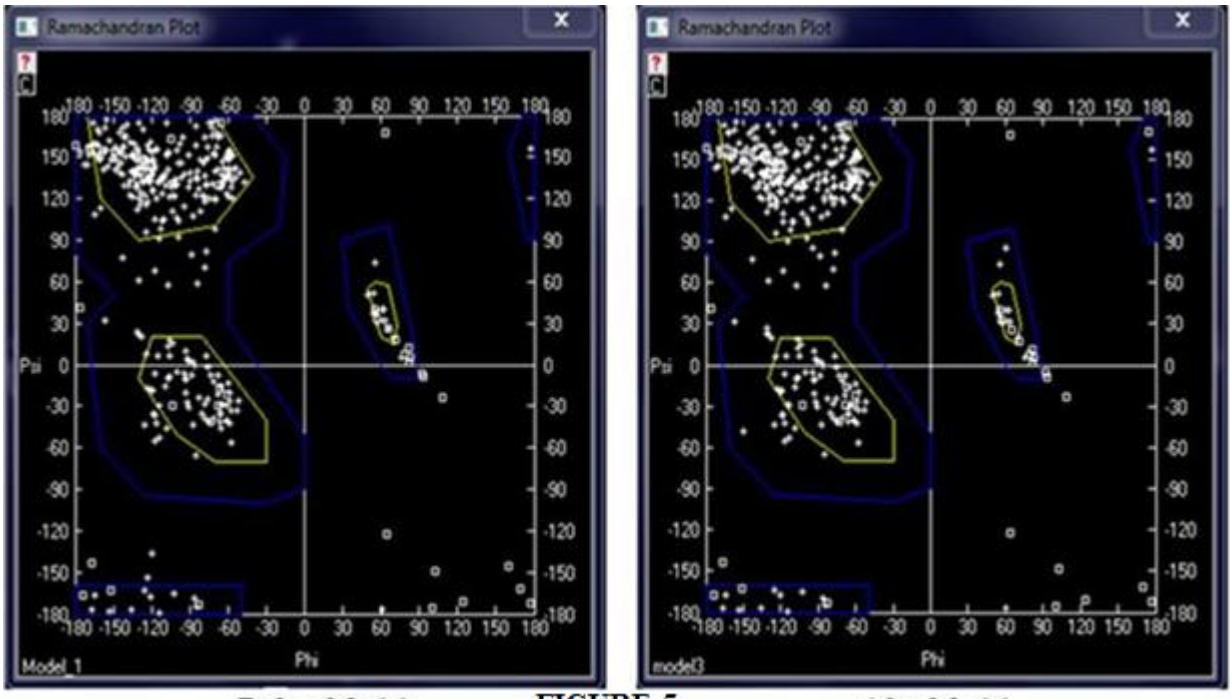
Before Model **FIGURE-3** After Model

Figure-4: The sequence Alignment of the query NA sequence (ACZ97471) of influenza A virus and the template NA (3B7E-A) of 1918-H1N1 virus. Secondary Structures of the query NA protein was predicted using program SOPMA at ExPASy Server.

```
>[Template]|3b7eA|1.45| Neuraminidase of A Brevig Mission 1 1918 H1N1
          strain in complex with zanamivir
          Length = 385

 Score =  713 bits (1841), Expect = 0.0,   Method: Composition-based stats.
 Identities = 342/385 (88%), Positives = 369/385 (95%)

Query: 83  VKLAGNSSLCPVSGWAIYSKDNSIRIGSKGDVFVIREPFISCSPLECRTFFLTQGALLND 142
           V L GN SLCP+SGWAIYSKDN IRIGSKGDVFVIREPFISCS LECRTFFLTQGALLND
Sbjct: 1   VILTGNSSLCPISGWAIYSKDNGIRIGSKGDVFVIREPFISCSHLECRTFFLTQGALLND 60

Query: 143 KHSNGTIKDRSPYRTLMSCPIGEVPSPYNSRFESVAWSASACHDGISWLTIGISGPDNGA 202
           KHSNGT+KDRSPYRTLMSCP+GE PSPYNSRFESVAWSASACHDG+ WLTIGISGPDNGA
Sbjct: 61  KHSNGTVKDRSPYRTLMSCPVGEAPSPYNSRFESVAWSASACHDGMGWLTIGISGPDNGA 120

Query: 203 VAVLKYNGIITDTIKSWRNNILRTQESECACVNGSCFTVMTDGPSDGQASYKIFRIEKGK 262
           VAVLKYNGIITDTIKSWRNNILRTQESECACVNGSCFT+MTDGPS+GQASYKI +IEKGK
Sbjct: 121 VAVLKYNGIITDTIKSWRNNILRTQESECACVNGSCFTIMTDGPSNGQASYKILKIEKGK 180

Query: 263 IVKSVEMNAPNYHYEECSCYPDSSEITCVCRDNWHGSNRPWVSFNQNLEYQIGYICSGIF 322
           + KS+E+NAPNYHYEECSCYPD+ ++ CVCRDNWHGSNRPWVSF+QNL+YQIGYICSG+F
Sbjct: 181 VTKSIELNAPNYHYEECSCYPDTGKVMCVCRDNWHGSNRPWVSFDQNLDYQIGYICSGVF 240

Query: 323 GDNPRPNDKTGSCGPVSSNGANGVKGFSFKYGNGVWIGRTKSISSRNGFEMIWDPNGWTG 382
           GDNPRPND TGSCGPVSSNGANG+KGFSF+Y NGVWIGRTKS SSR+GFEMIWDPNGWT
Sbjct: 241 GDNPRPNDGTGSCGPVSSNGANGIKGFSFRYDNGVWIGRTKSTSSRSGFEMIWDPNGWTE 300

Query: 383 TDNNFSIKQDIVGINEWSGYSGSFVQHPELTGLDCIRPCFWVELIRGRPKENTIWTSGSS 442
           TD++FS++QDIV I +WSGYSGSFVQHPELTGLDC+RPCFWVELIRG+PKENTIWTSGSS
Sbjct: 301 TDSSFSVRQDIVAITDWSGYSGSFVQHPELTGLDCMRPCFWVELIRGQPKENTIWTSGSS 360

Query: 443 ISFCGVNSDTVGWSWPDGAELPFTI 467
           ISFCGVNSDTVGWSWPDGAELPF+I
Sbjct: 361 ISFCGVNSDTVGWSWPDGAELPFSI 385
```

FIGURE-4

Figure-5: Ramachandran Plot of modeled NA protein of influenza A virus subtypes H1N1.



Before Model FIGURE-5 After Model

Figure-6: Binding Site Analysis of HA Protein.

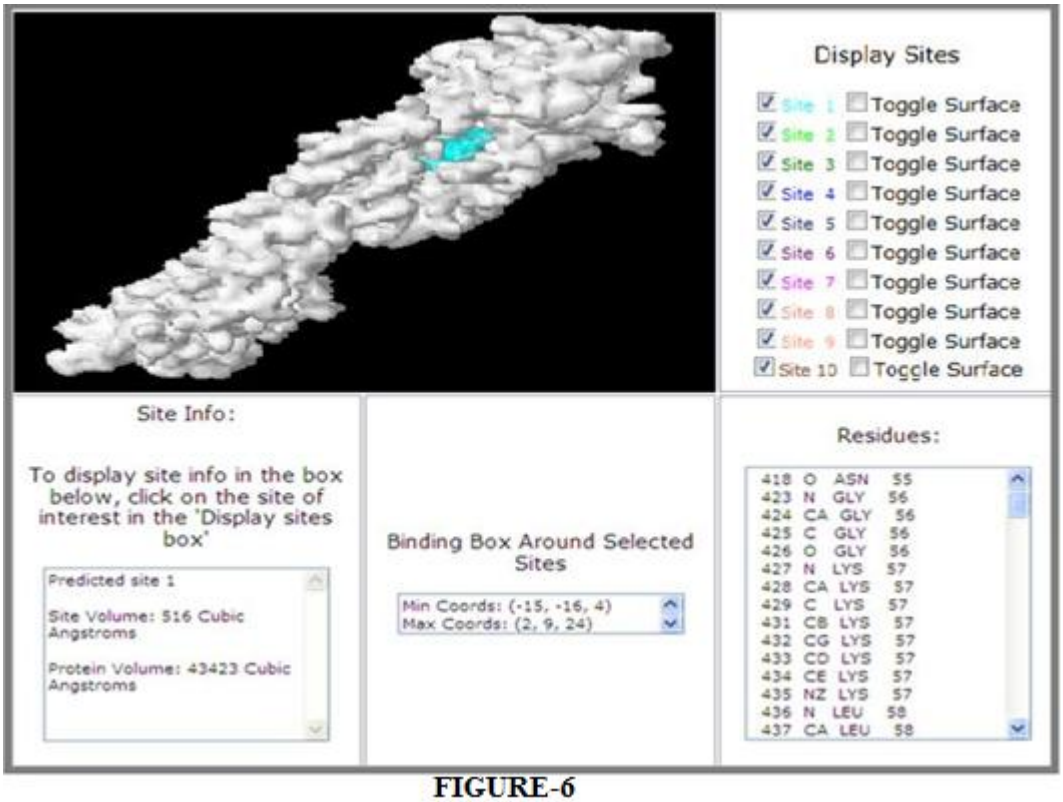


FIGURE-6

Figure-7: Binding Site Analysis of NA Protein.

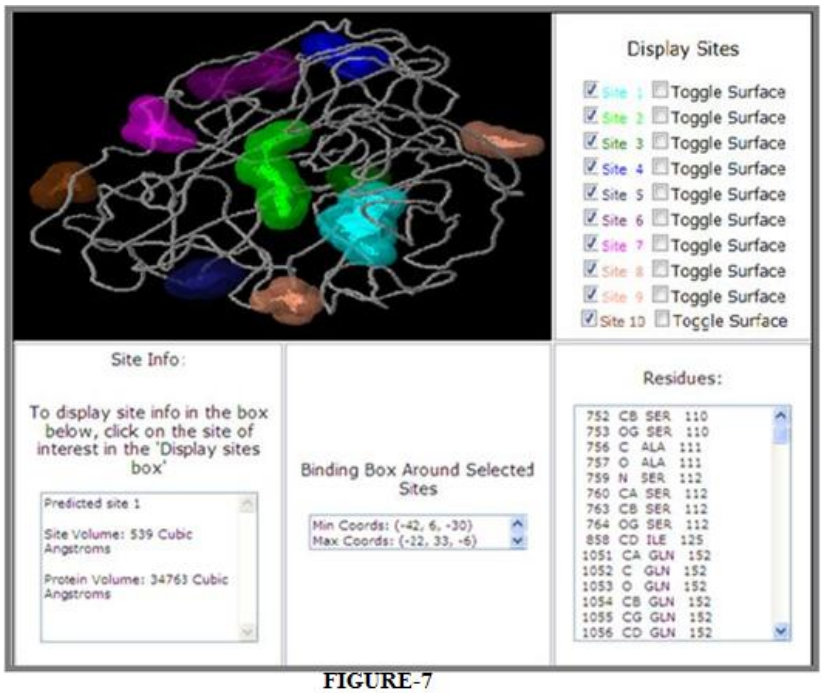


FIGURE-7

Figure-8: The chemical structures of all six candidates.

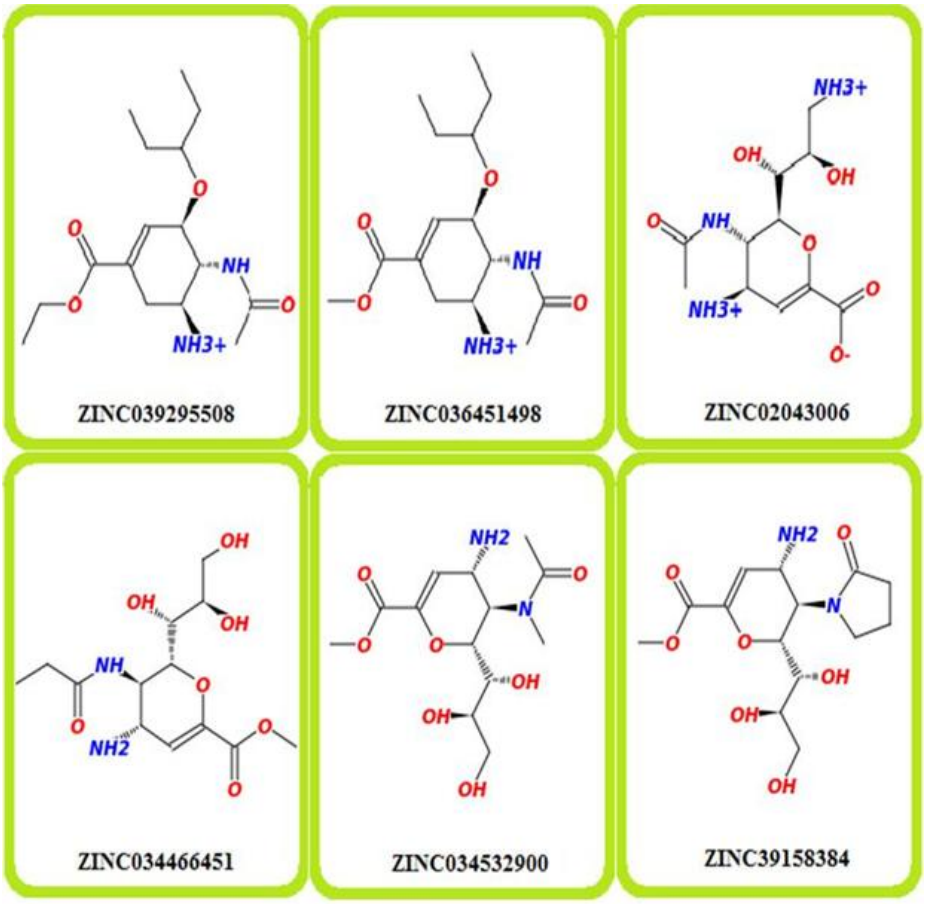


FIGURE-8

Figure-9: The docking poses of 6 candidates in HA.

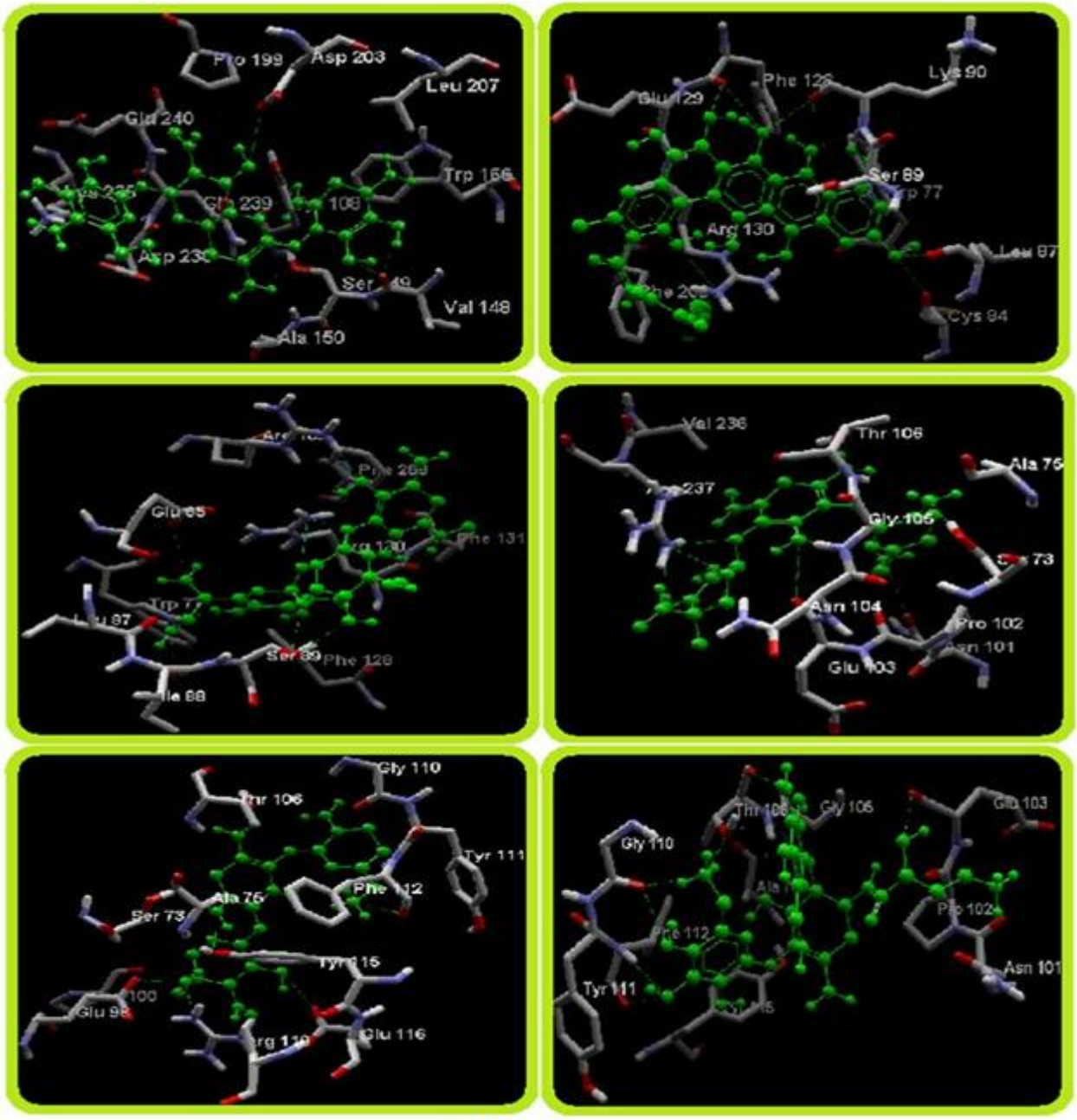
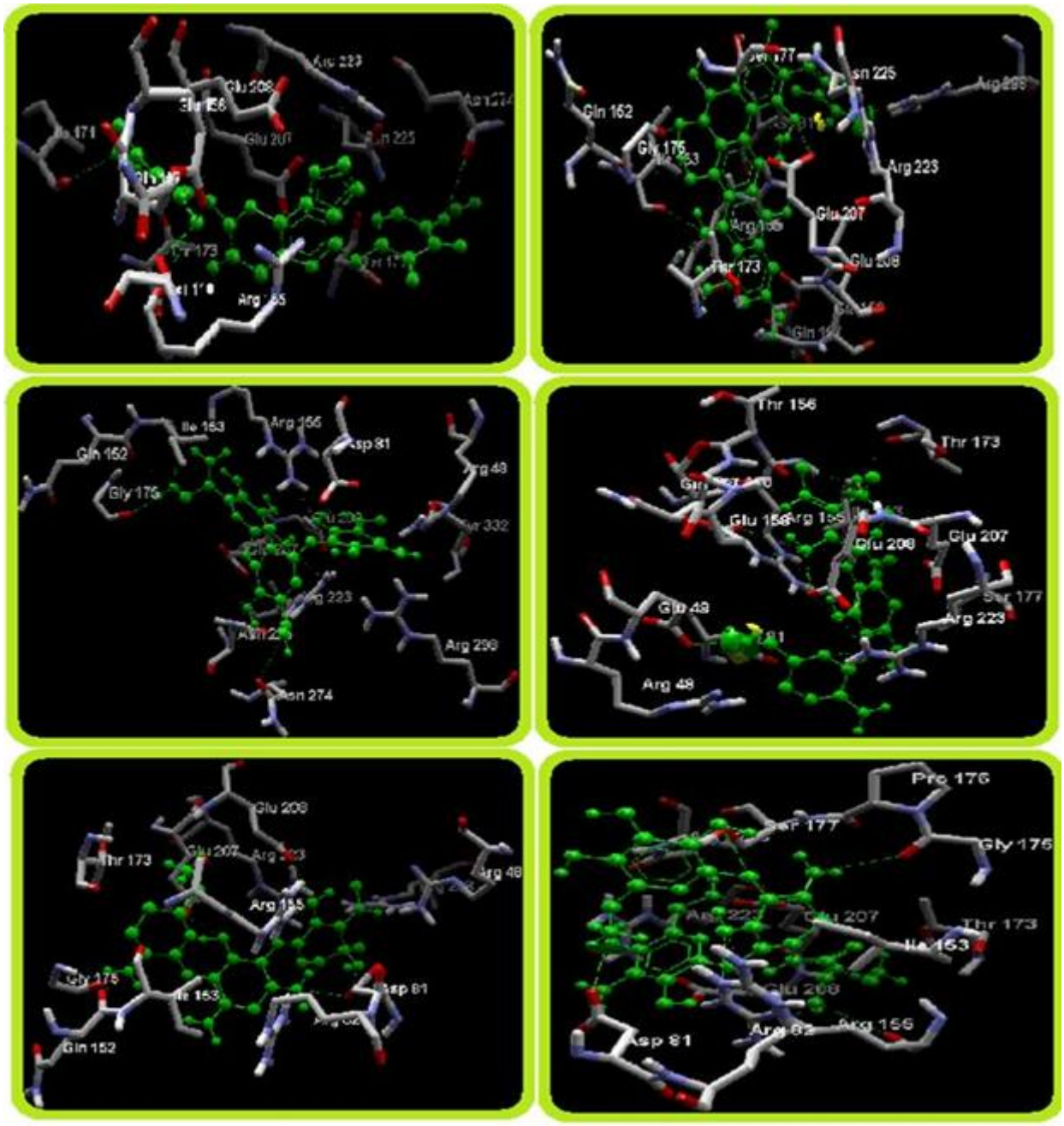


FIGURE-9

Figure-10: The docking poses of 6 candidates in NA.



The sequence identity and similarity of HA protein was 63% and 79% respectively. The result of alignment was employed to build new homology model. Reliability of new homology model for Hemagglutinin was identified by Ramachandran Plot. After optimization and energy minimization process, the best model was selected from 3D models generated for HA protein on the basis of Swiss Model.

Energy minimization of 3D structure is vital for providing the maximum stability to the protein. Ramachandran Plot drawn through Swiss Pdb Viewer program [16]validated the model with 90.7% of the total residues in most favoured region and residues in additional allowed region was 7.1 and 1.6% in the generously allowed region. This stipulates that protein that backbone dihedral angle PHI (ϕ) and PSI

(ψ) occupied reasonably accurate position in the selected 3D model as shown in **Figure-3**.

Only 3 residues, ALA138, GLN193 and TYR362 were located in the disallowed region, which constituted 0.6% of the total protein.

3.2 HOMOLOGY MODELING OF NEURAMINIDASE (NA) PROTEIN

The sequence alignment of the query NA sequence (ACZ97471) of 2009-H1N1 virus and template NA (3B7E-A) of 1918 H1N1 virus [21]. 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 sequence is modeled from 83 to 467 residues. The sequence identity and similarity of NA protein were 88% and 95% respectively as shown in **Figure-4**. The result of alignment was employed to build new homology model. The reliability of new homology model for Neuraminidase (NA) was identified by Ramachandran Plot. After optimization and energy minimization process the best model was selected from 3D models generated for NA protein on the basis of Swiss Model.

Energy minimization of three dimensional structures is vital for providing the maximum stability to the protein. Ramachandran Plot drawn through Swiss Pdb Viewer program[16] validated the model with 87.3% of the total residues in most favoured region and 1.2% in the generously allowed region and residues in additional allowed region was 11.1 as shown in **Figure-5**. This stipulates that protein that backbone dihedral angle PHI (ϕ) and PSI (ψ) occupied reasonably accurate position in the selected 3d model.

Only 2 residues, THR156 and SER330 were located in the disallowed region, which constituted 0.4% of the total protein.

3.3 ACTIVE SITE AND LIGAND BINDING SITE PREDICTION

The Ligand binding site of Hemagglutinin and Neuraminidase proteins were predicted using Q-Site Finder program which is energy based sites [22]. It uses the energy interaction between the simple Vander Waals probe and protein to locate energetically favorable binding sites.

3.3.1 BINDING SITE ANALYSIS OF HA PROTEIN

The result of Q-site finder shows that predicted binding site cavity volume modeled HA protein was 516 cubic angstroms and the coordinates of the binding box around predicted site had minimum coordinates is [-15,-16,4] and maximum coordinates is [2,9,24]. Binding site of HA was constituted by amino acid residues ASN55, GLY56, LYS57, LEU58, CYS59, LEU79, ASN81, PRO82, GLU83, GLU85, SER88, GLU98, THR99, SER102, ARG155, GLU207, ALA261, THR262, ASN264, GLY275, ARG298, ILE389, ASP390 as shown in **Figure-6**.

3.3.2 BINDING SITE ANALYSIS OF NA PROTEIN

The result of Q-site finder shows that predicted binding site cavity volume modeled NA protein was 539 cubic angstroms and the coordinates of the binding box around predicted site had minimum coordinates is [-42,6,-30] and maximum coordinates is [-22,33,-6].

Binding site of NA was constituted by amino acid residues SER110, ALA111, SER112, ILE125, GLN152, ILE153, LEU154, ARG155, THR156, GLN157, GLU158, SER159, GLU160, CYS161, VAL162, PHE169, THR170, ILE171, MET172, THR173, ASP174, PRO176, SER177, TYR205, TYR206, GLU207, GLU208, CYS209, SER210, CYS211, LYS277 as shown in **Figure-7**.

3.4 RESULTS OF VIRTUAL SCREENING

Docking results predicted the interaction of ligands with protein and residues involved in this complex. For such interaction, the most important requirement was the proper orientation and conformation of ligand which fitted to the enzymes binding site appropriately and formed protein ligand complex. Therefore, optimal interactions and best AutoDock score were used as criteria to interpret the best conformation among the 30 conformations, generated by AutoDock program.

All the 131compounds were docked into structures of HA and NA. The docking results of 77 drug compounds and one known drug Oseltamivir with HA and NA models were shown in Appendix-A. Among the 77 compounds ZINC039295508 and ZINC36451498 had the lowest docking energy with both HA and NA respectively as shown in **Table-1**.

Table-1: Two compounds had lowest docked energy comparing with one known drug Oseltamivir.

Drug Compounds	Mol Wt	X LogP	HBD	HBA	PSA	Net Charge	RB	Docked Energy		Ref RMS	
								HA	NA	HA	NA
Oseltamivir	312.40	1.10	2	5	90.6	0	8	-02.61	-03.95	34.13	24.78
ZINC03929508	313.41	0.85	4	6	92	1	8	-16.65	-17.45	27.69	32.97
ZINC36451498	299.39	0.48	4	6	92	1	7	-15.96	-17.75	26.98	33.14

Table-2: Four compounds had lowest docked energy comparing with 1 known drug Zanamivir.

Drug Compounds	Mol Wt	X LogP	HBD	HBA	PSA	Net Charge	RB	Docked Energy		Ref RMS	
								HA	NA	HA	NA
Zanamivir	332.30	-3.20	7	10	201	0	6	-14.6	-16.77	24.19	31.08
ZINC02043006	290.29	-1.22	9	9	174	1	5	-15.97	-18.00	23.93	34.96
ZINC34466451	318.32	-2.04	6	9	151	0	7	-15.17	-17.31	25.49	37.55
ZINC34532900	318.32	-2.64	5	9	143	0	6	-15.10	-17.74	25.68	37.11
ZINC39158384	330.33	-2.27	5	9	143	0	6	-16.02	-18.09	26.21	38.29

The docking results of 54 drug compounds and one known drug Zanamivir with HA and NA models were shown in Appendix-B. Among the 54 drug compounds ZINC34532900, ZINC34466451, ZINC02043006 and ZINC39158384 had the lowest docking energy with both HA and NA models than other docked compounds as shown in **Table-2**.

On screening the docked results on the basis of the docking energy, it predicts that there are 6 drug candidates which inhibit both HA and NA structures. These compounds had lower docked energy and even lower than the standard controls, Zanamivir and Oseltamivir. In fact Zanamivir and Oseltamivir were commonly used as inhibitors for NA drug for previous H1N1. The chemical structure of all six compounds as shown in **Figure-8**.

Docking poses of the best conformation of 6 compounds ZINC03929508, ZINC02043006, ZINC36451498, ZINC34466451, ZINC34532900 and ZINC29158384 in the binding site of modeled HA proteins were shown in **Figure-9**.

Residues of Hemagglutinin protein involved in the formation of hydrogen bonds with 6 compounds are ARG155, GLU207, ASN225, GLY275, and ARG298.

Docking poses of the best conformation of 6 compounds ZINC03929508, ZINC02043006, ZINC36451498, ZINC34466451, ZINC34532900 and ZINC29158384 in the binding site of modeled NA proteins were shown in **Figure-10**.

Residues of Neuraminidase protein involved in the formation of hydrogen bonds with 6 compounds are ILE153, ARG155, and GLU207.

CONCLUSION

The Hemagglutinin (HA) and Neuraminidase (NA) of influenza A virus are two drug targeting proteins for the drug discovery fighting with current influenza virus pandemic. Homology model built for HA [Accession No: ACZ97508] and NA [Accession No: ACZ97471] proteins of influenza A virus subtypes H1N1.Models built had high reliability show by Ramachandran Plot. There are 131 compounds screened from ZINC database were docked with homology model of HA and NA proteins respectively. After docking 6 compounds ZINC039295508, ZINC36451498, ZINC02043006, ZINC34466451, ZINC39158384 and ZINC34532900, ZINC39158384 were predicted as potent dual target candidate drug for H1N1.

Hopefully we have proposed some useful candidates for H1N1 diseases. Yet finally pharmacological studies have to confirm, which is the best target candidate drug is for H1N1 disease.

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